54th Annual Maize Genetics Conference

Program and Abstracts

March 15 – March 18, 2012

DoubleTree by Hilton Hotel
Portland, Oregon

This conference received financial support from:

National Science Foundation
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Dow AgroSciences
BASF Plant Science
KWS
National Corn Growers Association
Biogemma

This conference received other financial support from:

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We thank these contributors for their generosity!

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Cover art:
Woman offering an ear of maize to the heavens,
Valle de Banderas town plaza, Nayarit Mexico

Photo by Josh Strable
Department of Genetics, Development and Cell Biology
Iowa State University

General Information

Meeting Registration

Thursday:	3:00 PM to 6:00 PM:	Table in Lloyd Center Foyer.
	9:00 PM to Midnight:	Table in the Exhibit Hall, near the entrance to the Posters
Friday:	7:00 AM to 8:30 AM:	Table in Lloyd Center Foyer.

Meals

All meals will be served buffet style during hours listed in the Program.
Meals on Thursday, Friday, and Saturday will be held in the Broadway/Weidler/Halsey rooms (near the lecture hall), in the Alaska/Idaho rooms near the front desk, and in the Cascade Ballroom on the second level.
Sunday breakfast will be served in the Willamette Ballroom near the lecture hall, in Broadway/Weidler/Halsey and in the Cascade Ballroom.

Coffee, tea and soft drinks will be available at no charge during the beverage breaks.

Talks and Posters

All Talks will be presented in the Multnomah /Holladay sections of the Lloyd Center Ballroom on the First Level.

Posters will be presented in the Exhibit Hall, across the driveway from the main hotel entrance (First Level). 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 even number posters are asked to stand by their posters 1:30-3 PM on Friday and 3-4:30 PM on Saturday. Presenters of odd numbered posters should stand by their posters 3-4:30 PM on Friday and 1:30-3 PM on Saturday.

The maize meeting is a forum for presentation and discussion of unpublished material. **Photographing or recording of talks and posters is not allowed**. For authors who give permission to view electronic copies of oral and/or poster presentations, PDF files will be available at MaizeGDB shortly after the meeting at the following URL:

http://maizegdb.org/maize_meeting/2012/downloads.php

Hospitality

After the evening sessions on Thursday and Friday there will be informal socializing and poster viewing in the Exhibit Hall, with refreshments provided until 1 AM. On Saturday evening there will be informal socializing, music, dancing and refreshments in the Cascade Ballroom until 2 AM. Refreshments will also be provided in the Exhibit Hall for those wanting a quieter venue.

After 1 AM, a double suite (rooms 1551 and 1555) is available for continued socializing. This is a “private party room” and alcoholic beverages may be brought in; however, you must stay in this room if you are carrying drinks. Please be sure to dispose of any trash and bottles in the party room, as well.

Steering Committee

Please share your suggestions and comments about the meeting with the 2012 Steering Committee:

John Fowler, Chair (fowlerj@science.oregonstate.edu)
Phil Becraft , co-Chair (becraft@iastate.edu)
Paula McSteen, Treasurer (mcsteenp@missouri.edu)
Alice Barkan (abarkan@uoregon.edu)
Uta Paszkowski (uta.paszkowski@unil.ch)
Mary Alleman (alleman@duq.edu)
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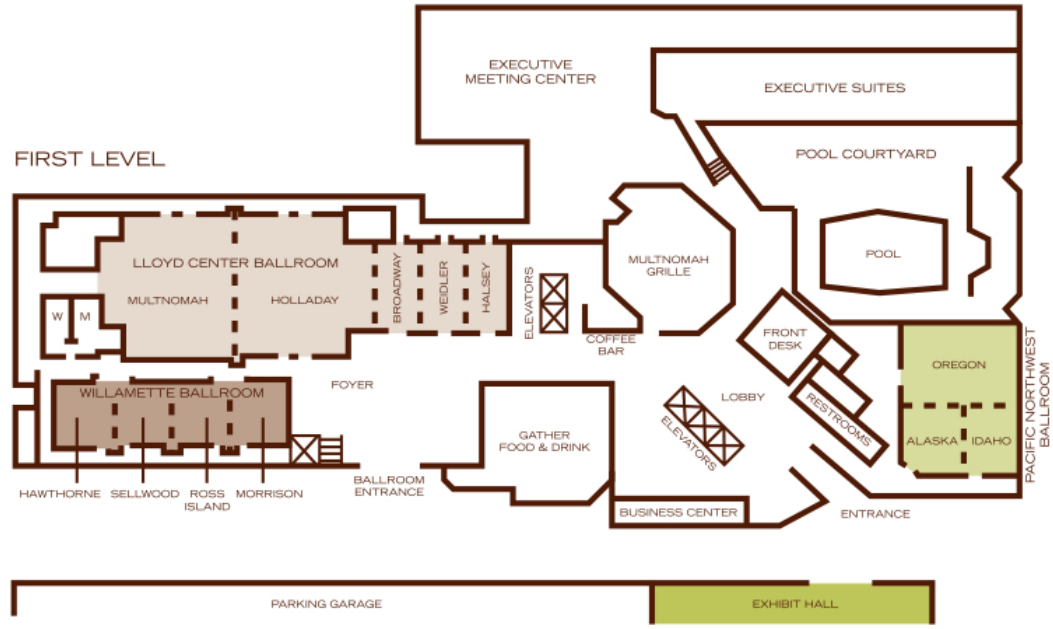
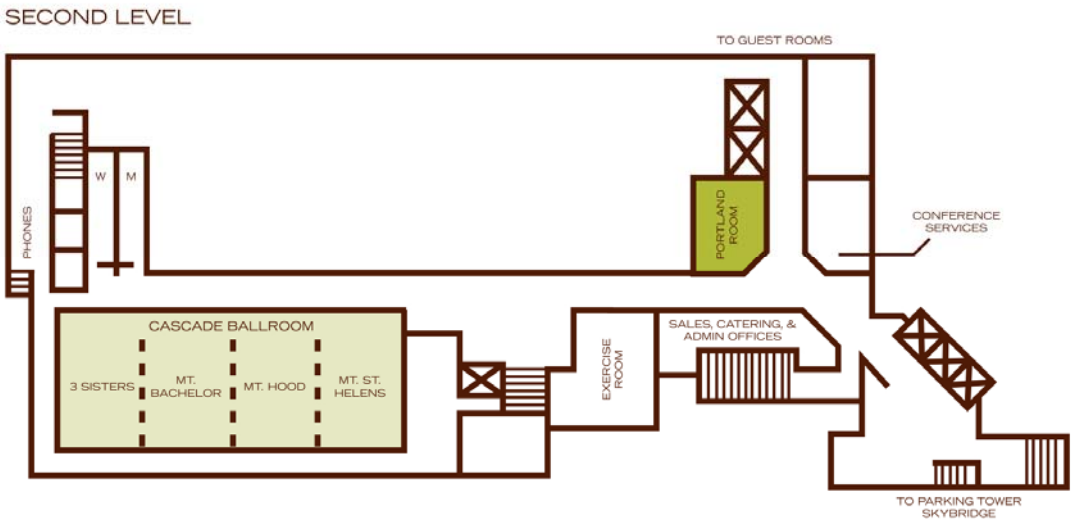
Mary Schaeffer, abstract coordinator, ex officio (Mary.Schaeffer@ars.usda.gov)
Carson Andorf, abstract coordinator, ex officio (Carson.Andorf@ars.usda.gov)

Acknowledgements

Many thanks go to Carson Andorf and Mary Schaeffer for their tremendous efforts in organizing, assembling, and advertising the conference program. We also thank Angela Freemyer and her team at the University of Missouri Conference Center for helping to organize the conference, handling registration and dealing with a multitude of other issues. Special thanks are also extended to Megan Haupt and the Doubletree staff for their help in organizing this conference, and to Darwin Campbell for providing AV and other support. Thanks go to Phil Becraft, Robert Bensen, Mark Cigan, Paula McSteen, Uta Paszkowski, Anne Sylvester, and John Fowler for their efforts in securing funding to support graduate student attendance at this meeting. An *ad hoc* Education, Outreach and Diversity committee is responsible for the MaGNET program and awards this year (page vii); in addition to the Chair, Co-Chair and Treasurer, thanks to Anne Sylvester and Brent Buckner for serving on this committee. Finally, many thanks go to Marty Sachs for his wisdom in all things related to the Maize Meeting.

Layout of the Doubletree Hotel- Lloyd Center





The MaGNET Program and 2012 Awards

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

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

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

2012 MaGNET Awardees

Undergraduate

Katarina Klusman, Oakland University	Poster #248
Juan Mendoza, San Jose City College & USDA/PGEC	
Jessica Wedow, Purdue University	Poster #75

Graduate Student

Dale Brunelle, University of North Dakota	Poster #94
Hema Kasisomayajula, University of South Carolina	Poster #250
Laura Morales, Cornell University	
Daniel Vera, Florida State University	Poster #292

Postdoctoral Researcher

Amy Hauth, University of Missouri	Poster #270
Thelma Madzima, Florida State University	Talk #29, Sunday 8:50 am
Stacey Simon, University of Delaware	

Faculty Mentor Accompanying Student

Bill Sheridan, University of North Dakota	Poster #311

The MaGNET program of the Maize Genetics Conference is supported by grant MCB-1212758 from the National Science Foundation.

Schedule of Events

Talks will be held in the Lloyd Center Ballroom.
Posters will be displayed in the Exhibit Hall.

Thursday, March 15

3:00 PM – 6:00 PM	**REGISTRATION** (Near Main Lobby)	
3:00 PM – 6:00 PM	**POSTER HANGING** (Exhibit Hall)	
3:00 PM – 6:00 PM	**OPTIONAL PRE-CONFERENCE WORKSHOPS** **MaizeGDB "How to" Tutorials** (Oregon Room) Three sessions, beginning on each hour, pre-registration recommended	
6:00 PM – 7:00 PM	**DINNER** (Broadway/Weidler/Halsey rooms, Cascade Ballroom, Alaska/Idaho rooms)	
7:00 PM – 7:15 PM	**WELCOME AND ANNOUNCEMENTS** (Lloyd Center Ballroom) John Fowler	
7:15 PM – 9:00 PM	**SESSION 1 – PLENARY TALKS** Chair: Paula McSteen	Pages 21 & 22
7:15 PM	**Bill Tracy, University of Wisconsin-Madison** *Plant Breeding: the Creative Power of Selection*	[Plen 1]
8:05 PM	**Jenny Graves, Australian National University** *Weird mammal genomics and sex; the power of evolutionary divergence*	[Plen 2]
9:00 PM – 12:00 AM	**REGISTRATION** (Exhibit Hall)	
9:00 PM – 1:00 AM	**INFORMAL POSTER VIEWING & HOSPITALITY** (Exhibit Hall)	

Friday, March 16

7:00 AM – 8:00 AM	**BREAKFAST** (Broadway/Weidler/Halsey rooms, Cascade Ballroom, Alaska/Idaho rooms)
7:00 AM – 8:15 AM	**REGISTRATION** (Foyer of Lloyd Center Ballroom)
8:00 AM – 8:15 AM	**ANNOUNCEMENTS** (Lloyd Center Ballroom)
8:15 AM – 10:15 AM	**SESSION 2 - CELL AND DEVELOPMENTAL BIOLOGY** Chair: Nathan Springer Talks 1-6. Pages 25-28
8:15 AM	**Thomas Hartwig, Purdue University** [T1] *The GRAS transcription factor upright leaf angle1 encodes a monocot-specific brassinosteroid function for leaf angle control in maize*
8:35 AM	**Carolyn Rasmussen, University of Wyoming** [T2] *Leaf boundaries: Live cell imaging of ligule development in mutants and non-mutants*
8:55 AM	**Fang Yang, Cold Spring Harbor Laboratory** [T3] *Abnormal phyllotaxy2 (Abphyl2): A new locus controlling phyllotaxy and shoot meristem size in maize*
9:15 AM	**Wei Li, Iowa State University** [T4] *Characterization and Cloning of tassels replace upper ear1 in Maize*
9:35 AM	**Claire Bendix, University of California, Berkeley** [T5] *GIGANTEA OF ZEA MAYS1A is a negative regulator of maize flowering time*
9:55 AM	**Timothy Kelliher, Stanford University** [T6] *Hypoxia triggers plant germline specification*
10:15 AM	**BREAK**
10:45 AM – 12:25 PM	**SESSION 3 – BIOCHEMICAL AND MOLECULAR GENETICS** Chair: Alice Barkan Talks 7-11. Pages 29-31
10:45 AM	**Xiaoguo Zhang, University of California, San Diego** [T7] *PANGLOSS2, A Receptor-Like Protein that Polarizes Cell Division in Maize Identified Via Quantitative Proteomics*
11:05 AM	**Leila Feiz, Cornell University** [T8] *RAF1, a new regulator of Rubisco biogenesis in maize*
11:25 AM	**Uta Paszkowski, University of Lausanne** [T9] *Essential factors for arbuscular mycorrhizal symbiosis from maize and rice*
11:45 AM	**Nick Carpita, Purdue University** [T10] *Defining expression networks in maize for secondary wall biogenesis*
12:05 PM	**Nathalie Bolduc, USDA-ARS and U. of California, Berkeley** [T11] *Unraveling the KNOTTED1 regulatory network in maize meristems*
12:30 PM – 1:30 PM	**LUNCH** (Broadway/Weidler/Halsey rooms, Cascade Ballroom, Alaska/Idaho rooms)

Friday, March 16 (continued)

1:30 PM – 4:30 PM **POSTER SESSION 1** (Exhibit Hall)
Presenters should be at even numbered posters from 1:30 PM to 3:00 PM.
Presenters should be at odd numbered posters from 3:00 PM to 4:30 PM.
Beverages will be available from 3:30 PM to 5:00 PM.

5:00 PM – 6:15 PM **SESSION 4 – NEW RESOURCES AND APPROACHES FOR MAIZE BIOLOGY** (Lloyd Center Ballroom)
Chair: Mark Cigan Talks 12-16. Pages 32-34

5:00 PM **Sanzhen Liu, Iowa State University** [T12]
Gene Mapping via Bulked Segregant RNA-Seq (BSR-Seq)

5:15 PM **Cinta Romay, Cornell University** [T13]
Maize inbred lines at the NCRPIS: a great public resource to explore maize genetics

5:30 PM **Taner Sen, USDA-ARS and Iowa State University** [T14]
MaizeCyc: Metabolic Networks in Maize

5:45 PM **Laurel Cooper, Oregon State University** [T15]
Annotating the Maize B73 Gene Expression Atlas in the Plant Ontology- A Tool for Plant Genomics

6:00 PM **Paul Zurek, Duke University** [T16]
Characterization of the root system architecture of the Nested Association Mapping (NAM) parent lines

6:30 PM – 7:45 PM **DINNER** (Broadway/Weidler/Halsey rooms, Cascade Ballroom, Alaska/Idaho rooms)

8:00 PM – 1:00 AM **INFORMAL POSTER VIEWING & HOSPITALITY** (Exhibit Hall)

Saturday, March 17

7:00 AM – 8:15 AM	**BREAKFAST** (Broadway/Weidler/Halsey rooms, Cascade Ballroom, Alaska/Idaho rooms)	
8:15 AM – 10:15 AM	**SESSION 5 – QUANTITATIVE GENETICS** (Lloyd Center Ballroom) Chair: Bob Bensen	Talks 17-22. Pages 35-38
8:15 AM	**Jason Peiffer, Cornell University** *The Genetic Architecture of Maize Height: A few loci can't explain the long and the short of it*	[T17]
8:35 AM	**Ivan Baxter, USDA-ARS** *Ionomics of the Maize Nested Association Mapping Population*	[T18]
8:55 AM	**Justin Gerke, University of Missouri** *The genomic response to reciprocal recurrent selection in BSSS and BSCB1*	[T19]
9:15 AM	**Yinping Jiao, China Agricultural University** *Pattern of genome-wide genetic changes throughout modern maize breeding*	[T20]
9:35 AM	**Juliana Teixeira, University of Delaware** *A Decade of Tropical to Temperate Maize Adaptation Reveals a Potential Mechanism for Broad Adaptation*	[T21]
9:55 AM	**Rob Martienssen, Cold Spring Harbor Laboratory** *The maize methylome: function, diversity, and inheritance* (Previously **Jim Holland** [T22] moved to Sunday at 10:20 AM)	[T32]
10:15 AM – 10:45 AM	**BREAK**	
10:45 AM – 12:25 PM	**SESSION 6 – COMPUTATIONAL APPROACHES TO MAIZE GENETICS** Chair: Peter Balint-Kurti	Talks 23-27. Pages 39-41
10:45 AM	**Camille Rustenholz, Iowa State University** *Identification, characterization and mapping of teosinte genes absent from the maize genome*	[T23]
11:05 AM	**Candice Hansey, Michigan State University** *Maize (Zea mays L.) Genome Diversity as Revealed by RNA-sequencing*	[T24]
11:25 AM	**Anja Paschold, University of Bonn** *Complementation contributes to transcriptome complexity in maize (Zea mays L.) hybrids relative to their inbred parents*	[T25]
11:45 AM	**Fangpu Han, Chinese Academy of Sciences** *A de novo centromere on Duplication 3a in the absence of canonical centromere sequence arrays*	[T26]
12:05 PM	**Donald McCarty, University of Florida** *Modeling of Mu transposon targeting reveals cryptic features of maize gene structure*	[T27]

Saturday, March 17 (continued)

12:30 PM – 1:30 PM **LUNCH** (Broadway/Weidler/Halsey rooms, Cascade Ballroom, Alaska/Idaho rooms)

1:30 PM – 4:30 PM **POSTER SESSION 2** (Exhibit Hall)
Presenters should be at odd numbered posters from 1:30 PM to 3:00 PM.
Presenters should be at even numbered posters from 3:00 PM to 4:30 PM.
Beverages will be available from 3:30 PM to 5:00 PM.

5:00 PM – 5:30 PM [Optional] **COMMUNITY SESSION - Maize Genetics Executive Committee**
MGEC Chair: Tom Brutnell (Lloyd Center Ballroom)

6:00 PM – 7:00 PM **DINNER**

7:15 PM – 9:00 PM **SESSION 7 – PLENARY TALKS** (Lloyd Center Ballroom)
Chair: John Fowler Pages 23 & 24

7:15 PM **Venkatesan Sundaresan, University of California, Davis** [Plen 3]
Female gametophytes and flowering plant reproduction: Green eggs and no SAM

8:05 PM **Thomas Dresselhaus, University of Regensburg** [Plen 4]
Germline Development and Function in Grasses

9:00 PM – 2:00 AM **INFORMAL POSTER VIEWING / HOSPITALITY** (Exhibit Hall)
DANCE (Cascade Ballroom)

Sunday, March 18

7:00 AM – 8:20 AM **BREAKFAST** (Broadway/Weidler/Halsey rooms, Cascade Ballroom, Willamette Ballroom)

Posters should be taken down by 9 am!

8:20 AM – 8:30 AM **ANNOUNCEMENTS**

8:30 AM – 9:50 AM **SESSION 8 – EPIGENETICS I**
Chair: Uta Paszkowski Talks 28-31. Pages 42-43

8:30 AM **Maike Stam, Universiteit van Amsterdam** [T28]
Mediator of paramutation 1 (Mop1) appears primarily involved in the establishment rather than maintenance of a silent chromatin structure

8:50 AM **Thelma Madzima, Florida State University** [T29]
Genome-wide effect of the mop1-1 mutation on chromatin structure in maize

9:10 AM **Steven Eichten, University of Minnesota** [T30]
Epigenetic variation among maize inbreds highlights complex genetic and transcriptional interactions

9:30 AM **Jonathan Gent, University of Georgia** [T31]
CHH Islands: a role for de novo methylation in near-gene chromatin regulation

9:50 AM **BREAK**

10:20 AM – 11:30 AM **SESSION 9 – EPIGENETICS II**
Chair: Phil Becraft Talks 32-34. Pages 44-45

10:20 AM **Jim Holland, USDA-ARS / North Carolina State University** [T22]
Genetic Control of Maize Photoperiod Response
(Previously **Rob Martienssen** [T32] moved to Saturday at 9:55 AM)

10:40 AM **Jay Hollick, University of California, Berkeley** [T33]
RNA polymerase IV regulates transcription of genes and transposable elements in maize

11:00 AM **Damon Lisch, University of California, Berkeley** [T34]
Recognition and inactivation of MuDR transposons involves multiple silencing pathways

11:30 AM **ADJOURNMENT**

Posters

Biochemical and Molecular Genetics

P1	**Haiming Zhao** <haiming223@163.com>	*β-tubulin6 involves in signaling of drought and hot stress in Zea mays*
P2	**Alberto Romero** <jar547@cornell.edu>	*A snapshot of the past: maize ancient DNA through targeted sequencing*
P3	**Anqi Xing** <Anqi.xing1984@gmail.com>	*A SNP mutation in Brachytic2 mildly influences maize plant height*
P4	**Sandeep Marla** <smarla@purdue.edu>	*Adult plant resistance in maize to a fungal pathogen: who would have guessed it?*
P5	**Jeff Gustin** <jgustin@ufl.edu>	*Advancing complex phenotype analyses through machine vision and computation*
P6	**Linlin Zheng** <linlin@wzw.tum.de>	*Analysis of benzoxazinoid biosynthesis in the maize lines B73 and Mo17, gene expression and QTL determination.*
P7	**Katja Machemer-Noonan** <machemer-noonan.1@osu.edu>	*Analysis of MYB Transcription Factors Involved in the Regulation of the Phenylpropanoid Pathway in Maize*
P8	**Alyssa Bagadion** <bagadiona@ufl.edu>	*Analysis of the mre*-594 maize seed development mutant with a maternal parent-of-origin effect*
P9	**Laura Matera** <lemmb2@mail.missouri.edu>	*bif42 Functions in Vegetative and Reproductive Development in Maize*
P10	**Vance Kramer** <vance.kramer@syngenta.com>	*Characterization And Partial Sequence Of The sh2-R Insertion*
P11	**Eliécer González Muñoz** <eliecergm070112@gmail.com>	*Characterization of Maize Group 1a-2 Purple Acid Phosphatases*
P12	**Robert Bruce** <rbruce@uoguelph.ca>	*Characterizing a wilted Zea mays mutant*
P13	**Jaime Hibbard** <hibbardj@missouri.edu>	*Characterizing Mutator insertions into sucrose transporters (SUTs) in maizeReinhold*
P14	**R. Frank Baker** <bakerrf@missouri.edu>	*Characterizing new maize genes that function in carbohydrate partitioning*
P15	**Kristen Leach** <leachka@missouri.edu>	*Characterizing the Biological Functions of and Genetic Redundancy among the Maize Sucrose Transporters*
P16	**Michael Swyers** <mjsc59@mail.missouri.edu>	*Characterizing the expression of TIE-DYED1, a novel protein that regulates carbohydrate partitioning in maize*
P17	**Ryan Gibson** <rgibson@dow.com>	*Classes of ABC Transporters in Maize (Zea mays L.) Genome*
P18	**Irina Makarevitch** <imakarevitch01@hamline.edu>	*Cloning and Characterization of brd1 – Maize Brassinosteroid C6 Oxidase Gene*
P19	**Brent Buckner** <bbuckner@truman.edu>	*Copy number variation (CNV) of genes involved in carotenoid biosynthesis in maize.*
P20	**Rosalind Williams-Carrier** <rozzz@uoregon.edu>	*Deep analysis of causal mutations in the Photosynthetic Mutant Library by Mu-Illumina sequencing: A progress report.*
P21	**Layton Peddicord** <laytonp@iastate.edu>	*Determining the genetic and environmental controls of surface hydrocarbon accumulation on maize silks*
P22	**Erin Finefield** <finefielde@missouri.edu>	*Determining the protein expression patterns of maize sucrose transporters*

P23	**Jay Hoch** <jayhoch@iastate.edu>	*Development of a strain of E. coli auxotrophic for Lysine, Methionine and Tryptophan and it's use for evaluation of amino acid balance in grains*
P24	**Cuixia Chen** <cxchen@sdau.edu.cn>	*Different resistance to southern corn rust of maize inbred lines in China*
P25	**Marna Yandeau-Nelson** <myn@iastate.edu>	*Dissecting the developmental and environmental effects on surface hydrocarbon accumulation on maize silks*
P26	**Nelson Garcia** <ngarcia@waksman.rutgers.edu>	*Divergence of gene regulation between maize and oat*
P27	**Kyle Logan** <kaillito@gmail.com>	*Early Endosperm Development in Maize*
P28	**Gretchen Spiess** <gemhdc@mail.umsl.edu>	*Evalutation of the role of IBA-derived IAA in Zea mays.*
P29	**Brent O'Brien** <bob2373@ufl.edu>	*Evolution and expression of the cellulose synthase (CesA) gene family in maize.*
P30	**Qinghua Wang** <qinghua@waksman.rutgers.edu>	*Evolution of bz Orthologous Regions in the Genus Zea and Relatives in the Andropogoneae*
P31	**Yuqing Xiong** <yqxiong@ufl.edu>	*Evolution of the maternally expressed gene1 (meg1) gene family in maize*
P32	**Allen Hubbard** <ahubbard@mail.smcvt.edu>	*Expression levels of several membrane transport proteins (Pip1, Pip2, and Sut1) and the photosynthetic enzyme Rubisco in the Zea Mays tie-dyed2 mutant*
P33	**Suchitra Chavan** <suchitra@uga.edu>	*Expression profiling and evolution of pathogenesis related genes in maize and teosinte in response to Ustilago maydis.*
P34	**Matt Estep** <mcestep@gmail.com>	*Foundations for Comparative Genomics: Preliminary Phylogeny of the Andropogoneae based on nuclear encoded markers.*
P35	**Federico Martin** <fmartin@ufl.edu>	*Functional analysis of RGH3/ZmURP domains suggests a splicing regulatory mechanism and uncovers multiple localization signals*
P36	**Wei Wu** <wuwei@iastate.edu>	*GBMAS: A Fast, Accurate and Cost-Effective Genotyping-by-Sequencing Technology using Ion Torrent*
P37	**Sivanandan Chudalayandi** <csiva@iastate.edu>	*Genetic and Biochemical analysis of Hairy Sheath Frayed mutation*
P38	**Weiwei Wen** <wenweiwei1982@gmail.com>	*Genetic basis and expression regulation underlying metabolite profiles in maize kernel*
P39	**Cromwell Kibiti** <ckibiti@purdue.edu>	*Genetic dissection of the temperature sensitivity of Rp1-D21, an autoactive R gene*
P40	**Ming Wang** <zhyl@hzau.edu.cn>	*Genome-wide Association Study of Resistance to Head Smut in Maize*
P41	**Sarah Hake** <hake@berkeley.edu>	*Identification and characterization of candy leaf1, a high glucan maize mutant*
P42	**Maria Casas** <casas.5@buckeyemail.osu.edu>	*Identification and Characterization of Gene Products Involved C-Glycosyl Flavones Formation in Maize*
P43	**Zhiming Zhang** <zhzhang@cshl.edu>	*Identification and prediction of miRNAs from maize seedling roots in response to lead (Pb) stress using deep sequencing*
P44	**Annett Richter** <annett.richter@pharmazie.uni-halle.de>	*Identification of herbivore-regulated plant defense pathways by Nested Association Mapping (NAM) and Genome Wide Association Study (GWAS)*
P45	**Claudia Lenk** <claudia.lenk@pharmazie.uni-halle.de>	*Identification of promoter regulatory sequences in the terpene synthases tps10 and tps23 in maize*

P46	**Tiffany Langewisch** <tllhw9@mail.missouri.edu>	*Identifying Candidates for the Restorer-of-Fertility Gene Rf3 in Maize CMS-S*
P47	**Stephanie Locke** <slocke@mail.smcvt.edu>	*Identifying Insertions in Phloem-Related Genes using UniformMu Maize*
P48	**R. Frank Baker** <bakerrf@missouri.edu>	*Laser-capture microdissection for the identification of genes involved in phloem function*
P49	**Bryan C. Gibbon** <bryan_gibbon@baylor.edu>	*Maize eukaryotic translation initiation Factor 5A is highly expressed in maize endosperm and is associated with an actin-rich cytoskeletal fraction*
P50	**Jake Withee** <jwithee@mail.smcvt.edu>	*Maize Sucrose Transporter Genes Display Distinct Circadian Expression Patterns*
P51	**Jun Zheng** <zhengjuncn@gmail.com>	*Maize ZmTCX8.1 gene contributes to abscisic acid signal transduction in transgenic Arabidopsis*
P52	**Martin Garcia** <masterfoodscience@live.com>	*Metabolic profiling of maize using DIESI-MS.*
P53	**Owen Hoekenga** <owen.hoekenga@ars.usda.gov>	*Metabolomic characterization of iron biofortified maize grain*
P54	**Michael Kolomiets** <kolomiets@tamu.edu>	*Modulating Lipid-Derived Signaling to Improve Corn Traits*
P55	**Sarit Weissmann** <SWeissmann@danforthcenter.org>	*Molecular genetic dissection of C4 malate transporter genes in maize*
P56	**Brady Barron** <bjb6x2@mail.missouri.edu>	*Molecular Mapping of a Carbon Partitioning Defective Mutant*
P57	**Chuck Dietrich** <charles.r.dietrich@monsanto.com>	*Monsanto Biotech Efforts Toward Doubling Yield of Corn in the US by 2030*
P58	**Clifford Weil** <cweil@purdue.edu>	*Natural Diversity and Genetic Modifiers of Expression Pattern in Leaf Mutants*
P59	**Karen Koch** <kekoch@ufl.edu>	*New releases from UniformMu in 2012: Seeds and sequences*
P60	**Rentao Song** <rentaosong@staff.shu.edu.cn>	*Opaque7 Encodes an Acyl Activating Enzyme-like Protein that Affects Storage Protein Synthesis in Maize Endosperm*
P61	**John Gray** <jgray5@utnet.utoledo.edu>	*Phylogenomic analysis of the Trihelix transcription factor family in grasses.*
P62	**Norman Best** <nbbest@purdue.edu>	*Propiconazole is a specific and accessible brassinosteroid biosynthesis inhibitor for Arabidopsis and maize*
P63	**Diwakar Dahal** <dahald@missouri.edu>	*Quantitative proteomics of total and mitochondrial proteins reveal specific changes that are associated with higher level of heterosis in maize hybrids*
P64	**Yongrui Wu** <yongrui@waksman.rutgers.edu>	*Regulation of the Nitrogen Sink in Maize Seed*
P65	**Yongrui Wu** <yongrui@waksman.rutgers.edu>	*Regulation of the Sulfur Sink in Maize Seed*
P66	**Montserrat Pages** <montse.pages@cragenomica.es>	*Role of plant specific N-terminal domain of maize CK2b1 subunit in CK2b functions and holoenzyme regulation.*
P67	**Elena Najar Duran** <elena.najar@cragenomica.es>	*SnRK2 family in maize: regulation of a Zinc-Finger transcription factor*
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P109 **Prem Chourey** <pschourey@ifas.ufl.edu>
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P110 **Andrea Gallavotti** <agallavotti@waksman.rutgers.edu>
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P111 **Amanda Wright** <amanda.wright@unt.edu>
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P113 **Gibum Yi** <gibumyi@gmail.com>
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P116 **Michael Pautler** <pautler@cshl.edu>
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P117 **Aaron Sluis** <asluis@berkeley.edu>
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P120 **Guo-Ling Nan** <gnan@stanford.edu>
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P123 **Awa N'Diaye** <awa8@hotmail.com>
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P125 **Michael Lewis** <mwlewis@berkeley.edu>
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P141 **Peter Rogowsky** <peter.rogowsky@ens-lyon.fr> — *PPR2263, a DYW-subgroup pentatricopeptide repeat protein, is required for mitochondrial nad5 and cob transcript editing, mitochondrion biogenesis and maize growth*

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P145 **Bailin Li** <Bailin.Li@cgr.dupont.com> — *Tasselless 1 (tls1) encodes a boron channel protein in maize*

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P150 **Peter Bommert** <bommert@cshl.edu> — *The interaction of fasciated ear2 and compact plant2 in controlling meristem size*

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Transcriptome and cytological profiling of maize anthers in the male sterile mutants ms32 and ms23 and other newly characterized middle layer and tapetal defective mutants

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Using Natural Variation to Identify Gene Modifiers of Three Developmental Mutations in Maize

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P158 **James Brewbaker** <brewbake@hawaii.edu>
Near-Isogenic Lines of Tropical Inbred Hi27

P159 **John Gray** <jgray5@utnet.utoledo.edu>
Using the maize TFome project to Foster the Integration of Research with Undergraduate Education (FIRE).

Quantitative Genetics & Breeding

P160 **Christopher Topp** <chris.topp@duke.edu>
3-dimensional imaging and high-throughput phenotyping of living root systems reveals dozens of QTL controlling cereal root architecture

P161 **Bode Olukolu** <baolukol@ncsu.edu>
A genome-wide association analysis identifies loci that modulate hypersensitive response caused by a maize auto-active resistance gene

P162 **Mingliang Xu** <mxu@cau.edu.cn>
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P164 **Willy Suwarno** <suwarno@wisc.edu>
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Dissecting maize genome complementation effects on hybrid vigor

P170 **Elhan Ersoz** <elhan.ersoz@syngenta.com>
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P171 **Latasha Smith** <lls3856@uncw.edu>
Drought and genotype affect phyllosphere communities

P172 **Lin Li** <lixx1601@umn.edu>
eQTL mapping of the maize shoot apex reveals complex gene regulation

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P182 **Zhengbin Liu** <Liuzhen@missouri.edu>	*Genetic Analysis of Kernel Traits in Maize-Teosinte Introgression Populations*
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P185 **Yuhe Liu** <yuheliu1@illinois.edu>	*Genetic architecture of nitrogen use efficiency traits in the maize IBM high-resolution genetic mapping population.*
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P187 **Jessica Bubert** <jbubert2@illinois.edu>	*Genetic Variation for Nitrogen Utilization in Historical and Improved Maize Germplasm*
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P223 **Sylvia Morais de Sousa** <smsousa@cnpms.embrapa.br>
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P224 **Sarah Hearne** <shearne@cgiar.org>
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Use of Public Germplasm Resources for the Research of the Genetic Inflorescence Architecture in Maize

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Computational and Large-Scale Biology

P232 **Darwin A. Campbell** <darwin.campbell@ars.usda.gov>
How well do you know YOUR maize research community?

P233 **Carson Andorf** <carson.andorf@ars.usda.gov>
Alpha.MaizeGDB.org: MaizeGDB's interface redesign

P234 **Lisa Harper** <ligule@berkeley.edu>
Learn How to Use MaizeGDB

P235 **Mary Schaeffer** <mary.schaeffer@ars.usda.gov>
MaizeGDB – use cases that leverage new data and tools.

P236 **Jack Gardiner** <jmgardin@iastate.edu>
Gene Expression Resources Available from MaizeGDB

P237 **Taner Sen** <taner.sen@ars.usda.gov>
MaizeCyc: Metabolic Networks in Maize

P238 **Ethalinda Cannon** <ekcannon@iastate.edu>
Diversity Data at MaizeGDB

P239 **Nathan Lai** <lain@onid.orst.edu>
A Bioinformatic Pipeline to Identify Transposon Flanking Sequences via High-Throughput Sequencing

P240 **Mingshu Huang** <mh728@cornell.edu>
A high resolution structural and proteomics atlas of the developing rice leaf

P241 **German Muttoni** <muttoni@wisc.edu>
A high-throughput stalk-core sampling device for the evaluation of maize biomass composition

P242 **Rajandeep Sekhon** <rsekhon@glbrc.wisc.edu>
An RNA sequencing and microarray-based gene atlas for the maize community

P243 **Brian Smith-White** <smtwhite@ncbi.nlm.nih.gov>
Analysis of the Zea mays gene-space

P244 **Gina Turco** <gturco@berkeley.edu>
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P254 **James Schnable** <jschnable@berkeley.edu>	*Fractionation mutagenesis: Natural Promoter Bashing in Maize*
P255 **Carol Rivin** <rivinc@science.oregonstate.edu>	*GA modulates ABA-regulated gene expression during maize embryogenesis*
P256 **Jeff Glaubitz** <jcg233@cornell.edu>	*Genetic architecture of maize and teosinte*
P257 **Margaret Woodhouse** <branwen@berkeley.edu>	*Genome Dominance in Maize and Brassica rapa*
P258 **Zachary Lemmon** <zlemmon@wisc.edu>	*Genome-Wide Allele Specific Expression Assays in Maize-Teosinte Hybrids*
P259 **Tanja Pyhäjärvi** <tpyha@ucdavis.edu>	*Genomics of adaptation in natural teosinte populations*
P260 **Tao Zuo** <taozuo@iastate.edu>	*Global gene expression profiling of maize copy number variation (CNV) with phenotypic impact*
P261 **Marcela Monaco** <mmonaco@cshl.edu>	*Gramene: a resource for comparative plant genomics*
P262 **Jeff Glaubitz** <jcg233@cornell.edu>	*Improving the B73 reference genome via genotyping by sequencing (GBS)*
P263 **Kelly Swarts** <kls283@cornell.edu>	*Inferences on Maize Biodiversity from Genotyping-by-Sequencing (GBS) American Landraces*
P264 **Wenbin Mei** <wmei@ufl.edu>	*Investigating tissue specific and genotype specific alternative splicing in maize*
P265 **Michelle Facette** <mfacette@ucsd.edu>	*Large-scale proteomic & phospho-proteomic analyses of growing maize leaves*
P266 **Gregory Downs** <gdowns@uoguelph.ca>	*Mapping microarray probe data to gene models reveals a low level of conservation between probes and maize gene models*
P267 **Felix Seifert** <felix.seifert@th-wildau.de>	*miRpp - A Plant microRNA Precursor Prediction Tool for Small RNA Reads on Genome Sequences and its application for 46k maize oligonucleotide array annotation*

P268 **Sabarinath Subramaniam** <shabari@berkeley.edu>
MotifView: A comparative genomics tool for analyzing motifs and their genes

P269 **Shohei Takuno** <showhey0119@yahoo.co.jp>
Parallel adaptation of maize landraces to highland environments

P270 **Toni Kazic** <kazict@missouri.edu>
Please Tell Us How You Think! or, Steps to Inferring Networks

P271 **Justin Walley** <jwalley@ucsd.edu>
Protein dynamics of the developing maize seed

P272 **Paul Bilinski** <pbilinski@ucdavis.edu>
Rapid Evolution of the Maize Centromere Repeat CentC

P273 **Dave Berger** <dave.berger@up.ac.za>
Re-annotation of the Agilent maize microarray based on the B73 genome sequence

P274 **Matt Evans** <mmsevans@stanford.edu>
RNA-Seq Analysis of Maize Gametophytic Transcriptomes

P275 **Andrea Eveland** <eveland@cshl.edu>
Systems approaches in maize inflorescence architecture

P276 **Joshua Stein** <steinj@cshl.edu>
The Maize Genome Project, an Update

P277 **John Gray** <jgray5@utnet.utoledo.edu>
The Maize Transcription Factor ORFeome (TFome) Project

P278 **Hank Bass** <bass@bio.fsu.edu>
Tissue-Specific Nucleosome Occupancy in the Promoter/TSS Region of 400 Classical Maize Genes.

P279 **Justin Fincher** <fincher@cs.fsu.edu>
Using Empirical Maize Chromatin Data to Train a Support Vector Machine to Predict Nucleosome Occupancy Likelihood (NOL)

P280 **George Chuck** <georgechuck@berkeley.edu>
Using the Corngrass1 gene to identify a grass specific juvenile transcriptome

Transposons & Epigenetics

P281 **Zidian Xie** <zidianx@hawaii.edu>
DNA binding properties of the ZmCENH3 nucleosome

P282 **Gernot Presting** <gernot@hawaii.edu>
The role and evolution of maize centromere repeats

P283 **Karen McGinnis** <mcginnis@bio.fsu.edu>
A two transgene system to induce DNA methylation and modify gene expression in maize.

P284 **Dafang Wang** <dwang@iastate.edu>
Ac Alternative Transposition Generates Segmental Duplications and New Chimeric Genes at the Maize p1 Locus

P285 **Thomas Peterson** <thomasp@iastate.edu>
Alternative transposition during DNA replication: a route to genome expansion?

P286 **Fei Wang** <lnwangfei@shu.edu.cn>
An Ac transposon system based on maize chromosome 4S for isolating long distance transposed Ac tags in maize genome

P287 **Yubin Li** <yubin@waksman.rutgers.edu>
An Ac/Ds-based reverse genetics resource for maize in the post-genomic era

P288 **Silvio Salvi** <silvio.salvi@unibo.it>
Bisulfite-based methylation analysis at the maize flowering time locus Vgt1

P289 **Fei Wang** <lnwangfei@shu.edu.cn>
Characterization of an Ac transposon system based on apt1-m1 (Ac) on long arm of chromosome 9 in maize

P290 **Kevin Cooper** <kevin.l.cooper@ufl.edu>
Characterization of the maternal rough endosperm (mre) mutant, mre-1014*

P291 **Kevin Ahern** <ka38@cornell.edu>
Creating novel alleles of target genes using the transposable element Dissociation

P292 **Daniel Vera** <dvera@bio.fsu.edu>	*Defining the chromatin domain organization of the maize genome*
P293 **Nathanael Ellis** <nellis@uga.edu>	*Differential modes of transposon regulation revealed by analysis of cytosine methylation*
P294 **Cristian Forestan** <cristian.forestan@unipd.it>	*Epigenetic mechanisms and environmental stresses in maize: a multiple approach to study epiallele formation and inheritance*
P295 **Elizabeth Buescher** <ebuesche@purdue.edu>	*Epigenetics controls seed development*
P296 **Charles Hunter** <jbe@ufl.edu>	*Evolution and diversity of Mutator transposable elements*
P297 **Ryan Douglas** <DouglasRN@missouri.edu>	*Examining epigenetic aspects of centromere specification in maize*
P298 **Mei Zhang** <zhangmei_2008_2006@126.com>	*Extensive genetic imprinting in the developing endosperm of maize*
P299 **Karen Vellacott-Ford** <kvellaco@purdue.edu>	*Half-leaf chimeric mutants in maize: can they be the works of gene silencing?*
P300 **Wenwei Xiong** <xiongwenwei@gmail.com>	*HelitronScanner: A general Helitron detection tool*
P301 **Yurii Baranov** <noise2004@inbox.ru>	*Maize waxy gene and Ds mobile element secondary structure polymorphism*
P302 **Ann Ferguson** <armeniaa@msu.edu>	*Mutator-like elements with multiple long terminal inverted repeats (TIR) in plants*
P303 **Laura Vann** <levann@ucdavis.edu>	*Natural Variation at the teosinte branched1 (tb1) Locus in Teosinte*
P304 **Jay Hollick** <hollick@berkeley.edu>	*Paramutation at the maize pl1 locus requires a pioneer protein encoded by required to maintain repression2*
P305 **Wesley Barber** <barber4@illinois.edu>	*Repeat associated small RNAs vary among parents and following hybridization in maize*
P306 **Amanda Waters** <water157@umn.edu>	*RNAseq based discovery of novel imprinted genes in maize*
P307 **Qing Li** <qingli@illinois.edu>	*Small RNA Profiling in Maize Hybrids and Their Parents with Reduced RNA-Dependent RNA Polymerase2 Function*
P308 **Dongyan Zhao** <Dongyan Zhao>	*The impact of Mutator-like transposable elements (MULEs) on the Illinois Long-Term Selection maize strains*
P309 **Vincenzo Rossi** <Vincenzo.Rossi@entecra.it>	*The many facets of nfc102 function during maize development*
P310 **Jay Hollick** <hollick@berkeley.edu>	*trans-generational epigenetic variation defined by maize Pol IV*
P311 **William Sheridan** <william.sheridan@email.und.edu>	*Transposition Fate of Seven Maize Activator (Ac) Elements Located on the Short Arm of Chromosome 1.*
P312 **Surinder Singh** <surinder.singh@mail.mcgill.ca>	*Utilizing the Maize Ac/Ds Transposons for saturation mutagenesis of important QTLs in Barley*

Plenary Talk Abstracts

Plenary 1

Plant Breeding: the Creative Power of Selection

(presented by William Tracy <wftracy@wisc.edu>)
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Plant breeding and plant genetics are often spoken of as two tightly aligned disciplines, sometimes even used as synonyms. Yet until relatively recently breeding and genetics have been quite disparate disciplines with very different goals, tools, and ways of understanding plant genetics. While genetics is a relatively new science, plant breeding is one of our oldest technologies. Distilled to its essence, plant breeding is human directed selection in genetically variable populations of plants. Historically, selection based on the phenotype has been the key feature of plant breeding programs. If successful, selection results in a population that is phenotypically and genetically different from the starting population. The direct response to selection can be remarkably predictable and precise. However, the underlying molecular, genetic, and physiological changes are often unpredictable and usually unknown. Selection does not simply cull out undesirable types; selection with genetic recombination creates new and useful phenotypes. In addition to being economically useful, these new phenotypes can be useful tools for geneticists to increase our understanding of underlying genetic and physiological relationships. I will discuss the results of a number of selection programs in maize and what we now know about the genetic basis of these changes. Our greatly increased understanding of biochemical pathways and our ability to transform plants and rapidly sequence genomes has now made genetics one of the major tools of the plant breeder.

Plenary 2

Weird animal genomes and sex; the power of evolutionary divergence

(presented by Jennifer A. Marshall Graves < j.graves@latrobe.edu.au>)
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The availability of genome sequence for animals distantly related to humans and mice has greatly advanced our understanding of how sex and sex chromosomes work, and how sex determination evolved. The genomes of some of Australia's iconic animals, including marsupial and monotreme mammals, including the kangaroo and platypus, devils (Tasmanian), dragons (lizards), snake and emus are proving a goldmine of new information that has revolutionized our understanding of sex in all vertebrates, including humans.

In humans and other mammals, females have two X chromosomes, but males have a single X, and a Y that bears a gene (*SRY*) that induces testis differentiation and switches on hormones that masculinize the embryo. The human X is a mid-sized chromosome, rich in "brains-and-balls" genes involved in reproduction and intelligence (often both), and thought to play a major role in human evolution. The small Y is a genetic wasteland, full of repetitive sequence and bearing only 45 genes, most active only in testis. How did human sex chromosomes get to be so weird?

Our strategy is to compare the chromosomes, genes and DNA in distantly related mammals and even birds and reptiles. Birds have completely different sex determining systems, snakes and lizards are different again (and some lack sex chromosomes, and do it by incubation temperature). Kangaroo sex chromosomes reveal the original mammal sex chromosomes, while the bizarre platypus sex chromosomes (more related to those of birds) tell us that our sex chromosomes are relatively young. The human X and Y evolved from an ordinary chromosome pair as the Y degraded progressively. The Y is predicted to disappear in just 5 million years. If humans don't become extinct, new sex determining genes and chromosomes will evolve, perhaps leading to the evolution of new hominid species.

Plenary 3

Female gametophytes and flowering plant reproduction: Green eggs and no SAM

(presented by Venkatesan Sundaresan< sundar@ucdavis.edu>)
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The reproduction of flowering plants involves the formation of gametic cells by the haploid male (pollen) and female (embryo sac) gametophytes, In most angiosperms, the female gametophyte contains just 7 cells including two gametes, the egg cell and the central cell, which give rise to the embryo and endosperm respectively. The embryo sac first develops as a syncytium of 8 nuclei, which are partitioned into 7 cells upon cellularization. Cell fates in the embryo sac are determined immediately depending upon nuclear positioning; there are no undifferentiated cells that typify sporophytic development, e.g., as in the shoot apical meristem. Signaling by the hormone auxin plays an important role in position based cell identity in the *Arabidopsis* embryo sac, through an asymmetric auxin distribution generated during female gametophyte development. The highest auxin levels are at the distal pole corresponding to the egg cell and the synergid cells, and these cell fates can be altered by manipulation of auxin signaling. The auxin distribution appears to be correlated with localized biosynthesis, rather than auxin efflux, which plays a more important role in sporophytic patterning. An asymmetric cytokinin distribution might also be acting to promote cell fates within the embryo sac. We have also begun to investigate the reprogramming of the differentiated egg cell to a totipotent zygote after fertilization. Preliminary results using rice suggests that the maternal to zygotic transition in plants is initiated as early as the first cell cycle of the zygote, much earlier than has been observed in animals.

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

Plenary 4

Germline Development and Function in Grasses

(presented by Thomas Dresselhaus <thomas.dresselhaus@biologie.uni-regensburg.de>)
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In contrast to animals a germline is not established during embryogenesis in flowering plants (angiosperms). Instead both male and female germ lineages are initiated from somatic cells during flower organ development. The female germline is initiated with the redifferentiation of a subepidermal cell of the L2 layer of the ovule primordium forming the archespore or primordial germ cell. This cell differentiates into the highly polar megasporocyte or megaspore mother cell, undergoes meiosis followed by three rounds of mitotic nuclei divisions before cellularization takes place resulting in the seven-celled female gametophyte (embryo sac). The mature embryo consists of four cell types including two female gametes (egg and central cell) and accessory cells (synergid and antipodal cells). While both female gametes are involved in the double fertilization process generating embryo and endosperm, respectively, accessory cells serve as signaling centers to attract and receive pollen tubes and likely to polarize central cell and developing endosperm, respectively. The male germline is already initiated during anther development. After completion of meiosis male spores generate a small germ cell dividing into two sperm cells and a large vegetative cell forming the tube cell during pollen germination. Establishment of polarity, cell identity, asymmetric division and germ cell cross-talk play key roles during germline development and function.

Using maize as a model we have investigated auxin responses, auxin fluxes and germ cell secreted peptide patterning during ovule development and double fertilization. We will report on the localization of *DR5* promoter activity and auxin efflux carrier ZmPIN1a as well as the role of small proteins secreted from the egg apparatus. Moreover, proteins leading to asymmetric nuclei positioning will be presented being involved in degradation and modification of target proteins during key processes of male and female germline development.

Short Talk Abstracts

SESSION 2 - CELL AND DEVELOPMENTAL BIOLOGY
Chair: Nathan Springer Friday, March 16. 8:15 AM – 10:15 AM

T1

The GRAS transcription factor *upright leaf angle1* encodes a monocot-specific brassinosteroid function for leaf angle control in maize

(presented by Thomas Hartwig <thartwig@purdue.edu>)
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Leaf angle is an important yield trait in maize that has been manipulated in elite hybrids since the 1970s (*1*). Although the influence of Brassinosteroid (BR) hormones on leaf inclination in monocots is well established, the underlying molecular mechanism is poorly understood.
We report the cloning and characterization of *upright leaf angle1* (*url1*). Mutants of *url1* produced semi-dwarf statures with shorter but wider, dark green and crinkled leaves. *url1* leaves also showed increased lamina inclination angles, changing from 60° in WT to more than 80° in *url1* plants. Distinct from the classical *liguleless* mutations, which are also implicated in leaf angle control in maize (*2*), *url1* causes little or no leaf collar aberrations. Cloning of the *url1* gene by transposon tagging revealed it encodes a member of the GRAS-transcription factor superfamily in maize. We found single gene copies of *url1* in all land plants, except for two copies in *Physcomitrella*, with a phylogeny that fits estimates of land plant relationships. However, multiple lines of evidence show a monocot-specific function of *url1* as a positive regulator of BR signaling. This suggests that speciation- rather than duplication events have led to functional divergence. Expression profiling showed that *url1* mRNA accumulates primarily in meristematic tissues and at lower amounts at the base of developing leaves. Given the distinct features of mono- and dicot leaves, *url1* seems to mediate a monocot-specific BR function in the establishment of leaf angle.

1. Duvick (2005) *Maydica* 50, 193-202; 2. Tian et al. (2011) *Nature Genetics* 43, 159-162.

Funding acknowledgement: National Science Foundation (NSF)

T2

Leaf boundaries: Live cell imaging of ligule development in mutants and non-mutants

(presented by Carolyn Rasmussen <crasmus8@uwyo.edu>)
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The ligule in the maize leaf defines the boundary between the blade and sheath and contributes to blade angle, thereby regulating an important agricultural trait. Upright blade angle has been selected to decrease plant spacing: genome wide association studies show that genes required for ligule formation strongly associate with varied blade angles. Mutations in genes that underlie quantitative trait loci for blade angle cause defects in ligule development, including *liguleless1* (*lg1*) and *liguleless2* (*lg2*). For these two mutants, the ligule is absent or reduced, but the boundary itself is retained. Thus, to understand how such an important leaf trait is determined, we seek to understand when and how the boundary is first established; and what signals and maintains the elaboration of the ligule. A preligule band is the first identifiable structure at the boundary and is recognized by a morphologically distinct group of cells characterized by increased cell division rates at the blade sheath border. Live cell imaging is used to identify the timing and position of Liguleless1 and Liguleless2 localization in relation to the cell division burst during preligule band formation and early ligule outgrowth. We are using *pLg1::Lg1-YFP*, *pLg2::Lg2-TFP*, and auxin-related and cytoskeletal markers in *lg1*, *lg2* and other liguleless mutants to compare with non-mutant plants. We show that the preligule band is geometrically stable (in B73 adult leaves), is ~60 µm high and recognized by a stack of ~4-10 epidermal cells that process through waves of longitudinal, transverse, then periclinal divisions. We identify the in vivo nuclear localization of LG1 and LG2 in relation to meristem-specific and auxin signals associated with the initiation of the ligule. A model for sequential signaling will be presented. These observations focus our attention to the precise region containing signaling and ligule-specific markers, which we show is both transitory and highly localized, to allow the identification of the panorama of ligule-specific gene expression through RNA sequencing.

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

T3

Abnormal phyllotaxy2 (Abphyl2): A new locus controlling phyllotaxy and shoot meristem size in maize

(presented by Fang Yang <fyang@cshl.edu>)
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Plant morphology and diversity are largely dependent on the establishment of phyllotaxy, which is initiated from groups of anlagen cells on the flanks of the shoot apical meristem (SAM). Auxin and cytokinin are crucial factors controlling phyllotactic patterns in maize and Arabidopsis. However, our depth of understanding is limited, because very few mutants defective in phyllotaxy have been characterized. In order to explore the mechanism by which phyllotaxy is determined by the SAM, we analyzed a novel abnormal phyllotaxy mutant, Abphyl2 (Abph2). The phyllotaxy of Abph2 changes from alternate, as seen in wild type, to decussate, after leaf ~ 4-5. In addition, Abph2 mutants form a larger shoot apical meristem, which might be the reason for the decussate phyllotaxy. A map-based cloning approach led us to map Abph2 to a 20kb region on the top of Chromosome 7. None of the genes in this interval had an obvious change in the dominant Abph2 mutant, nor in several EMS generated revertants. We therefore made and screened a BAC library from Abph2 mutants, and found a new 4.5kb-fragment inserted within the mapping interval that is not present at this position in the B73 genome. When this 4.5kb fragment was transformed into wild type maize plants, the transgenic plants phenocopied Abph2 mutants, indicating that the mutation is caused by sequences within the insertion. The 4.5kb insertion contains a gene encoding a predicted enzyme, and this gene is mutated in EMS revertants of Abph2, confirming that it corresponds to the Abph2 gene. Abph2 transcripts have a striking expression pattern in the P zero of the SAM, and the vasculature of young leaf primordia. This expression is highly dynamic, and differs in Abph2 mutants compared to wild type at ~30 days after pollination. We also found that ABPH2 protein physically interacts with other proteins involved in maize meristem size regulation, defining a new regulatory module for size control of plant shoot meristems.

T4

Characterization and Cloning of tassels replace upper ear1 in Maize

(presented by Wei Li <wli@iastate.edu>)
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Plant shoot architecture results from coordinated activities of shoot meristems. In maize, the typical architecture comprises a single shoot axis, and terminal and axillary inflorescence shoots (tassel and ear) derived from terminal and lateral meristems, respectively. The inflorescences produce more complex branching patterns to generate several branch axes and show selective elimination of male or female developmental processes. The tassels replace upper ear1 (tru1) gene of maize regulates shoot architecture by restricting various shoot meristem activities including tassel branch elongation and axillary branch (shank) elongation, and by affecting subsequent floral meristem activities in inflorescence identity. By map-based cloning with the original, tru1-WS allele (total 5,399 mutant individuals) we isolated the tru1 gene. Cloning was confirmed with five additional mutant alleles from an EMS noncomplementation screen. tru1 transcripts were detected in all tissues queried including root, vegetative shoot and leaves, embryo, and inflorescences at various developmental stages, consistent with a fundamental role of tru1 in regulating plant architecture. tru1 encodes a protein with a BTB/POZ domain and ankyrin repeats, highly similar to BLADE-ON-PETIOLE1 (BOP1) of Arabidopsis. In Arabidopsis, BOP1 has many functions, including in leaf morphogenesis and in shoot architecture through effects on floral meristem fate. In maize tru1 mutants, upper axillary branches are elongated and tassel-tipped, and lower branches are progressively reduced, revealing a key role for tru1 in regulating axillary branch growth. Double mutant analysis suggests that teosinte branched1 is epistatic to tru1. However, tru1 acts in an alternative pathway from grassy tillers1. As knotted1; tru1 double mutant resembles tru1 single mutant, tru1 is placed upstream of kn1, analagous to BOP-KNOX relationships in Arabidopsis. Interestingly, tassel seed1 and/or jasmonates might positively regulate tru1 in repressing ear branching, as the ts1; tru1 double mutant has a synergistic phenotype: a branched ear on an elongated shank. Other double mutants showed mostly additive effects. The proposed regulatory network among tru1, tb1, kn1 and ra1 in modulating axillary meristem activities is supported by expression studies.

Funding acknowledgement: National Science Foundation (NSF)

T5

GIGANTEA OF ZEA MAYS1A is a negative regulator of maize flowering time

(presented by Claire Bendix <clairebendix@googlemail.com>)
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Photoperiod is a key environmental cue many plants use to ensure that floral development occurs at the most favorable time of year. Temperate *Zea mays* (maize) is generally insensitive to photoperiod, but it was domesticated from photoperiod-sensitive teosinte. Understanding the activities of genes controlling flowering time in maize will help to reveal the genetic changes that produced this shift to photoperiod-insensitivity. One gene known to act in the signaling pathways that trigger photoperiod-sensitive flowering in rice and Arabidopsis is *GIGANTEA* (*GI*). GI protein is highly conserved at the amino acid level across angiosperms. In rice, OsGI controls leaf-specific expression of the Heading dates 1 (Hd1) transcription factor, which then regulates expression of Hd3a, the florigen that triggers floral development. We investigated the role of *gigantea of zea mays1a* (*gigz1a*) in flowering time to understand the regulatory architecture of this floral signaling module in maize. Two novel *Mutator* transposon insertions that disrupt *gigz1a* expression accelerate maize floral development in long day conditions, as plants with *gigz1a* mutant alleles produced markedly fewer leaves at maturity than did normal plants. Investigation of the expression levels of flowering-associated genes in the *gigz1a* mutants revealed elevated transcript levels from both *constans of zea mays1* (*conz1*), an *Hd1*-like gene, and *zea mays centroradialis8* (*zcn8*), a gene encoding an Hd3a-like floral activator. Therefore, GIGZ1A acts as a floral repressor in long days by suppressing *conz1* expression and, ultimately, that of *zcn8*. These findings indicate that GIGZ1A serves to repress the switch to floral development in long day conditions. At this point, GIGZ1A activity appears different from the positive role that GI has in promoting flowering in rice and Arabidopsis, which raises the intriguing possibility that orthologous flowering time components in maize and rice may have divergent functions.

Funding acknowledgement: United States Department of Agriculture (USDA)

T6

Hypoxia triggers plant germline specification

(presented by Timothy Kelliher <tkellih1@stanford.edu>)
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Despite the central importance of sexual reproduction, the mechanism(s) by which meiotically competent cells differentiate in floral primordia is unknown. Current models invoke simultaneous specification of germinal and supporting somatic niche cells from division of a singular, positionally defined precursor cell. Through confocal reconstruction of fertile, *mac1* (encoding a secreted signaling protein; absence results in excess germinal and fewer somatic cells), and *msca1* (encoding a glutaredoxin; absence results in loss of both somatic and germinal anther cell types) maize anthers we establish that germinal cells have a multiclonal origin within a field of pluripotent progenitors and that these cells subsequently utilize a MAC1-dependent pathway to direct cell fate setting in neighbors that differentiate as somatic support tissues. We demonstrate that cellular redox status determines germinal fate by manipulating the gas and chemical environment of immature anthers. Treatments that decreased oxygen and/or H_2O_2 – nitrogen gas or KI injection – significantly increased germ cell numbers with ectopic germinal cell formation nearer the anther surface. Conversely, oxidizing environments significantly inhibited germinal specification, delayed somatic development, and caused germinal differentiation in deeper tissues. Remarkably, we were able to correct the *msca1* phenotype chemically, restoring germinal differentiation and development of anatomically normal anthers. We propose a model in which a field of equivalent, pluripotent progenitors proliferates until the reductive environment activates MSCA1, which in turn induces germinal cell differentiation and increases MAC1 expression to direct somatic differentiation in neighboring cells.

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

SESSION 3 - BIOCHEMICAL AND MOLECULAR GENETICS

Chair: Alice Barkan Friday, March 16. 10:45 AM – 12:25 PM

T7

PANGLOSS2, A Receptor-Like Protein that Polarizes Cell Division in Maize Identified Via Quantitative Proteomics

(presented by Xiaoguo Zhang <xiz038@ucsd.edu>)
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Asymmetric cell division creates daughter cells with distinct cell fates and is therefore important for plant development. However, very little is known about regulation of asymmetric cell division. Maize stomatal subsidiary cells are generated by an asymmetric division of subsidiary mother cells (SMCs), and provide an excellent model for studies of asymmetric cell division. In *pangloss* (*pan1* and *pan2*) mutants, disruption of the asymmetric SMC division leads to formation of aberrantly shaped subsidiary cells. Genetic interaction between *pan1* and *pan2* suggests that they function cooperatively to promote the polarization and timely execution of SMC divisions. Previous results have shown that maize PANGLOSS1 (PAN1) is a leucine-rich repeat receptor-like kinase (LRR-RLK), which accumulates in SMCs at sites of guard mother cell (GMC) contact prior to the appearance of other polarity markers. However, PAN1 polar accumulation depends on PAN2, since PAN1 does not polarly accumulate in the *pan2* mutant. PAN2 has been recently identified via quantitative proteomics to be another LRR-RLK. PAN2 also polarly localizes at contact site of GMC and SMC at the same time as PAN1. This accumulation is not dependent on PAN1, suggesting PAN2 acts upstream of PAN1. Immunoprecipitation and yeast 2-hybrid experiments suggest PAN2 interacts with itself, however no evidence for an interaction with PAN1 has been observed. Like PAN1, the kinase domain of PAN2 exhibits no detectable kinase activity in vitro. PAN2 may interact with PAN1, and possibly also exhibit kinase activity, only upon activation by binding of an unknown ligand originating from the adjacent GMC.

Funding acknowledgement: National Science Foundation (NSF)

T8

RAF1, a new regulator of Rubisco biogenesis in maize

(presented by Leila Feiz <lf259@cornell.edu>)
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Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the photosynthetic rate- limiting enzyme which fixes carbon dioxide into life's building blocks. The inefficient catalytic properties of Rubisco and its lack of specificity for carbon dioxide as the sole substrate has adversely affected the crop yield. Attempts to improve the catalytic properties of higher plant Rubisco have met with little success mostly because of the lack of a comprehensive knowledge of its biogenesis and the absence of an in vitro reconstitution system. Here we report the identification of a new chloroplast protein required for Rubisco accumulation in maize, RAF1 (Rubisco Accumulation Factor), which lacks any characterized functional domains. Maize lines lacking RAF1 due to Mutator transposon insertions are Rubisco-deficient and seedling lethal. Analysis of transcripts and proteins showed that Rubisco synthesis in raf1 plants is not compromised, however newly-synthesized Rubisco large subunit appears in a high molecular weight form whose accumulation requires a specific Cpn60 isoform. The prominent accumulation of RAF1 in bundle sheath chloroplasts similar to Rubisco, is in agreement with our evidence that RAF1 participates specifically in Rubisco accumulation. Our results support the hypothesis that this trimeric protein acts as an assembly chaperone by releasing the large subunit from chaperonins early in the assembly process.

Funding acknowledgement: United States Department of Agriculture (USDA)

T9

Essential factors for arbuscular mycorrhizal symbiosis from maize and rice

(presented by Uta Paszkowski <uta.paszkowski@unil.ch>)
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The mutually beneficial arbuscular mycorrhizal (AM) symbiosis is the most widespread plant-fungal interaction between roots of terrestrial plants and fungi of the Glomeromycota. The association receives increasing scientific attention because of the nutritional benefit it confers to host plants, which is particularly pronounced for phosphate. Mutants defective in AM symbiosis resulted from a forward genetics screen in maize (PASZKOWSKI et al. 2006, Plant J. 47: 165-173). The *nope1* (*no perception 1*) mutant displayed loss of susceptibility, indicative of pre-symbiotic function to be affected. The mutation segregated as a monogenic recessive trait and was mapped to the peri-centromeric region of maize chromosome 10. Gene cloning efforts employed a synteny-based approach in rice and identified a candidate gene, whose disruption reproduced the maize *nope1* phenotype, thereby suggesting the successful cloning of *NOPE1*. Insertion alleles in the corresponding maize gene have been identified via *Ds* tagging and are currently examined for their impact on symbiotic properties. The gene is predicted to encode a protein of unknown function but assumed to be involved in transport processes across membranes as it groups with the major facilitator superfamily. Recently, we have made the exiting observation that wild-type root exudates complemented the mutant phenotype "*in trans*". It can therefore be hypothesized that NOPE1 participates in an efflux activity across the plasma membrane of root cells.

Funding acknowledgement: Swiss National Science Foundation

T10

Defining expression networks in maize for secondary wall biogenesis

(presented by Nick Carpita <carpita@purdue.edu>)
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We estimate that plants devote 10% of their genomes to cell wall biogenesis. We have classified cell wall-related genes of Arabidopsis, rice, and maize (*Zea mays* cv. B73) into gene families whose products function in six stages of wall biogenesis: substrate generation, polysaccharide synthesis, membrane trafficking, assembling and turnover, secondary wall formation, and signaling (http://cellwall.genomics.purdue.edu). Arabidopsis, rice and maize genes populate the same families, but exhibit divergence in sub-group membership responsible for construction of their distinctive type II cell walls of grasses. Illustrating these differences further are expression data that show that functionalization of genes involved in primary wall synthesis has diverged in such a way that the closest homologs are not necessarily orthologous in function. Deep sequencing technologies were used to profile expression patterns distinct from primary wall expression during the development of the rind tissues of maize internodes during normal growth under field conditions. These studies established the gene inventories responsible for distinct stages of carbohydrate and lignin formation. Expression profiles varied in internodes below the ear shoot compared to upper internodes, underscoring different lignin compositions between them. Expression profiles of B73 and Mo17 internode development also showed differences responsible for their distinctive wall compositions. Alterations of expression in the *Bk2* mutant promise to enlighten us to compensation mechanisms when lignocellulosic matrices are compromised by a cell growth integration factor. Results have identified key targets for the tailoring energy grasses for optimizing biomass for conversion to biofuels.

Funding acknowledgement: Grant no. DE–FG02–08ER64702 from the Office of Science, U.S. Department of Energy.

T11

Unraveling the KNOTTED1 regulatory network in maize meristems

(presented by Nathalie Bolduc <nath.bolduc@gmail.com>)
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Understanding of gene regulation by transcription factors requires the dissection of the cis-regulatory elements that they act upon. In maize and other plant species, KNOTTED1(KN1)-like homeobox (KNOX) transcription factors are required for meristem maintenance, but how their DNA binding activities correlate with changes in gene expression and observed phenotypes is poorly understood. Using ChIP-seq, we defined the KN1 cistrome in maize inflorescences and found that KN1 binds to at least 6,500 loci. To understand how these binding activities correlate with changes in transcriptional regulation, we performed RNA-seq on immature ears and tassels, and compared expression profiles between normal and loss-of-function kn1 plants, in addition to immature leaves from normal and gain-of-function Kn1 plants. We found that 643 of the bound genes were modulated in one or multiple tissues, indicating that they represent biologically relevant direct targets. Among the bound and modulated genes, those involved in the auxin hormonal pathway as well as transcription factors (including other homeobox genes) show the most significant enrichment. Our results show that KN1 regulation of hormonal pathways is not restricted to gibberellins and cytokinin, contrarily to previously thought, and that KN1 works in large part by regulating other transcription factors. Thus, KN1 occupies a high position in the maize gene regulatory network, in agreement with its role as a master regulator of development.

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

SESSION 4 – NEW RESOURCES AND APPROACHES FOR MAIZE BIOLOGY

Chair: Mark Cigan Friday, March 16. 5:00 PM – 6:15 PM

T12

Gene Mapping via Bulked Segregant RNA-Seq (BSR-Seq)

(presented by Sanzhen Liu <liu3zhen@iastate.edu>)
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Bulked segregant analysis (BSA) is an efficient method to rapidly and efficiently map genes responsible for mutant phenotypes. BSA requires access to quantitative genetic markers that are polymorphic in the mapping population. We have developed a modification of BSA (BSR-Seq) that makes use of RNA-Seq reads to efficiently map genes even in populations for which no polymorphic markers have been previously identified. Because of the digital nature of next-generation sequencing (NGS) data, it is possible to conduct de novo SNP discovery and quantitatively genotype BSA samples by analyzing the same RNA-Seq data using an empirical Bayesian approach. In addition, analysis of the RNA-Seq data provides information on the effects of the mutant on global patterns of gene expression at no extra cost. In combination these results greatly simplify gene cloning experiments. To demonstrate the utility of this strategy BSR-Seq was used to clone the glossy3 (gl3) gene of maize. Mutants of the glossy loci exhibit altered accumulation of epicuticular waxes on juvenile leaves. By subjecting the reference allele of gl3 to BSR-Seq, we were able to map the gl3 locus to an ~2 Mb interval. The single gene located in the ~2 Mb mapping interval whose expression was down-regulated in the mutant pool was subsequently demonstrated to be the gl3 gene via the analysis of multiple independent transposon induced mutant alleles. The gl3 gene encodes a putative myb transcription factor, which directly or indirectly affects the expression of a number of genes involved in the biosynthesis of very-long-chain fatty acids.

Funding acknowledgement: National Science Foundation (NSF)

T13

Maize inbred lines at the NCRPIS: a great public resource to explore maize genetics

(presented by Cinta Romay <mcr72@cornell.edu>)
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The USDA-ARS North Central Regional Plant Introduction Station (NCRPIS) of Ames, Iowa, preserves 2,711 maize inbred lines including some of the key inbred lines for maize breeding history and breeding programs from all over the world in its germplasm bank. To take advantage of this resource, we genotyped 3,379 of lines from the NCRPIS and other sources, using Genotyping-By-Sequencing (GBS), a low cost, high-throughput sequencing technology. The method produced more than 1.3 million SNP markers distributed across the entire genome, with ability to call rare alleles at high confidence levels. The analysis of this data shows that the collection presents high levels of genetic diversity. However, the extent of variation is dependent on the different subpopulations. For example, there are a few clusters of very similar inbred lines, especially within the subset corresponding to Ex-PVP materials. This contrasts with the tropical inbreds, which contribute a large number of the rare alleles. Additionally, the data show that the LD decays rapidly when analyzing the entire collection, although we detected big LD blocks when examining specific groups of germplasm. Accordingly, when the data is used to map a simple trait like yellow versus white kernels, the associated SNP is found within the Y1 gene. But, the associated SNP for a trait like sweet corn is about one Mb away from Su1. We also explored the power of this resource to study a complex trait like flowering time. In our GWAS, the best associations overlap with regions identified in previous QTL studies, providing better resolution. To date, analyses of GBS data have helped identify errors in germplasm identity, and we hope these data can be used to better understand the collection and assist in curatorial management. It also gives the maize community a new tool to choose materials and perform their genetic studies.

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

T14

MaizeCyc: Metabolic Networks in Maize

(presented by Taner Sen <taner.sen@ars.usda.gov>)
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MaizeCyc is a Pathway Genome Database, and represents a catalog of known and predicted metabolic and transport pathways for corn (Zea mays), which enables plant researchers to study and graphically represent the metabolome of this cereal, thereby supporting integrated systems-biology analysis. MaizeCyc is accessible from the Gramene and MaizeGDB websites, and was created using Pathway Tools software version 15. Analyses and cross-species comparisons are supported for a variety of data, including genetic and phenotypic profiles, transcriptomics, proteomics, and metabolomics data sets. Pathways, reactions, and genes in the catalog are based on the electronic and manual annotations of 39,656 maize gene models in the RefGen_v2 filtered set (Maize Genome Sequencing Project, release 5b.60), and phylogenetically-derived projections from related plant models, including Arabidopsis and rice. This community resource includes sequence-based associations provided by Gramene, MaizeSequence, and MaizeGDB to external database entries from EntrezGene, UniProt-SwissProt, and Gene Ontology. Manual annotations of genes include mapping of classical phenotype genes to sequenced genomic loci (Schnable and Freeling, 2011), proteomics-supported functional annotations (Friso et al, 2010), and enzyme commission code mappings from literature mining. Using expression profiling data from the B73 Maize Gene Atlas transcriptomics set (Sekhon et al, 2010), we created exemplar visualizations of spacio-temporally regulated global gene expression in maize, and will provide these graphic representations freely to the community. This work is supported by the NSF (Gramene: A Platform for Comparative Plant Genomics), and the USDA-ARS (The Maize Genetics and Genomics Database).

Funding acknowledgement: United States Department of Agriculture (USDA), National Science Foundation (NSF), and the National Corn Growers Association (NCGA).

T15

Annotating the Maize B73 Gene Expression Atlas in the Plant Ontology- A Tool for Plant Genomics

(presented by Laurel Cooper <cooperl@science.oregonstate.edu>)
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The Plant Ontology (www.plantontology.org) is a structured vocabulary and database resource for all plant scientists that links plant anatomy, morphology, and development to the rapidly expanding field of plant genomics. The primary purpose of the PO is to facilitate cross-database querying and to foster consistent use of vocabularies in annotation. An essential feature of the PO is the set of freely accessible web links from terms to associated annotations, which are structure- or development-specific genes, proteins, and phenotypes sourced from numerous plant genomics datasets. In collaboration with MaizeGDB (www.maizegdb.org), we have recently added approximately 1.5 million new associations between maize (*Zea mays*) gene models and Plant Ontology terms. These associations are based on a large NimbleGen microarray data set profiling genome-wide transcription patterns in 60 tissues, representing 11 distinct organs over the life cycle of a maize plant of the inbred line B73 (Sekhon, et al, Plant Journal, 2011). The microarray data was associated with ~35,000 maize gene models developed from the recent sequencing of its genome and updated to the current assembly, B73 RefGen_v2, as a collaboration between MaizeGDB and PLEXdb (www.plexdb.org). PO association files in gaf 2.0 format (www.geneontology.org) were further enhanced by the inclusion of classical gene names, mapped by CoGe (www.genomevolution.org/CoGe/). The maize gene atlas associations were made public in the Plant Ontology Release #16 in October 2011. They are available for download, and can be viewed in various browser modes, both at the PO and at MaizeGDB. The addition of the maize gene atlas annotations to the PO represents an example of how ontologies provide access to large genomics data sets. Currently, the PO includes over 2 million such annotations from 17 species associated with over 1,300 terms. Other recent additions include annotations to cotton (*Gossypium*) and the moss *Physcomitrella patens*, with plans for the future inclusion of grape (*Vitis*) and potato (*Solanum*). The PO is a valuable resource for both research and teaching that can be used as a guide to plant structures and growth and developmental landmarks in life cycles of plants across many taxa.

Funding acknowledgement: National Science Foundation (NSF)

T16

Characterization of the root system architecture of the Nested Association Mapping (NAM) parent lines.

(presented by Paul Zurek <prz@duke.edu>)
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Plant root systems function both in plant anchorage, as well as in nutrient and water acquisition. How a plant distributes its root system should then affect the efficiency of nutrient and water acquisition. As such it's important to understand how the root system architecture (RSA) is organized, as well as the underlying genetic factor controlling it. This has proven to be quite a challenge, as current technologies have emphasized either high throughput or high resolution data acquisition. Here we present data acquired using a gel system that bridges the gap, and allows the collection of high resolution data in a high throughput fashion. In particular, the data were collected on the 26 NAM parent lines. The RSA was described using both 2D traits (ones based on a set of images of the root system), as well as 3D traits (ones based on 3D reconstruction of the root system). The data show a wide spectrum in traits scored. Some varieties, such as B73, have shallow and compact root systems while others, such as Ki3, have much deeper and spread out roots systems, demonstrating just some of the variability observed among the whole population.
Funding acknowledgement: National Science Foundation (NSF)

SESSION 5 - QUANTITATIVE GENETICS
Chair: Bob Bensen Saturday, March 17. 8:15 AM – 10:15 AM

T17

The Genetic Architecture of Maize Height: *A few loci can't explain the long and the short of it*

(presented by Jason Peiffer <jap333@cornell.edu>)
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Plant height is one of the most heritable and, with the exception of yield, most complex traits in maize. Analogous to findings in recent studies of human height, geneticists have been baffled by this apparent incongruity. How can something so heritable, explained so well by simple models of parent-offspring regression, be nearly irreducibly complex in natural populations? Where is the missing heritability? As plant geneticists, we have several tools in our research arsenal that allow us to tackle this inquiry in a more rigorous manner than that afforded to human geneticists. The most noteworthy of these are an ability to regulate allele frequencies, empirically control for population structure, and replicate genotypes across unique environments. By measuring the nested association mapping panel (NAM) and a panel of diverse inbred lines from the USDA-ARS repository located in Ames, IA, we have collected phenotypic data for more than 8,000 inbred lines across three to eight environments. These lines have also been genotyped for over 26 million SNPs through construction of the Second-Generation Maize HapMap. Using joint linkage mapping and GWAS we estimated the distribution of significant effects, epistasis, environmental conditionality, and pleiotropy of loci capturing heritable variation in plant and ear height, node counts, flowering time, and traits derived from these measures. Comparisons with more than 35 published loci associated with plant height by positional cloning and molecular methods revealed little overlap. However, current positional cloning efforts are validating our mapping results. In addition to dissecting the natural genetic diversity for plant height, genomic prediction was implemented. Genetic relatedness inferred using all genotypic data simultaneously explains a substantial portion of heritable phenotypic variation. Our analyses further reveal the genetic architecture of maize height and provide requisite empirical information to enrich our understanding of evolutionary phenomena applicable to the optimization of crop improvement.

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

T18

Ionomics of the Maize Nested Association Mapping Population

(presented by Ivan Baxter <ivan.baxter@ars.usda.gov>)
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Heterogeneity in the elemental composition of soils is among the major causes of plant stress worldwide. In order to adapt to these conditions, plants frequently alter their elemental content. We have developed a high-throughput elemental profiling platform to focus on the effect of genetic architechture and soil environment on a plants elemental profile or "ionome". We have used this platform to measure the concentration of 20 elements in ~10,000 single kernel samples from the Nested Association Mapping panel (NAM). Applying this high precision phenotyping technique to the NAM leads to fundamental insights on the genetic regulation of physiology in maize. We identified >200 joint linkage QTLs for the elemental traits. Demonstrating the power of this approach, we found a major molybdenum (Mo) QTL on maize chromosome 1. Genome-wide association mapping with the 1.6M HapMapv1 SNPs localized the QTL to an ortholog of *Mot1*, the mitochondrial molybdenum transporter from *Arabidopsis*. Examining this locus further, we sequenced part of the *ZmMot1* coding region in the Buckler/Goodman Diversity Panel and 16 teosinte accessions. Allelic diversity among wild and cultivated accessions suggests that *ZmMot1* was under selection during domestication or modern crop improvement. Genetic analysis of teosinte introgression lines and IBM RILs confirm that variability in the *Mot1* region is driving variation in Mo accumulation and reciprocal crosses confirm that the phenotype is maternal. Our studies of the maize NAM population will rapidly lead to the discovery of genes important for control of the ionome, assist the development of varieties adapted to stressful soil environments and promote global food security.

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

T19

The genomic response to reciprocal recurrent selection in BSSS and BSCB1

(presented by Justin Gerke <justin.gerke@pioneer.com>)
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The Iowa State reciprocal recurrent selection (RRS) experiment on the Iowa stiff stalk synthetic (BSSS) and Iowa synthetic corn borer 1 (BSCB1) populations represents one of the longest standing models of selection for combining ability in maize. We used the Illumina MaizeSNP50 high-density SNP array to determine genotypes of progenitor lines and over 300 individuals from BSSS and BSCB1 across multiple cycles of selection. Our findings show that divergence between the heterotic groups has been gradual, and began prior to the initiation of active selection, indicating a critical role for genetic drift in establishing the heterotic groups. In addition, our dense SNP coverage reveals large genomic regions where all genetic diversity has been lost within a population. By use of simulations we demonstrate that these patterns of fixation and loss of diversity are unlikely to occur by genetic drift alone. Of particular note, we find several regions of fixation spanning tens of megabases in regions of low recombination near the centromeres of several chromosomes. These regions can be traced back largely intact to individual founders, bearing little evidence of recombination in these selected regions in over 60 years of crossing. These results provide new hypotheses to test regarding the genetic basis of phenotypic gain in the RRS populations, and highlight the challenges that genetic drift and variable recombination impose on maize breeding efforts.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Justin Gerke received support as a Merck Fellow of the Life Sciences Research Foundation

T20

Pattern of genome-wide genetic changes throughout modern maize breeding

(presented by Yinping Jiao <jiaoyinping@cau.edu.cn>)
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The success of modern maize breeding has been well demonstrated by remarkable increase of productivity over the last decades. Yet the underlying genetic changes correlated with the genetic gain throughout the breeding process remain largely unknown at genome-wide level. We report here the sequencing of 278 temperate maize inbred lines (90 Ex-PVP lines, 36 public US lines and 152 elite Chinese lines), including deep resequencing of a set of inbred lines with known pedigree information. A total of 1.3 Tb data composed of 13 billion 100bp reads was generated; with an average of ~2X sequencing depth for each line. Analyzing these data resulted a total of 27,818,705 SNPs. Using the CLR method, we found that the breeding selection has affected on thousands of targets genes, leading to apparent reduction of nucleotide diversity and increasing of proportion of rare alleles. Additionally, through the analysis of the 4 deep sequencing lines with known pedigree information, we found that genetic changes during breeding happen rapidly, with extensive variation (SNPs, Indels and CNVs) even within the identity-by-descent regions. Our results of genome-wide assessment of genetic changes along the modern maize breeding provide new strategies as well as practical targets for future crop breeding and biotechnology.

Funding acknowledgement: 973 program of China

T21

A Decade of Tropical to Temperate Maize Adaptation Reveals a Potential Mechanism for Broad Adaptation

(presented by Juliana Teixeira <juliana@udel.edu>)
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Despite the availability of diverse germplasm for maize improvement, 'exotic' sources represent only ~3% of the total U.S. maize parentage, and these sources are themselves a very narrow sample; tropical sources comprise a meager 0.3% of the total U.S. parentage (Goodman 1998). Public efforts are underway to incorporate exotic maize germplasm into N. American maize to help address growing challenges associated with climate change and an increasing human population. In a USDA-NIFA funded project, called Maize ATLAS (Adaptation Through Latitudinal Artificial Selection; www.maizeatlas.org/), our team is developing approaches to study the genetic basis of response to artificial selection underlying the crop adaptation. These approaches are being applied to study a tropical landrace, Tusón, adapted to a temperate environment after ten generations of artificial selection for earliness. A population comprised of 300 families, derived from samples of the generations, were evaluated in replicated trials conducted across nine environments that represent a latitudinal transect from Wisconsin to Puerto Rico. Dissection of the environmental variables associated with variation in flowering time across environments revealed a dual role of increased temperature use efficiency (the primary factor) and decreased photoperiod sensitivity (the secondary factor) for the response to artificial selection. The response to selection across environments suggests temperature use efficiency as a common mechanism for broad adaptation.

Goodman, M.M. 1998. Research Policies Thwart Potential Payoff of Exotic Germplasm. Diversity 14:30-35.
Funding acknowledgement: United States Department of Agriculture (USDA)

T22

Genetic Control of Maize Photoperiod Response

(presented by Jim Holland <james_holland@ncsu.edu>)
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Teosinte, the progenitor of maize, is restricted to tropical environments in Mexico and Central America. The pre-Columbian spread of maize from its center of origin in tropical Southern Mexico to the higher latitudes of the Americas required post-domestication selection for adaptation to longer daylengths. Flowering time of teosinte and tropical maize is delayed under long daylengths, whereas temperate maize evolved a reduced sensitivity to photoperiod. We measured flowering time of the maize nested association mapping population and a diverse maize association panel in the field under both short and long daylengths, and of a maize-teosinte mapping population under long daylengths. Flowering time in maize is a complex trait affected by many genes and the environment, of which photoperiod response is one component with simpler genetic architecture controlled by fewer genes with larger phenotypic effects. Genome-wide association and targeted high resolution linkage mapping identified *ZmCCT*, a homolog of the rice photoperiod response regulator *Ghd7*, as the most important gene affecting photoperiod response in maize. Functional analysis of this gene revealed that it is expressed at high levels and confers later flowering under long daylengths in teosinte compared to a temperate maize inbred lines. Many maize inbred lines, including some adapted to tropical regions, carry *ZmCCT* alleles with no sensitivity to daylength. Prehistoric plant breeders were remarkably successful at selecting on genetic variation at key genes affecting the photoperiod response to create maize varieties adapted to vastly diverse environments despite the hindrance of the geographic axis of the Americas and the complex genetic control of flowering time.

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

SESSION 6 - COMPUTATIONAL APPROACHES TO MAIZE GENETICS

Chair: Peter Balint-Kurti Saturday, March 17. 10:45 AM – 12:25 PM

T23

Identification, characterization and mapping of teosinte genes absent from the maize genome

(presented by Camille Rustenholz <crusten@iastate.edu>)
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During maize (*Zea mays* sp *mays*) domestication the genomes of teosinte (*Zea mays* sp *parviglumis*), maize's wild ancestor, went through a genetic bottleneck. Despite numerous introgressions some teosinte genes were probably not transmitted to the maize genome. To test this hypothesis a combination of RNA-seq, comparative genomic hybridization and sequence capture was used to identify, characterize and map teosinte genes absent from the B73 maize genome and from a maize diversity panel. Several thousand of RNA-seq contigs were found to be present and expressed in two teosinte accessions but lacking in the B73 maize genome. Out of them ~100 RNA-seq contigs were also missing in a diversity panel composed of 92 maize inbreds. Moreover these non-maize genes tend to cluster in the teosinte genome. This result suggests that complete regions of the teosinte genome were not transmitted to maize due to either genetic drift or selective sweep. The frequency of these genes is currently being assessed in a teosinte diversity panel composed of 85 accessions. Finally our study suggests that the teosinte genomes contain regions carrying not only alleles but also whole functional genes that could be useful to improve maize.

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

T24

Maize (Zea mays L.) Genome Diversity as Revealed by RNA-sequencing

(presented by Candice Hansey <hansey@msu.edu>)
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Maize is rich in genetic and phenotypic diversity. Understanding the sequence, structural, and expression variation that contributes to phenotypic diversity would facilitate more efficient varietal improvement. RNA based sequencing (RNA-seq) is a powerful approach for transcriptional analysis, assessing sequence variation, and identifying novel transcript sequences, particularly in large, complex, repetitive genomes such as maize. In this study, we sequenced RNA from whole seedlings of 21 maize inbred lines representing diverse North American and exotic germplasm. Single nucleotide polymorphism (SNP) detection identified 351,710 polymorphic loci distributed throughout the genome covering 22,830 annotated genes. Tight clustering of two distinct heterotic groups and exotic lines was evident using these SNPs as genetic markers. Transcript abundance analysis revealed minimal variation in the total number of genes expressed across these 21 lines (57.1% to 66.0%). However, the transcribed gene set among the 21 lines varied, with 48.7% expressed in all of the lines, 27.9% expressed in one to 20 lines, and 23.4% expressed in none of the lines. De novo assembly of RNA-seq reads that did not map to the reference B73 genome sequence revealed 1,321 high confidence novel transcripts, of which, 564 loci were present in all 21 lines, including B73, and 757 loci were restricted to a subset of the lines. RT-PCR validation demonstrated 87.5% concordance with the computational prediction of these expressed novel transcripts. Intriguingly, 145 of the novel de novo assembled loci were present in lines from only one of the two heterotic groups consistent with the hypothesis that, in addition to sequence polymorphisms and transcript abundance, transcript presence/absence variation is present and, thereby, may be a mechanism contributing to the genetic basis of heterosis.

Funding acknowledgement: Department of Energy (DOE)

T25

Complementation contributes to transcriptome complexity in maize (*Zea mays* L.) hybrids relative to their inbred parents

(presented by Anja Paschold <paschold@uni-bonn.de>)
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F_1-hybrids are more vigorous than their homozygous, genetically distinct parents, a phenomenon known as heterosis. Despite its large agronomic importance, the molecular mechanisms underlying the manifestation of heterosis are only poorly understood. In the past it was demonstrated that in addition to above-ground traits such as plant size and yield which emerge late in development, already the young root system shows heterosis. In the present study the transcriptomes of the reciprocal maize (*Zea mays* L.) hybrids B73xMo17 and Mo17xB73 and their parental inbred lines B73 and Mo17 were surveyed in primary roots early in the developmental manifestation of heterosis. The application of novel and robust statistical approaches and a suitable experimental design established that 35,202 (i.e., 89%) of all high-confidence maize genes were expressed in at least one genotype. Nearly 60% of all expressed genes were differentially expressed between the two parents and 34% to 47% of expressed genes were differentially expressed between one of the parents and one of the hybrids. In both hybrids, ~12% of expressed genes exhibited non-additive gene expression. Consistent with the dominance model (i.e., complementation) for heterosis 865 genes were expressed in only one of the two parents, but in the hybrids. For 50 genes it was shown that this was a consequence of complementation of presence/absence variation. For dozens of other genes, alleles from the inactive inbred were activated in the hybrid, presumably via interactions with regulatory factors from the active inbred. As a consequence of these types of complementation, both hybrids expressed more genes than did either parental inbred. Finally, in hybrids >10% of expressed genes exhibited allele-specific expression (ASE) levels that differed significantly from the parental-inbred expression ratios, providing further evidence for interactions of regulatory factors from one parental genome with target genes from the other parental genome.

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

T26

A de novo centromere on Duplication 3a in the absence of canonical centromere sequence arrays

(presented by Fangpu Han <fphan@genetics.ac.cn>)
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The centromere is the part of the chromosome that organizes the kinetochore, which mediates chromosome movement during mitosis and meiosis. In most multicellular organisms, the centromere region is composed of species-specific sequence repeats arranged in large tandem blocks. Maize centromeres contain two basic types of DNA elements. One is the centromeric satellite referred to as CentC, a 156 base pair unit that is present at all of the primary constrictions of the chromosomes. Another one is a retrotransposon family referred to as Centromeric Retrotransposon of Maize (CRM). A small fragment from chromosome 3 was created by irradiation by Stadler and Roman and named Duplication 3a. Its genetic behavior is reminiscent of a ring chromosome but their cytological images as well as our own failed to clearly show such a structure. Indeed, telomere FISH results indicate that Dp3a contains telomeres suggesting a linear chromosome. However, this small chromosome does not contain any detectable CentC and CRM sequences, but when molecular features of functional centromeres such as CENH3 and CENP-C were examined, they were present. Immunolocalization analysis of phosphorylation of Ser-10 of histone H3 levels on Dp3a shows a pattern typical of a functional centromere. Meiotic analysis revealed that sister chromatids divided equationally at meiosis I as do all small chromosomes examined to date in maize. To examine the sequences associated with CENH3, chromatin immunoprecipitation (ChIP) was carried out with anti-CENH3 antibodies and material from young seedlings with and without Dp3 chromosome as the tissue source. The ChIPed DNA sample was labeled for FISH detection and prepared for Illumina sequencing. Using BWA to map the ChIP-Seq reads, 76% of total reads matched the B73 genome. There are several peaks detected in the Dp3a sample that span 350 kb of the long arm of chromosome 3, which is the candidate region for association with CENH3. Collectively, the results suggest the formation of a de novo centromere on this fragment. These observations add further evidence for the epigenetic nature of centromere function in maize.

Funding acknowledgement: National Science Foundation (NSF), NSFC

T27

Modeling of Mu transposon targeting reveals cryptic features of maize gene structure

(presented by Donald McCarty <drm@ufl.edu>)
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We have used statistical modeling methods to probe the mechanism of gene targeting by the Robertson's Mutator transposon. Our findings suggest that Mu exploits novel long-range correlations in gene structure to optimize targeting of the putative plus-one nucleosome position at the 5'-end of maize genes. The Mu transposon is a simple device (2 genes) that is able to recognize gene sequences within the complex milieu of the maize chromatin with remarkable efficiency. Thus, over 80% of the 41,000 germinal Mu insertions contained in the UniformMu collection are located within or near gene models included in the MaizeSequence.org Filtered Gene Set (39,450 genes). The distribution of Mu insertions in maize chromosomes is in turn highly correlated with gene density (R^2 >0.9). The uniform density and broad diversity of genes targeted implies that Mu recognizes a widely conserved or possibly universal feature of genic chromatin in maize. The finding that Mu insertions within genes are strongly concentrated in a narrow 200 bp band immediately downstream of transcription start supports a hypothesis that the universal target may be the specialized nucleosome that typically occupies the plus-one position. However, genes exhibit a wide spectrum in frequencies of Mu insertions. Moreover, although Mu preferentially targets a compact region at the 5'-end, refined modeling of biases in Mu insertion frequencies and hotspots in the UniformMu dataset implies that Mu targeting is influenced by a larger region extending up to 4 kbp downstream from the transcription start. Efforts to identify the physical bases for the model led to discovery of cryptic structural features of maize genes that correlate with Mu targeting. We propose that long-range correlations are in part a consequence of optimization for target size.

Funding acknowledgement: National Science Foundation (NSF)

SESSION 8 - EPIGENETICS I
Chair: Uta Paszkowski Sunday, March 18. 8:30 AM – 9:50 AM

T28

Mediator of paramutation 1 (*Mop1*) appears primarily involved in the establishment rather than maintenance of a silent chromatin structure

(presented by Maike Stam <m.e.stam@uva.nl>)
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We investigate the role of epigenetic mechanisms and chromosomal interactions in gene regulation. Both levels of gene control are essential for normal growth and development. As a model system we study paramutation, a mitotically and meiotically heritable change in gene expression induced by allele interactions *in trans*. We examine paramutation at *b1*, a regulatory gene of the maize pigmention pathway. The low expressed *B'* epiallele imposes its low transcription rate onto the high expressed *B-I* epiallele *in trans*. Recent data indicate a role for siRNAs in paramutation, but also suggest they may not be sufficient. Seven tandem repeats, ~100 kb upstream of the *b1* coding region, are essential for *trans*-inactivation and tissue-specific enhancement of *b1* expression. High *b1* expression is associated with H3ac, nucleosome depletion and decreased H3K27me2 at the *B-I* hepta-repeat (Haring et al. 2010 Plant J; Bader & Stam, unpublished). The repeats of *B'* show tissue-independent DNA hypermethylation, and H3K9me2 and H3K2me2. *Mediator of paramutation 1* (*Mop1*), the predicted orthologue of *RDR2* of Arabidopsis, is required for paramutation, and the maintenance of silencing at paramutated loci (Arteaga-Vazquez and Chandler, Curr.Opin.Genet. Dev. 2010). We show that consistent with the release of silencing, regulatory sequences within the repeats are active in a *mop1* mutant, illustrated by higher *b1* transcript levels, H3ac at the repeats, and the formation of multiple chromatin loops at the *b1* locus. Despite the presence of epigenetic features associated with activation, however, the tandem repeats remain DNA methylated and associated with the silencing H3K9me2 and H3K27me2 marks, indicating the maintenance of repression in the mutant. These results indicate that the function of MOP1 in paramutation is primarily in the establishment of a silent chromatin structure *in trans*, rather than maintaining it.

Funding acknowledgement: Royal Netherlands Academy of Arts and Sciences

T29

Genome-wide effect of the *mop1-1* mutation on chromatin structure in maize.

(presented by Thelma Madzima <tmadzima@bio.fsu.edu>)
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The *Mop1* gene encodes a putative RNA-dependent RNA polymerase required for several examples of epigenetic regulation of endogenous genes and transgenes in maize. Its orthology to the Arabidopsis *RDR2* and pleiotropic mutant phenotypes suggest that Mop1 influences chromatin structure via an RNA-dependent silencing pathway and plays an important role in maize development. Nucleosome occupancy and higher order chromatin structure have been characterized using micrococcal nuclease (MNase) protection and sensitivity assays. We developed two types of Nimblegen microarray-based assays to characterize chromatin structure responses in maize. One assay (nucleosome occupancy) produces data on promoter/TSS architecture. The other assay (nuclease sensitivity) produces genome-wide data on global chromatin accessibility. Here we describe new findings from our chromatin accessibility assay used to characterize the global changes in chromatin structure in response to the *mop1-1* mutation. Using the *mop1-1* mutation segregating in a B73 genetic background we observed two large trends in genomic response, an increase in chromatin accessibility in gene-rich areas, especially near telomeres, and an intriguing decrease in accessibility around centromeres, which are enriched in LTR retroelements. These observations are consistent with Mop1's proposed role in global regulation of chromatin structure. This work demonstrates the utility of genome-wide, microarray-based nuclease sensitivity assays to determine changes in chromatin structure.

Funding acknowledgement: National Science Foundation (NSF)

T30

Epigenetic variation among maize inbreds highlights complex genetic and transcriptional interactions

(presented by Steven Eichten <eicht021@umn.edu>)
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Epigenetic variation describes heritable differences that are not solely attributable to changes in DNA sequence. There is the potential for both 'pure' epigenetic variation that occurs in the absence of any genetic change as well as more complex situations that involve both genetic and epigenetic differences. Methylation of cytosine residues provides one mechanism for the inheritance of epigenetic information. Genome-wide profiling of DNA methylation in several diverse maize genotypes allowed the identification and characterization of examples of natural epigenetic variation. An initial comparison of the DNA methylation levels in two genotypes, B73 and Mo17, allowed for the identification of approximately 700 differentially methylated regions (DMRs). Several of these DMRs occur in genomic regions that are identical by descent in B73 and Mo17 suggesting that they may be examples of pure epigenetic variation. The methylation levels of the DMRs were further studied in a panel of near-isogenic lines to evaluate the stable inheritance of the methylation levels and to assess the contribution of cis- and trans- acting information to natural epigenetic variation. The majority of DMRs that occur in genomic regions without genetic variation reflect local inheritance in the absence of genetic differences and exhibit relatively stable inheritance. However, we have also found evidence that specific classes of LTR retrotransposons can influence the chromatin state of adjacent sequences reflecting genetic control of DNA methylation. The comparison of methylomes and transcriptomes for diverse maize genotypes and tissues provide examples of epigenetic variation that may control gene expression variation in maize.

Funding acknowledgement: National Science Foundation (NSF)

T31

CHH Islands: a role for de novo methylation in near-gene chromatin regulation

(presented by Jonathan Gent <gent@uga.edu>)
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Transcription can play a major role in controlling intergenic chromatin structure, with effects on diverse cellular phenomena including gene expression. We have found that RNA-directed DNA methylation (de novo DNA methylation) marks intergenic chromatin near genes, primarily upstream and to a lesser extent downstream. We call these regions CHH islands because of their enrichment for cytosine methylation in the CHH sequence context. CHH islands suppress transposon activity close to genes but have either a positive or neutral effect on expression of the nearby genes themselves except in cases where CHH islands are especially close to genes (within one kb). In these cases, compromising CHH islands by mutation of de novo methylation components resulted in elevated expression of both genes and transposons. We conclude from these studies that a specialized form of RNA-mediated chromatin regulation suppresses transposons at the boundaries of intergenic regions; and while demonstrating a tradeoff between robust transposon suppression and gene expression in some contexts, these results also highlight one means by which cells effectively separate wanted (genic) from unwanted (intergenic) genetic activity.

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

SESSION 9 - EPIGENETICS II
Chair: Phil Becraft Sunday, March 18. 10:20 AM – 11:30 AM

T32

The maize methylome: function, diversity, and inheritance.

(presented by Rob Martienssen <martiens@cshl.edu>)
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The maize genome has undergone dramatic changes in sequence and organization following domestication and improvement, and is one of the most diverse crop genomes. Here we describe the cytosine methylation map of two maize inbred lines, B73 and Mo17, at single nucleotide resolution. Methylation is highest in symmetric CG (65%) and CHG (50%), rather than non-symmetric CHH (5%) contexts, and is highly enriched in transposons, repeats and intergenic regions, but not in centromeric repeats. Within genes, symmetric CG methylation (8%) and CHG methylation (5%) is found in some exons, while CHH methylation is found at splice sites within introns. Alignment with RNA sequences indicates that some of these patterns are correlated with small RNA, gene expression and alternate splicing. Diversity in cytosine methylation patterns was observed in TEs and especially in genes, and was found to be largely heritable in recombinant inbred lines (RILs). However, significant deviations from heritability were observed, many of which were conserved in different RILs. We conclude that directed changes in DNA methylation, resembling paramutation, are frequent events and may account for at least some of the "hidden" variation in maize breeding programs.

Funding acknowledgement: DuPont

T33

RNA polymerase IV regulates transcription of genes and transposable elements in maize

(presented by Jay Hollick <hollick@berkeley.edu>)
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Plants use RNA Polymerase IV (Pol IV), a Pol II-related complex defined by distinct catalytic subunits, to generate small interfering RNAs (siRNAs) highly enriched for transposable elements (TEs). Pol IV-dependent siRNAs target homologous sequences for chromatin modifications, indicating Pol IV plays a primary role in the epigenetic regulation of TEs. Pol IV is dispensable in *Arabidopsis*. However, the largest subunit of maize Pol IV (RMR6/ZmRPD1) is required for normal plant development (Erhard *et al*., 2009). It is likely this distinction is related to the different repeat contents of the *Arabidopsis* and maize genomes (Hale *et al*., 2009). Maize Pol IV controls expression of long terminal repeat (LTR) retrotransposons by interference with normal Pol II transcription (Hale *et al*. 2009). We hypothesize that maize co-opted Pol IV to regulate specific alleles of endogenous genes, a scenario that predicts TEs act as regulatory elements for such alleles. To identify primary genomic targets of Pol IV transcriptional regulation, we employed global run-on sequencing (GRO-seq) (Core *et al*. 2008) using wild-type (WT) and Pol IV (*rpd1*) mutants. Computational and statistical analyses indicate that Pol IV represses transcription rates of both endogenous genes and specific families of TEs. The WT and *rpd1* GRO-seq profiles are similar at transcription start sites and termination sites of genes, indicating that Pol IV does not affect Pol II transcription of gene-coding regions on a genome-wide level. Our analyses indicate that, despite the abundance of Pol IV targets in the maize genome, the loss of Pol IV increases the transcription rates of only a subset of LTR retrotransposons at the seedling stage. These data represent the first genome-wide transcription-based analysis in plants and provide the first indication of the extent of Pol IV transcriptional regulation of alleles of endogenous genes and TEs.

Funding acknowledgement: National Science Foundation (NSF)

T34

Recognition and inactivation of MuDR transposons involves multiple silencing pathways.

(presented by Damon Lisch <dlisch@berkeley.edu>)
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A fundamental problem facing all eukaryotes is the proliferations of transposable elements (TEs). This problem is addressed by a sophisticated nuclear immune system whose function is to recognize these selfish genetic elements, epigenetically silenced them and then them keep that way. This system is efficient enough so that the vast majority of TEs in plants are epigenetically inactive and therefore much of what we understand about TE silencing refers to its maintenance rather than its initiation. Fortunately, maize is host to a number of currently active TEs, the most virulent of which are Mu transposons, which are regulated by the autonomous MuDR element. Active MuDR elements can be silenced using Muk, a naturally occurring rearranged variant of MuDR that expresses a hairpin transcript that triggers heritable silencing of this element. Here we provide evidence that silencing of MuDR by Muk likely involves movement and amplification of small, mostly 22 nt RNAs whose production is sensitive to the availability of transcript from lbl1, a key component to the tasi-RNA pathway. We also show that the hairpin encoded by Muk is competent to trigger both H3K9 dimethylation of a homologous target and H3K27 trimethylation of a linked target in cis. Together with previous work, these data show that silencing of a single TE can exhibit aspects of multiple silencing pathways, including RNA-directed DNA methylation and heterochromatin formation, lbl1-dependent tasi-RNA silencing and even polycomb-mediated chromatin modification. The convergence of multiple distinct pathways on a single target suggests that although systems of epigenetic silencing in plants have functionally diverged, TE silencing remains the single unifying theme that ties them together.

Funding acknowledgement: National Science Foundation (NSF)

Poster Abstracts

P1

β-tubulin6 involves in signaling of drought and hot stress in *Zea mays*

(submitted by Haiming Zhao <haiming223@163.com>)
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Microtubule dynamics has been hypothesized to have played critical role in regulating plant response to abiotic stress; however the molecular mechanism underlying this process is largely unknown. Here we present the characterization and map-based cloning of a maize dominant maize Wilty mutant (Wi2). The mutant plants are hypersensitive to drought and hot stress with induced ABA and H_2O_2 accumulation. Map-based cloning result shows that Wi2 encodes β-tubulin6 with a point mutation within a conserved domain. Through immunofluorescence staining it is shown that the mutant Zmtub6 had affected the dynamic of microtubules. To our knowledge, this is the first genetic demonstration that microtubules have functioned in response to the drought and high temperature stress.

Funding acknowledgement: 973 program

P2

A snapshot of the past: maize ancient DNA through targeted sequencing

(submitted by Alberto Romero <jar547@cornell.edu>)
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Domestication is a very interesting example of evolution under high artificial selective pressure, and has given rise to many species today, some of which hardly resemble their wild ancestors. The widespread application of sequencing technology has greatly enhanced the study of domestication at a molecular level. Furthermore, the recent availability of high throughput sequencing technologies has made possible archaeological genomics, or sequencing genomes of historical samples.

Maize was domesticated in the central highlands of Mexico by ~8700 calibrated years before the present, and because the archaeological record traces back as early as maize domestication itself, the molecular process of domestication could potentially be studied in "real time" through ancient DNA extraction and sequencing. Despite the recent advances in ancient DNA methodology, several challenges still limit its use, including the low genome-wide coverage and depth of coverage, the nucleotide substitutions product of the natural degradation process, low sample DNA concentration compared to contaminant and modern DNA, and errors generated during the sequencing process itself. Furthermore, because maize has a very complex genome, with many repetitive sequences, correct mapping of ancient sequences given their small size is also an issue.

I present research demonstrating the plausibility of ancient maize kernel's DNA capture through the use of two independent in-solution target enrichment methods. DNA capture is contrasted with shotgun sequencing of the same samples, and several observation and recommendations are suggested for the use of such methods in similar experiments.

Funding acknowledgement: Danish Council for Independent Research 'Skou/Sapere Aude', The Danish Basic Research Foundation 'GeoGenetics'

P3

A SNP mutation in Brachytic2 mildly influences maize plant height

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Plant height has always been an important agronomic trait in maize breeding. A great many plant height QTL were reported but so far none had been cloned. In this study, a major QTL, designated as QPH1, associated with both plant and ear heights was mapped in a 1590bp interval on chromosome1 using BC6F2 population. Five SNPs were identified within the region between iso-genetic lines, and only one of which is non-synonymous, SNP5259. Out of analyzed 500 inbred lines, this mutant only occurred in five lines, indicating that is a rare mutation present exclusively in temperate germplasms. This SNP mutant is located on the 5th exon of the maize brachytic2 (Br2). The nucleotide changes from G (wild type) to T (dwarf parent) result in an amino acid substitution from R to L in the 9th α-helix in the trans-membrane domain of Br2 and may be crucial for capturing IAA-. Compared with other Br2 mutants, this mutation influences plant and ear heights less severely resulting in a decrease of 60% in homozygous state. In heterozygous state the mutation slightly decreases plant and ear heights and has a little effect on yield. This mutant could be used to decrease plant and ear heights in maize improvement.

P4

Adult plant resistance in maize to a fungal pathogen: who would have guessed it?

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Adult plant resistance (APR) is an important but unknown form of resistance in plants. We are exploring this form of resistance using the maize – *Cochliobolus carbonum* race1 (CCR1) pathosystem. This interaction is characterized by the disease Northern leaf spot (NLS), a key agent of which is HC-toxin. This potentially devastating disease is prevented from becoming economically important by *Hm1*, a disease resistance gene present widely in the maize germplasm. *Hm1* confers resistance to NLS by encoding an NADPH-dependent reductase (HCTR) that inactivates HC-toxin. Resistance conferred by *Hm1* is complete and operates globally in the plant. In contrast, *Hm1A*, an allele of *Hm1*, and *Hm2*, a duplicate of *Hm1*, confer full protection only at maturity. Cloning of these APR genes has revealed that *Hm1A* encodes an HCTR with five amino acid substitutions, while *Hm2* encodes a truncated HCTR without the last 52 amino acids. These are relatively weak changes and likely cause HM1A and HM2 to lose their function only partially. Given these findings, our working hypothesis is that the APR nature of *Hm1A* and *Hm2* stems from their weak HCTR activity. To address this hypothesis, we made use of a targeted mutagenesis approach in which ears of a susceptible inbred (*hm1hm1hm2hm2*) were fertilized with EMS-treated pollen from B73 (*Hm1Hm1hm2hm2*). Out of 7 mutants found in the M1, 2 were APR and 5 null. All null mutants had sustained drastic changes such as premature stop codons or disrupted intron-exon splicing. The APR alleles, on the other hand, suffered relatively minor changes, with one undergoing a T to M change at amino acid 90, and the other V to M substitution at residue 210. These findings are clearly in line with our hypothesis, although we are still uncertain as to why weak *Hm* genes exhibit APR.

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P5

Advancing complex phenotype analyses through machine vision and computation

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Phenotyping methods frequently limit functional genomic studies. Phenotypes are currently not studied with the same degree of sophistication or throughput as genomes or processes more proximate to the genome such as gene expression. Because phenotypes are such an important source of information about gene function, we are integrating multiple machine vision platforms to study seed and seedling phenotypes. Machine vision utilizes information contained in an image or other optoelectronic signal, such a reflectance spectroscopy, to collect quantitative measures of phenotypes. We are focusing on the interrelationships of maize kernel traits with seedling growth traits. We have developed a semi-automated pipeline to collect kernel weight, near infrared reflectance (NIR) kernel spectra, kernel color and 3D shape, and dynamic seedling root growth. Serial phenotyping on indexed kernels will provide greater statistical power to detect interrelationships that have a physiological basis. Computational workflows are being developed to automatically extract biologically relevant data from each phenotyping platform and to interrelate the machine collected data. The combination of high-throughput serial phenotyping and diverse genetic resources available in maize allow access to a wide variety of relationships between seed and seedling characteristics. As an example, preliminary studies show that aspects of the NIR spectra of a kernel are correlated with seedling root gravitropism response. We are also using this pipeline to identify quantitative trait loci (QTL) underlying the phenotypes and physiological relationships within the maize Nested Association Mapping (NAM) population.

Funding acknowledgement: National Science Foundation (NSF)

P6

Analysis of benzoxazinoid biosynthesis in the maize lines B73 and Mo17, gene expression and QTL determination.

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Benzoxazinoids (DIMBOA) are secondary metabolites primarily found in maize and other grasses. DIMBOA serves as a natural pesticide and reduces damage by controlling a wide range of pathogens and herbivores. Although the biosynthesis of DIMBOA has been elucidated, nothing is known about the regulation of the pathway. The IBM population has been used for QTL analysis to identify elements in *Bx*-gene regulation. The parental lines of IBM population, Mo17 and B73, have significant differences in DIMBOA content in leaves of 24 days old plants. The analysis of *Bx1* transcript levels in Mo17 and B73 shows a correlation between gene expression and benzoxazinoid content. Two consistent QTLs have been detected; one is on the short arm of Chromosome 4 (QTL4), the same position as the *Bx*-gene cluster. The Mo17 allele increases the value of the trait. The other QTL is at chromosome 6 (QTL6). The inbred line IBM38, that has Mo17 genotype at QTL4, and B73 genotype at QTL6, was backcrossed with B73. The F2 progeny was used to generate the fine mapping population. 20 recombinants help to delimit the regulator of *Bx1* expression.

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P7

Analysis of MYB Transcription Factors Involved in the Regulation of the Phenylpropanoid Pathway in Maize

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Many products of the phenylpropanoid pathway play a crucial role in multiple aspects of our everyday lives. Anthocyanins and phlobaphenes serve as powerful antioxidants and display antimycotic properties, whereas lignins and lignin precursors play an important role in cell wall composition, especially in the area of renewable fuel production. Grasses, including maize, are a major source of agricultural biomass, offering significant opportunities for the increasing demand for sustainable resources. The efficiency of biofuel production is influenced by lignin content which can significantly reduce the amount of extractable sugars. To explore the possibility of altering lignin content we are investigating transcriptional regulators of the phenylpropanoid pathway. The two R2R3-MYB transcription factors *ZmMYB40* and *ZmMYB95* are members of the R2R3-MYB^{PtoA} clade, which is closely related to *ZmC1* and *ZmP1*. In maize, C1 and P1 control different branches of the phenylpropanoid pathway (anthocyanins and flavonoids, respectively), and based on the mechanisms by which they regulate gene expression, we hypothesized that closely related R2R3-MYB^{PtoA} members would control further branches of the phenylpropanoid biosynthetic pathway. In accordance with this assumption, *ZmMYB40* overexpression in maize cells induces the accumulation of phenylpropanoids (ferulic acid, chlorogenic acid, and caffeic acid), but not flavonoids, suggesting the regulation of a different branch of the phenylpropanoid pathway. In order to identify direct targets for ZmMYB40 and ZmMYB95, maize transgenic lines harboring RNAi constructs to silence the respective genes have been generated. Characterization of these MYBs in combination with investigation of negative regulators of lignin biosynthesis (ZmMYB31 and ZmMYB42) will help explain the intricate regulation of lignin and lignin precursor production in maize cells. These studies will also help identify approaches for genetic improvement of maize and other important grass species as feedstocks for biofuel production.

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P8

Analysis of the *mre*-594* maize seed development mutant with a maternal parent-of-origin effect

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Maize is a central crop to agriculture with the seed being the primary product for food and industrial applications. Mature maize seeds contain an embryo and an endosperm. The endosperm accumulates seed reserves of starch and protein and accounts for more than 80% of seed weight. Epigenetic regulation, specifically imprinting within the endosperm, is thought to play an important role in determining endosperm and seed size. However, imprinted genes that regulate maize endosperm size have not been identified. We are screening for defective kernel mutants with maternal parent-of-origin effects. We present preliminary analysis of one locus, maternal rough endosperm isolate 594 (mre*-594). When the female gametophyte is mre*-594, seeds develop a rough, etched, or pitted endosperm surface regardless of pollen genotype. The mre*-594 mutant fully transmits through the pollen and does not cause seed phenotypes when fertilizing wild-type plants suggesting it confers a maternal parent-of-origin effect on seed development. Laser confocal microscopy of pre-pollinated female gametophytes suggests mre*-594 alters antipodal cell morphology, and 4 days after pollination mre*-594 seeds showed delayed endosperm development. These data suggest the Mre gene is required prior to pollination and help explain the reduced size of mre*-594 seeds. We mapped mre*-594 to the short arm of chromosome 4 using SSR markers and a backcross mapping population.

P9

bif42 Functions in Vegetative and Reproductive Development in Maize

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barren inflorescence 42 (bif42) mutants have vegetative and reproductive defects. *bif42* mutants exhibit a narrow leaf phenotype. Measurement of leaf width at different stages of development demonstrated that the *bif42* mutant produces progressively narrower leaves than the wild type siblings, while still maintaining leaf symmetry. Mutant tassels also have a sparse appearance. Quantification indicates that *bif42* mutants produce fewer branches and spikelets. To investigate the reason for fewer tassel branches, immature tassels were dissected from five week old mutants and viewed by Scanning Electron Microscopy (SEM). The SEM images showed that the mutants had consistently longer, thinner tassels with fewer branches indicating that *bif42* may function in the apical meristem. In order to clone *bif42,* SSR markers were used to map the *bif42* mutation to bin 3.04. The next step will be to fine map and finish cloning the bif42 gene.

Funding acknowledgement: National Science Foundation (NSF)

P10

Characterization And Partial Sequence Of The sh2-R Insertion

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The sh2 gene (GRMZM2G429899) encodes the ADP-glucose pyrophosphorylase large subunit and is located on Chr3, position 216,414,684. The enzyme catalyses the reversible synthesis of ADP-glucose and pyrophosphate from ATP and glucose-1-phoshphate. It is one of the main regulatory steps in the biosynthesis of starch in plants. The sh2-R allele defined the sh2 locus and was first identified by E. B. Mains in 1949. The interval from GRMZM2G316635 (CL11820) to sh2 contains in order the five genes GRMZM2G316635 (CL11820), a1, yz1, x1 and sh2 in an interval of 159 kb. The sh2-R allele is a complex re-arrangement whereby the genes from GRMZM2G316635 (CL11820) to x1, and possibly others upstream, have been inserted into the first exon of sh2. The four genes in the insertion are in the opposite orientation in the sh2-R allele versus B73. We have cloned 32.46 kb of the 5' end of the insertion and 69.76 kb of the insertion on the 3' end. The insertion is at least 102.22 kb in length. The next gene in the sequence, GRZM2G316635 (upstream of GRMZM2G316635 (CL11820)), is missing as confirmed by PCR and hybridization. Southerns have been non-informative due the size of the insertion and lack of unique enzymes for mapping. The true size of the sh2-R insertion is unknown at this time.

P11

Characterization of Maize Group 1a-2 Purple Acid Phosphatases

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In agricultural systems, the essential element phosphorus (P) is largely locked away in organic forms that are not directly accessible to plants. To access P from organic sources, plants require that inorganic P (Pi) is released: one key strategy plants have to promote this process is the secretion of acid phosphatases (APs) to the surface of roots and into the soil. Recent study has shown that in Arabidopsis the major secreted AP activities are encoded by the *AtPap10*, *AtPap12* and *AtPap26* purple acid phosphatases (PAP) Group 1a-2 genes.

Maize is the second most consumed cereal in the world and, like all crop grasses, it has a high P requirement. To uncover genes that encode secreted AP activities in maize, we are characterizing four candidates identified on the basis of similarity to the known Arabidopsis sequences. We will present analysis of the accumulation of transcripts encoded by our candidates, in different seedling tissues, and in response to changing phosphate availability. We will present also identification of putative transposon insertions in our candidate genes, and describe future plans for functional testing through mutant analysis and by heterologous expression of maize sequences in bacteria and Arabidopsis mutant backgrounds.

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P12

Characterizing a wilted *Zea mays* mutant

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In maize, leaves iteratively initiate and elongate until the vegetative to reproductive transition. We have identified an incompletely dominant and incompletely penetrant *Zea mays* mutation in which this process has been disrupted. Plants that express the mutant phenotype develop normally until the onset of wilting in upper leaves. The expression of the phenotype may occur early (V2) or late (V10) in vegetative development. Wilting spreads downwards, stem elongation ceases, and wilted leaves senesce and die. Water content is decreased in wilted leaves, but the phenotype is not initiated by water stress, and plants remain wilted regardless of water availability. Wilted plants also have normal metaxylem development. We have localized the mutation to a single locus within a circa. 7 Mb region on the long arm of chromosome 7, which does not overlap with a known, mapped wilted mutant. Of plants expressing the mutant phenotype, over 80% are homozygous for this region. Nonetheless, not all homozygous plants express the mutant phenotype, and a small proportion of heterozygous plants wilt. We hypothesize that the wilting phenotype is due to a mutation triggering wilting and senescence.

P13

Characterizing Mutator insertions into sucrose transporters (SUTs) in maize

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Carbon partitioning, the movement of sugars from photosynthetic tissues to sink tissues (e.g., developing leaves, ears), is essential for plant growth and development. In maize and many other plants, sucrose transporters (SUTs) are thought to control the movement of sucrose across the plasma membrane and into phloem cells. Despite the importance of these transporters, the details of carbon partitioning and the function of the maize *Sut* genes are poorly understood. From analyzing the maize genome sequence, we have identified six maize *Sut* genes in addition to the previously described ZmSut1. To ascertain their biological functions, we obtained Mutator transposable element insertions into each *Sut* gene. Using gene-specific primers, we are in the process of genotyping these mutant maize lines to verify the genomic locations of the insertions and to determine the consequences of each mutation. Once viable mutants are identified, we plan to characterize the biological functions of these *Sut* genes by analyzing the mutant phenotypes (growth, sugar and starch profiling, maturity, yield, etc.), as well as examining gene expression patterns, and measuring photosynthesis and carbon transport rates in mutant and wild type plants. These analyses will determine the contributions of each SUT to whole-plant carbohydrate partitioning.

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P14

Characterizing new maize genes that function in carbohydrate partitioning

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Movement of fixed carbon (e.g., sucrose) from photosynthetic leaves to sink tissues (e.g., ears, roots) is crucial to plant growth and development and central to crop yield and the production of biomass for biofuels. However, despite the importance of this process in plants, little is known about its genetic regulation. In an effort to identify genes controlling carbon partitioning in plants, we screened for mutants, termed *carbohydrate partitioning defective* (*cpd*) mutants, that accumulate excessive levels of carbon in the leaves. We have identified a number of mutants that show variegated chlorotic regions with anthocyanin accumulation and altered starch accumulation patterns in the leaves. Moreover, the mutant plants vary in overall severity (brachytic to normal height) and appear to fall into discrete classes. A number of mutations are currently being mapped to chromosome regions/bins, will be complementation tested if they map to the same region, and will facilitate the map-based cloning of the mutated genes. These mutants will serve as a valuable tool for elucidating the genetic control of this essential process in plants and thereby allow the manipulation of carbon flux in plants for improving crop yields and increasing plant biomass.

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P15

Characterizing the Biological Functions of and Genetic Redundancy among the Maize Sucrose Transporters

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Sucrose is the primary sugar used by plants for the long-distance transport of carbohydrates. However, knowledge about the regulation and mechanisms controlling this transport is limited. Multiple sucrose transporters (*Sut*s) have been identified for several different plant species, including Arabidopsis, maize, sorghum, pea, and potato, and phylogenetic analyses have divided these *Sut*s into five different clades. Clade 2 is dicot specific, while Clades 1 and 5 are monocot specific. Seven *Sut*s, belonging to four of the five clades, have been identified in maize. To date, *ZmSut1* is the only maize sucrose transporter to have been functionally characterized. Meanwhile, a previously conducted time course experiment examined the expression of *ZmSut2*, *ZmSut5*, and *ZmSut7* during a 24-hour period. This study found that each *Sut* displayed a unique expression profile and that the gene expression profiles differed according to tissue type. To determine the carbon transport functions of the different *Sut*s in maize, we set out to examine their gene expression profiles in a more extensive time course experiment. Using the information obtained from these experiments, we will select time points at which *ZmSut1*, *ZmSut2*, and *ZmSut4* display maximal gene expression. From these data, we will analyze tissues from mutants in these three *Sut* genes to determine if changes in the expression of the other *Sut* genes compensate for the mutation. Information gathered from these experiments will address the role of *Sut* functional redundancy and aid in the assignment of a biological function (e.g. physiological role) for each *Sut*.

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P16

Characterizing the expression of TIE-DYED1, a novel protein that regulates carbohydrate partitioning in maize

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Carbon transport is essential for plant growth and development. Photosynthesis occurs primarily in the leaves and leads to the accumulation of fixed carbon (e.g., sucrose, starch). For sustaining the metabolism and growth of non-photosynthetic tissues, plants principally transport fixed carbon in the form of sucrose. In maize, sucrose is loaded into the phloem of the leaves, transported through the veins, and unloaded into the sink tissues (e.g., roots, tassels, ears). We have analyzed genes involved in regulating the transport of fixed carbon throughout the plant. Through these analyses, we have identified a collection of mutants, called the *tie-dyed* (*tdy*) loci, that hyperaccumulate carbohydrates in sectors of their leaves. *Tie-dyed1* (*Tdy1*) is a novel gene expressed in the phloem. To better understand the biological function of this gene, we sought to determine where the TDY1 protein localizes in the plant. In previous studies, we translationally fused a yellow fluorescent protein (YFP) to TDY1 and transiently expressed the TDY1 fusion protein in onion epidermal cells. Based on these assays, TDY1 was determined to be localized to the endoplasmic reticulum and the nuclear lamina. To determine the TDY1 localization in the native environment, we created two unique TDY1-YFP fusions under the control of the endogenous regulatory sequences and stably transformed these into maize. Preliminary investigations on these lines suggest the gene is expressed in the phloem. Ongoing work on localizing TDY1-YFP will be presented. After determining the TDY1 expression pattern under normal conditions, future research will investigate changes in TDY1 expression and carbohydrate partitioning in response to environmental stress conditions and in different genetic backgrounds.

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P17

Classes of ABC Transporters in Maize (*Zea mays* L.) Genome

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ABC transporters are proteins that play important role in various cellular activities, which broadly include nutrient uptake and secretion of toxic products into the surrounding medium. Plant ABC transporters have been associated with such important traits as tolerance to diseases, insects and heavy metals, fiber development, light signaling etc. ABC transporters have been well described in the model plant *Arabidopsis thaliana*. However, very little is known about the classes of these proteins in major crops, including maize. In this study, we mined internally validated maize SNP collection for the sequences that would putatively encode ABC transporters. Our objectives were to investigate the distribution of these genes in maize genome, identify their classes and detect gene-based SNP assays associated with ABC transporters.

P18

Cloning and Characterization of *brd1* – Maize Brassinosteroid C6 Oxidase Gene

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Brassinosteroids (BRs) are a group of plant steroid hormones that regulate growth and development. Plants defective in brassinosteroid biosynthesis and regulation exhibit dwarf phenotype and alteration of the cell structure and growth. To date, the role of brassinosteroids has not been well studied in maize. A maize mutant with severe dwarfing was identified and used for positional cloning of the causative mutation. A single base nonsense substitution in the *Zea mais* brassinosteroid-deficient (*brd1*) gene was identified. This gene is a homolog of the rice OsDwarf and tomato Dwarf gene and encodes a BR C6 oxidase enzyme that is responsible for the last step in the brassinosteroid biosynthesis. The maize genome contains only one homolog for BR C6 oxidase. Maize plants homozygous for the mutation in maize brd1 are sterile, exhibit severe dwarf phenotypes, no etiolation response, alterations in the morphology of leaves and floral structures. The expression of maize *brd1* is negatively regulated by brassinosteroids such that low levels of brassinosteroids result in high gene expression. Supplementing growth media with 10^{-6}M brassinolide partially rescues the mutant phenotype and lowers down the expression of maize *brd1* in the homozygous mutant plants. Characterizing maize mutants defective in brassinosteroid biosynthesis will lead to better understanding of the brassinosteroid biosynthesis and biosignaling pathways might help in developing new semi-dwarf maize varieties.

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P19

Copy number variation (CNV) of genes involved in carotenoid biosynthesis in maize.

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Carotenoids are yellow-orange pigments that, in part, protect chlorophyll from photooxidation in leaf tissue. Carotenoids can also accumulate in both the embryo and endosperm of the maize kernel. Some carotenoids have pro-vitamin A activity, which is nutritionally important for animals. Vitamin A is essential for prevention of xerophthalmia and blindness as well as proper immune function. We have used qPCR to determine gene copy number relative to B73 for four carotenoid biosynthetic genes: *Geranyl-geranyl diphosphate synthase 1* (*Ggpps1*; AC194970.5_FG001), *phytoene synthase 1* (*Psy1/y1*; GRMZM2G300348), *lycopene β-cyclase* (*Lycβ*; GRMZM5G849107), and *Lycopene ε-cyclase* (*Lycε*; GRMZM2G012966). The expression and enzymatic activity of GGPPS1 and PSY1 are generally regarded as being important to regulating the flow of precursors into the carotenoid biosynthetic pathway. The two lycopene cyclases function at a branch point in the carotenoid pathway leading to either β or α carotene. The regulation of the flow down these two branches of the carotenoid pathway impacts the amount of pro-vitamin A carotenoids present in the tissue. Copy number was determined for Mo17 and 8 inbred lines that are members of the NAM parent population and thus well represent the genetic diversity of maize. We find that *Psy1* and *Ggpps1* exhibited the greatest degree of CNV among the lines investigated. The correlation between kernel carotenoid content and CNV at these four genes is presently under investigation.

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P20

Deep analysis of causal mutations in the Photosynthetic Mutant Library by Mu-Illumina sequencing: A progress report.

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Forward genetic analyses of chloroplast biogenesis have led to the recognition of unanticipated players and the establishment of new paradigms. However, the power of phenotype-driven gene discovery for understanding chloroplast biogenesis has not been thoroughly exploited. Our collection of non-photosynthetic maize mutants, the "Photosynthetic Mutant Library" (PML), provides an opportunity to discover most genes in maize that are required for photosynthesis. The PML collection consists of ~2000 independently-arising Mu transposon-induced mutants. We estimate that the collection represents ~600 genes, each with several mutant alleles. Each mutant has an accompanying pedigree, and many have characterized chloroplast protein and RNA "fingerprints" that elucidate gene function.

The high Mu-copy number has hampered efforts to use Mu for forward genetics. To fully exploit the PML collection for phenotype-driven forward genetics, we developed a method that uses Illumina sequencing to identify Mu insertions causing phenotypes-of-interest in high copy Mu lines (Williams-Carrier et al, 2010). The approach entails sequencing (virtually) every Mu flanking sequence in individuals of known genotype, and identifying insertions that cosegregate with mutations-of-interest. The rate-limiting step in this process is the development of the two-generation pedigree from which the DNAs are obtained. In the ~two years since developing this method, we have used it to identify 76 insertions (representing 43 genes) underlying specific phenotypes of interest. Our success rate is ~85%. Failures are likely due to gaps in the maize genome sequence, incorrect gene models, and untagged alleles. ~1/3rd of the genes identified are conserved in Arabidopsis but have not been studied in any organism. ~1/3rd are orthologs of genes that have been superficially described in Arabidopsis, but for which our phenotypes suggest distinct or more specific functions. ~1/3rd are orthologs of well-characterized genes in other organisms, and our phenotypes match those previously described. A byproduct of the approach is the identification of numerous heritable insertions that are unrelated to the targeted phenotype. We are making such bystander insertions available for community use via a search interface at http://teosinte.uoregon.edu/mu-illumina/.

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P21

Determining the genetic and environmental controls of surface hydrocarbon accumulation on maize silks

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Reproductive success, and ultimately yield and seed quality in maize require that its stigmatic silks withstand significant abiotic and biotic stresses during the critical period of pollen reception. Like many plant organs, silks are coated with surface lipids that protect against desiccation, UV radiation, pathogen invasion and insect feeding. The surface lipid coat of maize silks is unique because it contains mostly (>90%) simple non-isoprenoid hydrocarbons. Presently, neither the biochemical mechanisms nor the genetic and environmental regulators that determine the biosynthesis of these surface hydrocarbons (SHCs) are sufficiently defined. In order to understand the biochemistry and regulation of these SHCs, we have employed the IBMRIL and IBMDH populations in a genetic approach that capitalizes on differences in SHC amounts and constituents between B73 and Mo17 [Plant J. 64: 618-632 (2010)]. Silk samples were collected three days after emergence from ~4,000 field grown plants (660 isolines, 6 samples/line), using only the plants that represented the mid-silk cohort in each line. The samples were flash-frozen, lyophillized, ground, and used for hydrocarbon extraction. Metabolomic phenotypes have been assayed by GC-MS for the IBMRIL samples, allowing estimation of SHC constituent heritabilities and metabolite-QTL analyses. Genotype explains 46.5% of the phenotypic variance for total SHC quantity ($p<0.0001$), and its effects on individual SHC constituents range from 20-78% ($R2>0.55$ for more than half the individual SHC constituents). These determinations suggest that a mix of genetic and environmental effects control these traits. Based on these heritability estimates, we expect our full-scale experiment to robustly detect chromosomal positions controlling specific steps in the biochemical pathways involved in SHC accumulation. Also, we are building a modeling framework to utilize the meteorological data from the ~3-week period over which the ~4,000 samples were collected. We expect this multifaceted experiment to produce new insights into SHC biochemistry and its regulation.

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P22

Determining the protein expression patterns of maize sucrose transporters

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Whole-plant carbohydrate partitioning is a physiological process vital for plant development and growth. During this process, photosynthetically assimilated carbon is translocated in the form of sucrose from leaves to non-photosynthetic sink tissues through the phloem. The maize *Sut1* gene is essential for loading sucrose into the phloem, since it functions as a sucrose transporter that moves sucrose from the apoplast into the cytoplasm of phloem cells. Previous research in our lab has identified other potentially important *Sut* genes (*Suts2–7*). Currently, we are determining whether several transposable element-insertion lines obtained from Pioneer carry knock-out mutations for each *Sut* gene, since loss-of-function mutants allow the assessment of biological functions. As one approach to characterizing *Sut* function, we are raising antibodies specific for each SUT protein. In combination with antibodies to various subcellular markers, the antibodies against SUTs 1–7 will be used to investigate the expression of the SUT proteins via western blotting. Additional experiments will determine in which tissues each SUT protein is expressed, which alleles correspond to null mutations, and if the expression of a particular SUT protein is changed in a genetic background deficient for a different *Sut* gene. Details of our progress will be presented.

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P23

Development of a strain of E. coli auxotrophic for Lysine, Methionine and Tryptophan and it's use for evaluation of amino acid balance in grains

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The nutritional value of grain is determined partly by the amino acid balance. Current methods of determining the amino acid balance of grains are expensive and time consuming due to low throughput. A method enabling low-cost quantitative measurement of amino acid balance would allow researchers to develop crop varieties with higher nutritional value. In plant-based diets, three of the ten essential amino acids are more often limiting than the other seven: lysine, tryptophan and methionine. We engineered an E. coli strain that is auxotrophic for these three essential amino acids. We identified optimal conditions for using this bacterial strain to evaluate deficiencies of these three amino acids in grain extracts. The auxotrophic strain displayed different growth patterns when grown on extracts from different corn varieties or other grains known to contain varied amino acid levels. Grains were tested on the basis of total protein content so observed differences were likely to be due to different amino acid balances within the grain. Among the corn varieties, those known to contain mutations that increase lysine levels tended to supported higher growth of the auxotrophic strain. By supplementation of the growth media with known amino acid solutions, the bacterial strain enabled identification of a single limiting amino acid for each grain. We next tested binary combinations of grains using the auxotrophic bacterial strain to identify combinations with optimal amino acid balance. Sorghum combined with wheat or soybeans produced the best growth of the auxotrophic strain. This new analytical test enables quantitative analysis of grain amino acid balance, which may be useful to researchers who focus on improving amino acid balance. While developing this analytical tool, nutritional information on grains consumed by humans was compiled, and these data can inform grain consumers on how to build healthier diets.

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P24

Different resistance to southern corn rust of maize inbred lines in China

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Southern Corn Rust (SCR), caused by *Puccinia polysora* Underw, is a destructive disease in maize. In China, it did not occur until 1970, which was first observed in the most southern area of Hainan and Taiwan. In 1998, SCR was first widespread into north maize belt of China and caused serious maize yield losses. Since then, it has been a main maize disease in China.

We selected 14 elite inbred lines, which were wide used germplasms of maize breeding in China, and performed SCR inoculation on seedling leaves. The results showed that most of the inbred lines were susceptible to SCR. Eight out of 14 inbred lines were highly susceptible, three were moderately susceptible, and two lines showed partial resistance. Only inbred line Qi319 gave a high resistance to SCR. The disease pustules developed rapidly after inoculation. At flowering stage, all leaves of susceptible plants were covered with pustules and become chlorotic and dry. The susceptible plants died around 60 days after inoculation. There were almost no yields in those susceptible corn inbred lines.

For the high SCR resistance inbred line of Qi319, twenty hybrids were obtained by crossing Qi319 with five susceptible inbred lines. The responses of F1, F2 and BC1F1 progenies to *P. polysora* were observed. All of F1 progenies showed highly resistant to this disease, F2 and BC1F1 progenies segregated in 3:1 and 1:1 ratio of resistance to susceptible, respectively. These results indicated that the resistance gene of Qi319 to SCR was conditioned by a single dominant gene *RppQ*. SSR analysis showed that this resistance gene was mapped on the short arm of chromosome 10 in maize.

P25

Dissecting the developmental and environmental effects on surface hydrocarbon accumulation on maize silks

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During the critical period that maize silks are exposed to the environment for pollen reception, the silk's cuticle protects the tissue from abiotic and biotic stresses (e.g., UV radiation, insect damage, desiccation). The cuticle also mitigates mechanical stresses imposed on silks encased within the husks (e.g. surface-to-surface interactions with the cob and among adjoining silks). Lipids that comprise the cuticle are >90% simple, non-isoprenoid hydrocarbons ranging from C23 to C33. These surface hydrocarbons (SHC) are likely produced via elongation of fatty acids with subsequent conversion to hydrocarbons. Notably, in the B73 inbred the emerged silks express ~5-fold more SHCs than the encased silks. Moreover, B73 silks have ~5-fold more SHCs and a more complex array of constituents as compared to Mo17, indicating that genotype, development and environment affect SHC traits [Perera et al., *Plant J.* **64:** 618-632 (2010)]. Recently, we have dissected the developmental gradients that affect SHC accumulation on silks in inbreds B73, Mo17 and their reciprocal hybrids. These analyses establish that phenotypic variation observed in the inbred B73 and the reciprocal hybrids is largely attributable to the developmental gradient along the length of the silk. To separate the developmental component from the environmental impact(s) of silk-emergence, we tested the effects of the silk development gradient solely among the encased samples. These latter analyses establish that SHC accumulation in the encased silks is also affected by the developmental gradient, which is independent from the environmental modifier associated with silk emergence. Responses to these gradients do differ among genotypes. Finally, emerged silks that were subjected to light versus dark treatments showed no discernible differences in total hydrocarbon accumulation. This suggests that external cue(s), other than illumination status, govern the strong response to the emergence of the silk from the husk.

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P26

Divergence of gene regulation between maize and oat

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Oat-maize addition (OMA) lines have single maize chromosomes in addition to their own. One interesting question that arises is how expression of the maize genes is affected in the oat background, and conversely, whether they will effect a change in phenotype of oats. As an example, we selected the maize prolamin seed storage protein genes, called zeins, and their know regulators, *Prolamin-box binding factor (Pbf)* and *Opaque2 (O2)*, which are present on different maize chromosomes. In each OMA, the specific maize chromosome was identified with simple sequence repeat (SSR) markers. Knowing the chromosome, presence of each zein locus was confirmed using specific primers for gene amplification. Indeed, zein genes were confirmed for OMAs with maize chromosomes 2, 4, 6, 7, and 9. As expected the oat genome does have diverged prolamin gene sequences that were not amplified. Expression of the regulators *Pbf* and *O2* and *zein* genes was tested with RT-PCR of OMA immature endosperm. Although *O2* and the 27- and 50- kda gamma zeins were expressed in the OMA line with maize chromosome 7, none of the alpha zeins on chromosome 7 are. Also *Pbf* in OMA line with maize chromosome 2 is expressed, but no zein genes in other OMA lines are. Based on previous work, gamma zeins are the only ones requiring solely PBF for transcription, whereas the other ones require an additional transcriptional activator. Given that in OMA with maize chromosome 7 maize *Pbf* is absent and maize gamma zeins are expressed, it appears that *Pbf* is the only prolamin gene regulator conserved between oats and maize. Furthermore, regulators of *Pbf* and *O2* must have been conserved as well. In contrast to oats, maize *Pbf* and *O2* can trans-activate the expression of the alpha prolamin genes in sorghum, when introduced into maize.

P27

Early Endosperm Development in Maize

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Endosperm is one of the defining characteristic of Angiosperms. Resulting from a second fertilization event in the central cell of the female gametophyte, endosperm plays an essential biological role for the developing embryo inside the seed by providing nutrients as it grows and germinates. The agricultural importance of endosperm is global. Nearly 60% of all calories consumed by humans comes from endosperm. Maize endosperm contributes to this figure significantly. Although later developmental stages of endosperm have been well studied in Maize, very little is known about early events of endosperm development. In order to better understand morphological and molecular aspects of endosperm development in the Maize genomic model B73, a developmental staging system was developed along with the generation of transcriptional profiles at each stage using high through put sequencing. Next, transcription factors (TFs) were identified and clustered to characterize novel expression patterns. Currently, a set of TFs are being tested for tissue specific expression using In Situ Hybridization, which will aid in prioritizing the TFs that will be functionally analyzed either through available mutants or by generating RNAi knockdowns. Additionally, non-TF genes are being assayed with In Situ Hybridization in order to generate a large set of tissue specific markers that can potentially be used during mutant analysis. Together, these data not only provide new insights into the regulation of early endosperm development but will also provide the Maize community with a valuable set of new resources and tools that can be utilized for both biological and agricultural studies.

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P28

Evalutation of the role of IBA-derived IAA in *Zea mays*.

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Auxin is a phytohormone involved in cell elongation and division. In monocots, auxin regulates the development of adventitious and lateral roots, vascular tissues, leaf number, and leaf blade area. Because of the importance of auxin, levels of indole-3-acetic acid (IAA), the primary auxin, are tightly regulated through biosynthesis, degradation, sequestration, and transport. IAA is sequestered in reversible processes by adding amino acids or sugars, forming IAA-conjugates, methylation, or via a two carbon elongation forming indole-3-butyric acid (IBA). These sequestered forms of IAA reduce the ability of the hormone to act as a signal. IAA-conjugates can be hydrolyzed back to active IAA by enzymes ILR1, IAR3, and ILL2 while MES17 converts Methyl-IAA back to IAA. Plants also maintain IAA levels by a reversible conversion to IBA. IBA to IAA conversion is similar to fatty acid β-oxidation and involves the enzymes IBR1, IBR3, IBR10, and ECH2 in Arabidopsis. We are translating the current knowledge of IBA-derived IAA from Arabidopsis to maize. To gain a better understanding of how auxin homeostasis is maintained, we have made Arabidopsis higher-order mutants that combine disruptions in the IAA-conjugate, methyl IAA, and IBA pathways in Arabidopsis. These mutants show phenotypes indicative of low auxin levels such as decreased apical dominance, rolled leaves, and abnormal vein patterning. Phenotypic analysis of these mutants enables us to dissect the roles of each pathway in growth and development in Arabidopsis. I have identified maize genes similar to *AtECH2, AtIBR1, AtIBR3*, and *AtIBR10* with 78, 82, 80, and 68% identity. Mutants in *ZmECH2, ZmIBR3*, and *ZmIBR10* show phenotypes including random embryo placement and chlorosis. Examining IBA metabolism in maize will lead to increased understanding of auxin homeostasis in maize.

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P29

Evolution and expression of the cellulose synthase (*CesA*) gene family in maize.

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Cellulose is the most abundant, renewable biopolymer on earth. Understanding how this compound is synthesized at both molecular and genetic levels is central to better utilizing this valuable resource for fuel, fiber, feed, structural material, and other diverse applications. Here we show that the *CesA* gene family in maize is distributed across the genome with a bias towards chromosomal segments that have retained functional genes following the diploidization of this ancient tetraploid. This observation follows the model of Schnable et al. (2011) in which one "copy" of a genome or genome-segment is more resistant to gene loss after a duplication event. Additionally, we present evidence, using qPCR of numerous tissues at key developmental stages, that *CesA 10, CesA 11,* and *CesA 12* are strongly associated with tissues developing secondary cell walls. Results are further supported by expression analyses of developing kernels and a suspension culture that was induced to differentiate tracheary-element-like cells, rich in secondary cell walls. In developing kernels *CesA 10, CesA 11*, and *CesA 12* were highly upregulated, but only in the "woody" pedicel tissue. In suspension culture, these genes showed a peak in expression that coincided with induction of secondary cell wall synthesis. Analysis of coordinately-expressed genes showed that *CesA 10, CesA 11,* and *CesA 12* are the only family members that exclusively and consistently group together at the seedling-, vegetative-, and anthesis-stages. These data are consistent with current models for potential association of CesA proteins in the same tripartite heterohexameric complex. In summary, our results show that the maize CesA gene family predominantly maps to genome segments associated with retention of functional genes. Furthermore, *CesA 10, CesA 11,* and *CesA 12* are strongly associated with secondary cell wall biosynthesis.

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P30

Evolution of *bz* Orthologous Regions in the Genus *Zea* and Relatives in the *Andropogoneae*

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Genome structure exhibits remarkable plasticity within *Zea mays*. To examine the extent of haplotype structure variation across the genus *Zea* and more distant relatives in the *Andropogoneae* tribe, we have analyzed the *bz* gene-rich region of seven species, including maize, the teosintes *Zea mays* ssp. *mexicana*, *Zea luxurians* and *Zea diploperennis*, *Tripsacum dactyloides*, *Coix lacryma-jobi*, and *Sorghum propinquum*. We have sequenced and annotated 10 BAC clones from these species and reannotated the orthologous *Sorghum bicolor* region. Our analysis has revealed that gene colinearity in the *bz* region is well conserved within the genus *Zea*. However, the orthologous regions of *Coix* and *Sorghum* exhibited several micro-rearrangements relative to *Zea*, including addition, truncation, and deletion of genes. As in *Zea*, different patterns of interspersion between genes and retrotransposons are observed in *Sorghum*. The *stc1* gene, involved in the production of a terpenoid insect defense signal, is evolving particularly fast. It is mutated in *Tripsacum*, truncated in *Coix*, and deleted to barely detectable fragments in the two *Sorghum* haplotypes. We estimate that the average divergence time between maize and *Tripsacum*, *Coix* and *Sorghum* is around 8.5, 12.1, and 12.4 million years ago (MYA), respectively, and that between *Coix* and *Sorghum* is 9.3 MYA. Common transposon insertion sites were identified among haplotypes belonging to different *Zea mays* subspecies, but not outside of the species. As in maize, very few solo LTRs occur in its *Andropogoneae* relatives, suggesting that genome size reduction by homologous recombination between repeats may be rare in the tribe. A comparison of the *bz* orthologous regions of *Zea*, *Sorghum*, and *Coix* with those of *Brachypodium* and *Oryza* allows us to infer how the region has evolved by the addition and progressive deletion of genes in the approximately 50 MY since these genera diverged from a common progenitor.

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P31

Evolution of the *maternally expressed gene1* (*meg1*) gene family in maize

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The *maternally expressed gene1* (*Meg1*) gene encodes a small cysteine-rich peptide with a cysteine motif CX{6}CX{4}CYCCX{14}CX{3}C that play important roles in the development of the basal endosperm transfer cells (BETCs) in maize. Its clustered paralogous genes, however, can complicate the investigation of the function of the gene. Here we analyzed a 690-kb region on chromosome 7 of maize, which contains all loci with high similarity to *Meg1* genes in maize. Twelve loci show the complete reading frame and one appears to be a pseudogene. Except *Meg1*, all genes are flanked by homologous sequences at both ends; 7 show the truncated flanking sequences with varying sizes at 5' ends and 4 copies have truncated flanking sequences at 3' ends. The expansion of the gene family was subject to unequal crossing over. Phylogenetic analysis suggests *Meg1* as the oldest copy of the gene family. The major duplication events are dated to 7-10 million years ago based upon estimated synonymous distance (*Ks*), and took place on the homologous region of one of the two ancestral species that formed the modern maize genome by allotetetraploid.

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P32

Expression levels of several membrane transport proteins (Pip1, Pip2, and Sut1) and the photosynthetic enzyme Rubisco in the Zea Mays tie-dyed2 mutant

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As a major food crop, Zea Mays L. is of great significance to the global food supply, and an improved understanding of its metabolism could bring about increased crop yields. However, little is known about the molecular underpinning of one of the major physiological processes in Zea Mays L.: phloem loading. Phloem loading is the process whereby sugars produced by photosynthesis, such as sucrose, are moved from leaves to the plant vasculature system for distribution to other tissues in a source-to-sink fashion. Seven sucrose transporter proteins have been identified, named Sut1-7, and at least some of these are known to participate in phloem loading. Moreover, phloem loading likely requires other membrane proteins, such as those of the aquaporin subtype. Aquaporins are a class of proteins that may regulate water transport into and out of cells. This study focused on the expression levels of sucrose transporters and two major families of aquaporin proteins—pip1 and pip2—in the tdy2 maize mutant. The leaves of tdy2 mutants exhibit yellow and green leaf patterning. This patterning is due to a sugar hyper-accumulation thought to be associated with impaired sucrose transport in the yellow sectors. Because excess sugar buildup usually leads to a down-regulation of photosynthesis (Paul and Foyer, 2001), we also decided to examine the expression of the major carbon-fixing enzyme, Rubisco , in mutant and wild-type leaves to determine whether its expression was downregulated in the yellow leaf regions.

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P33

Expression profiling and evolution of pathogenesis related genes in maize and teosinte in response to Ustilago maydis.

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Ustilago maydis is the causal agent of corn smut and is responsible for significant yield losses of approximately $1.3 billion annually in the U.S. Several methods including crop rotation, fungicide application and seed treatments are currently used to control corn smut. However, host resistance is the only practical method for managing smut. Interestingly, maize and teosinte are infected by U. maydis that turns infected grains into rapidly growing shapeless galls. We inoculated maize and teosinte with a strain of U. maydis and identified two teosinte lines (Zea diploperennis and Zea luxurians) with a high level of resistance and a phenotypic response similar to maize. To identify the genes underlying resistance, microarrays were used to profile gene expression changes that occur in response to U. maydis infection. A total of 5,639 genes were differentially expressed in resistant and susceptible maize plants inoculated with U. maydis. From this data set 529 genes were up regulated (>1.5 fold change), whereas 5,110 were down regulated (< 1.5 fold change) in inoculated resistant and susceptible maize plants. Differentially expressed genes were grouped into 18 functional categories. The majority of the categories were classified as pathogenesis related (PR) genes (PR1-like, chitinases, Thaumatin like, proteinase inhibitor and Lipid transfer protein) and genes coding for proteins related to stress, defense response, heat shock proteins and peroxidases. The expression data and morphological/genetic similarity of maize and teosinte indicate that these two species may share key components in plant defenses and the activation of PR genes. Therefore, we will select PR genes from the microarray dataset and identify and characterize the genes in teosinte and teosinte x maize introgression lines (NILs) responsible for resistance to U. maydis infection. This work will provide incite as to the expression pattern, genetic diversity and evolution of PR genes in maize and teosinte.

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P34

Foundations for Comparative Genomics: Preliminary Phylogeny of the Andropogoneae based on nuclear encoded markers.

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The tribe Andropogoneae (sub-family Panicoideae, family Poaceae) is a morphologically diverse clade of grasses that contains some of our most economically important taxa (maize (*Zea*), Sorghum, and Sugarcane (*Saccharum*)). Generic relationships among the 90 genera in the tribe are currently unresolved. Previous work has suggested a rapid radiation within the tribe and many examples of hybridization are known. In order to understand these relationships and provide the foundation for future comparative genomic analyses, new phylogenetic markers are needed. The genetics community has conducted extensive studies to identify genes that influence agriculturally important traits. Many of these loci also influence plant and inflorescence architecture, the same traits used by botanists to define groups and species. Combining these resources allowed us to identify and design primers for 10 new single to low copy nuclear loci that amplify across the diversity of the tribe. These markers allow us to identify allopolyploid taxa or clades and the ability to identify their likely parental donors. A preliminary analysis using a sample of these markers and over 100 taxa within the tribe allowed us to identify possible progenitors for the maize/*Tripsacum* tetraploid event. Future taxon sampling within the subtribe Rottboelliinae is required to verify and expand these findings.

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P35

Functional analysis of RGH3/ZmURP domains suggests a splicing regulatory mechanism and uncovers multiple localization signals

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Alternative RNA splicing produces multiple mRNA species from individual genes increasing protein diversity and regulating gene expression. Genome sequencing projects have shown that about 42% of intron containing genes in plants are alternatively spliced, but little is known about how alternative splicing is controlled. The *rough endosperm3* (*rgh3*) mutant causes developmental defects that are either seed or seedling lethal. *Rgh3* encodes a U2AF35 related protein (ZmURP), which is a predicted RNA splicing factor. U2AF35 proteins identify splice acceptor sites during RNA processing and function through protein-protein interactions by creating complexes with U2AF65 and other SR proteins. Semi-quantitative RT-PCR analyses of alternatively spliced genes showed that *rgh3* affects splicing in a subset of genes supporting a role for ZmURP in alternative splicing. ZmURP is alternatively spliced, producing at least 19 different spliced variants. Interestingly, only one variant is predicted to encode a full-length URP ortholog containing an N-terminal acidic domain followed by two zinc fingers flanking a UHM domain and a C-terminal RS-like domain. Several *Rgh3* splice variants produce truncated proteins missing one to several domains. GFP fused to full-length ZmURP localized to the nucleolus and nuclear speckles. Functional analysis with the endogenous truncated protein variants and artificial domain deletions within the UHM domain fused to GFP showed that while the acidic domain contains a nuclear localization signal, and the RS-like domain is important for its localization to speckles, the UHM domain may contain a cryptic nuclear export signal. These results suggest ZmURP is regulated by splicing creating truncated variants that render the protein unstable and excluded from splicesomal speckles.

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P36

GBMAS: A Fast, Accurate and Cost-Effective Genotyping-by-Sequencing Technology using Ion Torrent

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Genotyping-by-Sequencing (GBS) technologies are revolutionizing genetics and breeding experiments. It is, however, often necessary to generate genotyping data very quickly (e.g., to make decisions about which plants to cross in a winter nursery). The two-day turn-around time of the Ion Torrent instrument makes it an attractive platform to quickly generate GBS data on moderate numbers of markers and samples. We have developed a two-step GBS protocol that allows inbreds/RILs to be cost-efficiently genotyped at up to a few hundred pre-defined SNPs. In the first step of GBMAS (Genotyping by Multiple Amplicon Sequencing) targets are amplified via multiplexed PCR. In the second step unique barcodes are added to the PCR products from each individual RIL. All PCR products are then pooled for ion library construction and sequencing. GBMAS genotyping experiments exhibit a high degree of accuracy and are simple in execution, labor efficient, and easily scalable. Further, for experiments involving only a few hundred markers per inbred/RIL the cost of genotyping per RIL is comparable to other GBS platforms, but without the need to enter long sequencing queues and without the need for imputation.

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P37

Genetic and Biochemical analysis of *Hairy Sheath Frayed* mutation

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We have initiated a genetic and biochemical analysis of the maize *Hairy Sheath Frayed mutation* (*Zea mays Histidine Kinase 1*). This gene encodes a cytokinin (CK) signaling protein that is involved in a highly conserved "two component" signal transduction pathway via downstream response regulators that control several plant developmental pathways. We have analyzed several EMS alleles of this mutation and have shown that certain amino acids in key positions in the CHASE domain are changed in the *Hsf* mutant. We will present results from RNA expression analysis of *ZmHK1* and downstream response regulators in normal and *Hsf* mutant tissue. CK binding assays on normal and mutant protein (from three different alleles of *ZmHK1*) expressed in *E. Coli* reveal that mutant proteins appear to have altered CK binding affinity. In addition, we have also begun work on isolating tissue along different growth axes in normal and *Hsf1* mutant leaf primordia. In the near future, we plan on using laser capture micro dissection to perform a transcriptome wide expression analysis. This will provide us with a global view of gene expression changes occurring in the *Hsf* mutant. Our studies are aimed at helping us understand the hormonal control mechanisms involved in maize leaf patterning and development.

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P38

Genetic basis and expression regulation underlying metabolite profiles in maize kernel

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The various compounds produced by plants play a vital role for the existence of the plant itself, which are also very important in our lives. The advances of broad-spectrum metabolite profiling technologies are enabling a more intensive investigation into the influence of quantitative genetic variation on the plant metabolome. Using a genome-wide association study performed on a collection of 500 maize inbred lines, we are trying to reveal the genetic architecture of metabolome of mature maize kernels. We identified and measured metabolite levels using non-targeted LC-MS in maize kernels harvested in different locations. Large differences were observed among metabolite profiling conducted from various environments. Genomic regions of genotype–metabolite associations were identified by testing >800 metabolites against 1 million single nucleotide polymorphisms obtained by deep RNA-sequencing of kernels 15 DAP. A large number of metabolite QTLs with major effects were identified. The results with a combination of transcriptome data will reveal the genes and network responsible for the regulation of metabolism in maize.

P39

Genetic dissection of the temperature sensitivity of Rp1-D21, an autoactive R gene

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Temperature is a major environmental factor that constrains plant growth, as well as their ability to effectively respond to stresses, including those imposed by phytopathogens. Many R genes are known whose ability to mount an effective immune response is inhibited at higher temperatures; the underlying mechanisms however remain unknown. We are seeking to address this question using an autoactive R gene, Rp1-D21, which triggers a bona fide hypersensitive response (HR) in the absence of pathogens. This autoimmune phenotype of Rp1-D21 is temperature dependent, as it is completely suppressed at temperatures 30°C and above. However, the heat-suppressed Rp1-D21 plants do manifest HR lesions after return to normal temperature, although the speed with which it happens is genotype dependent. To explore the detailed kinetics of Rp1-D21 lesion development following transfer to permissive temperatures, we planted F1 progenies of Rp1-D21 with A632, B73, Mo17 and Tx303 at 30°C for 8 days before lowering the temperature to 26°C. Rp1-D21 lesions started forming on hybrids with Tx303 on day 2 after the temperature drop, on day 3 on hybrids with Mo17, on day 7 on hybrids with B73, and not even after 9-days on hybrids with A632. These results clearly demonstrate that the temperature-dependent phenotype of Rp1-D21 can be converted to an easy-to-measure quantitative trait by simply controlling the growth conditions. Given that Rp1-D21 lesion development is delayed by as many as four days on mutants having a background of B73 versus Mo17, an experimental set-up like this has important implications for the discovery of QTL capable of impacting the temperature-sensitivity of Rp1-D21 using testcrosses of Rp1-D21 with various RIL populations that exist in maize, including the IBM RILs.

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P40

Genome-wide Association Study of Resistance to Head Smut in Maize

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Head smut, caused by the fungus Sphacelotheca reiliana (Kühn) Clint, is a devastating global disease in maize, leading to severe quality and yield loss each year. Here, we performed the first genome-wide association study of resistance to head smut using the Illumina MaizeSNP50 array. Our result showed that out of 45,868 SNPS in a panel of 144 inbred lines, 18 novel candidate genes were found to be associated with resistance to head smut in maize, which were classified into three groups including R genes, disease response genes and other genes seem to function in plant disease resistance.
A gene encoding a leucine rich repeat (LRR)-containing protein, which may function in known plant disease-resistance pathways in responses to a variety of external stimuli from pathogens. A tubby-like gene was located in 4.08 bin; tubby-like proteins have been identified to be associated with the plant defense response. A gene encoding a protein containing a nucleotide binding site (NBS) was identified on chr.8.

P41

Identification and characterization of candy leaf1, a high glucan maize mutant

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Recently, plant cell walls and their constituent polymers have received increased attention as a potential highly abundant renewable resource for biofuel production. In particular, multiple members of the order Poales have been proposed as potential bioenergy feedstocks as they combine multiple desirable traits: C4 photosynthesis, large biomass yield and fast growth. Examples include switchgrass, Miscanthus and sugar cane. Also, crop residues like corn stover or wheat straw could be utilized.
Using a forward genetic approach, an EMS population in the A619 genetic background was performed. Two hundred families were screened for lignocellulosic wall sugar composition to identify mutants with alterations in their cell wall monosaccharide composition. Multiple lines with altered monosaccharide composition were identified. In particular, one mutant termed candy-leaf 1 (cal1), was found to have a 246% increase in hemicellulosic glucan content in its leaves. Standard saccharification assays on leaf and stalk material demonstrated an increase in glucose yield of 34%. We used positional cloning to identify a candidate gene. Insertions in this gene obtained from the TUSC program revealed a few individuals with similar increases in cell wall glucose, suggesting we have identified the correct gene. Complementation crosses are in progress as is ongoing screening to find additional mutants.

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P42

Identification and Characterization of Gene Products Involved C-Glycosyl Flavones Formation in Maize

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C-glycosyl flavones are specialized compounds that belong to the flavonoid family of specialized compounds and they have been shown to confer natural resistance against corn earworm (*Helicoverpa zea*), a devastating pest of maize. They accumulate in silks where they exert their biocidal action when ingested by the worm, and to a lesser extent in pericarps, the outermost layer of the maize kernel.

This research focuses on the biosynthesis of the *C*-glycosyl flavone (CGF) maysin, in which the R2R3-MYB transcription factor P1 (or its paralog P2) and the loci *salmon silks 1* (*SM1*), *salmon silks 2* (*SM2*) and *recessive enhancer of maysin 1* (*REM1*) have been proposed to be responsible for the formation and accumulation of CGFs in silks. Salmon silks mutants present a salmon silk phenotype and are positive for the silk browning reaction, which is evident only in the presence of P1 or P2, supporting their involvement in the control of maysin biosynthesis. Even though mutant analyses suggested *SM2* has rhamnosyl transferase activity, and that *SM1* is involved in the last dehydration step, the identification of the genes responsible for these reactions has yet to be determined. In addition, there has been no activity proposed for *REM1* and where it could be placed in the pathway.

Starting-off with the hypothesis that P1/P2 regulates these loci, we are searching for candidate genes by analyzing RNA-Seq derived from *P1-rr* and *P1-ww* pericarps and silks focusing on genes that are highly expressed in *P1-rr* tissues in the mapping intervals for each of these loci. Candidate gene analysis and biochemical characterization will help us elucidate the products of the *SM1*, *SM2* and *REM1* loci, and expand our knowledge of the branch of the flavonoid pathway that leads to C-glycosyl flavones formation. This Project is funded by NSF DBI-0701405 and IOS-1125620.

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P43

Identification and prediction of miRNAs from maize seedling roots in response to lead (Pb) stress using deep sequencing

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MicroRNAs (miRNAs) are a class of endogenous non-protein coding small RNAs that negatively regulate target mRNAs by post-transcriptional cleavage in plant developmental processes, cell proliferation and stress responses. Among heavy metals, lead (Pb) is a non-essential element that gets easily absorbed and accumulated in different plant parts, and is highly toxic to plants. To investigate the responsive functions of miRNAs under Pb stress, a deep sequencing approach was used for genome-wide prediction of conserved and novel miRNAs in Pb stressed maize seedling roots. A total of 243 miRNA and miRNA* belonging to 25 conserved and 43 novel, non-conserved maize miRNA families were discovered. On the basis of sequence complementarity, 516 potential target genes were predicted, which encoded transcription factors, metal transporters and proteins associated with metabolic processes or stress responses. QRT-PCR analysis of mature miRNA and predicted target mRNAs were confirmed in maize roots in response to Pb stress. The mRNA levels of several targets were negatively correlated with the corresponding miRNAs under Pb stress. In addition, a new stress-induced miRNA family, which target genes for metal transport, was predicted and confirmed. These results suggested that miRNAs and miRNA* play an important role in Pb tolerance in maize roots, and highlighted a novel molecular mechanism of heavy metal tolerance in maize.

P44

Identification of herbivore-regulated plant defense pathways by Nested Association Mapping (NAM) and Genome Wide Association Study (GWAS)

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Plant secondary metabolites can serve as plant defensive compounds or mediators of chemical communication, e.g. as attractants for natural enemies of herbivores. Maize plants attacked by caterpillars release a mixture of mono- and sesquiterpenes that attracts parasitic wasps, which are specific enemies of the herbivores. In our effort to study the molecular base of these indirect defense mechanisms, we want to identify the genes responsible for volatile terpene biosynthesis as well as their regulatory elements.

About 5000 recombinant inbred lines of a Nested Association Mapping (NAM) population derived from 26 inbred lines were screened for herbivore-induced volatile production. The variation of volatile emission within the NAM population enabled us to identify a set of important quantitative trait loci for volatile terpene production by nested association mapping (NAM). Genome wide association study (GWAS) utilizing a large SNP population resulted in close mapping of several QTLs. We identified a QTL for the trait '*(E)*-nerolidol emission' which is close to two putative terpene synthases, *tps2* and *tps3*. After fine mapping, we localized a SNP marker directly in the promoter of *tps2*. Biochemical characterization of TPS2 verified that this enzyme is a *(E)*-nerolidol synthase. The next step of the pathway, the conversion of *(E)*-nerolidol into the homoterpene 3,8-dimethyl-1,4,7-nonatriene (DMNT), maps to a P450 enzyme with similarity to the CYP92 group. Heterologous expression and characterization demonstrated that this P450 enzyme is indeed capable of converting nerolidol into DMNT by oxidative degradation. Thus, mapping of terpene metabolites by NAM enables us to characterize the pathways and their regulatory mechanisms.

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P45

Identification of promoter regulatory sequences in the terpene synthases *tps10* and *tps23* in maize

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The production of volatiles, especially terpenes, is essential for the indirect defense of plants. Maize plants emit terpene volatile blends when attacked aboveground by the lepidopteran larvae *Spodoptera littoralis* or belowground by the larvae of the coleopteran *Diabrotica virgifera virgifera*. These blends consist mostly of mono- and sesquiterpenes and can attract natural enemies of the herbivores which are parasitic wasps aboveground and entomophatogenic nematodes belowground, respectively. The enzymes responsible for the production of these volatile signals are the terpene synthases TPS10 and TPS23 [1, 2]. Both terpenes synthases are expressed after herbivory, but in response to a different set of cues and in different herbivore-infested organs.

To identify regulatory elements in the promoters of maize *tps10* and *tps23*, fragments of these promoters were fused to the beta-glucuronidase (GUS) reporter gene and transformed into *Arabidopsis thaliana*. Interestingly, a 1.5 kb fragment of both maize promoters conveyed herbivore-induced expression in Arabidopsis, indicating that the signal transduction pathways are highly conserved among plants. Promoter function was dissected by a deletion analysis and differential expression in response to mechanical damage, herbivore regurgitate and plant hormone treatment. Although an area with a WUN-motif is crucial for the regulation of both promoters, they respond to different herbivore cues. This analysis indicates parallel signal transduction pathways, of which some are dependent on a jasmonic acid intermediate.

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[2] Schnee C.; et al, PNAS, 106; (2003), 1129-1134

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P46

Identifying Candidates for the Restorer-of-Fertility Gene *Rf3* in Maize CMS-S

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Cytoplasmic male sterility (CMS) is a maternally inherited trait that prevents normal pollen development. In maize CMS-S, the pollen grains develop normally until the starch-filling stage, when they rapidly disintegrate and collapse. Cleavage of the sterility-associated mitochondrial transcript, *orf355-orf77*, mediated by the nuclear restorer *Rf3*, reverses male sterility in CMS-S. *Rf3* was previously mapped between *white pollen1* (*whp1*) and *BNL17.14* on the long arm of chromosome 2. The goal of this project is to fine-map the locus and to identify *Rf3* using a candidate gene approach. SNP-genotyping of near-isogenic lines (NILs) mapped *Rf3* to a 3 Mb region of 2L. Organelle targeting programs were automated to search for genes in this region that could encode mitochondrial proteins containing pentatricopeptide repeats (PPR). These criteria were chosen because nearly all known CMS restorer genes encode mitochondrially targeted PPR proteins. Six candidate genes were chosen to be PCR-amplified, sequenced, and compared from multiple *Rf3*-containing and non-restoring *rf3* inbreds, as well as from near-isogenic *Rf3* and *rf3* plants. RNA-Seq of pre-emergent tassels is also underway to compare gene expression in CMS-S sterile and restored plants which help identify coding regions that could function as *Rf3* alleles during pollen development.

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P47

Identifying Insertions in Phloem-Related Genes using UniformMu Maize

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In plants, phloem is the tissue responsible for transporting photosynthate from source to sink tissues, as well as reassimilated nitrogen from senescing leaves. We hypothesize that knocking out function of genes potentially involved in phloem transport will lead to phenotypes indicative of their physiological roles. Genes tested here were selected based on their potential to alter C-partitioning through effects on long-distance transport. The transposon-mutagenic UniformMu population provides a powerful resource for conducting research using both forward and reverse genetics. The advantages of using this population include uniform plants that assist in identifying new phenotypes and the ability to map the genetic location of Mu insertions. Furthermore, Mu elements produce new mutations that can often be associated with a phenotype when inserted into individual genes. Reverse genetics was used to analyze seven genes possibly involved in transporting sugars and nutrients through the phloem. These include: Phloem protein 2 A13 (PP2 A13), Glutamate dehydrogenase 2 (GDH2), Plant neutral invertase, P-protein (GRMZM2G141273, Solute carrier family 23, Sucrose Non-Fermenting 1 (SNF1), and Glucose-6-phosphate isomerase (G6PI). Families segregating for phenotypes possibly associated with deficient phloem transport were analyzed using a forward genetics approach. These included: Adult-stage wilty (co-segregation analyses done with 10 genes and so far, none cosegregate) and Dwarf mutant (and empty pericarp [ep]) co-seg with solute carrier family 2 appears negative. Thus far, we have negated our hypothesis for each of the lines selected however; this material may be valuable to other researchers since the presence of Mu insertions in these families has been confirmed.

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P48

Laser-capture microdissection for the identification of genes involved in phloem function

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The loading of photosynthetically derived sucrose into the phloem and its subsequent translocation to sink tissues is imperative for plant growth and development. However, the genetic regulation of sucrose movement in plants remains poorly characterized. One approach to identify genes involved in the loading, translocation, and unloading of sucrose from the phloem tissues is to isolate and characterize the phloem transcriptome. Laser-capture microdissection (LCM) is a method that permits the isolation of a specific population of cells from a sample. The RNA can be subsequently isolated from the harvested tissue, and NextGen sequencing used to determine which genes are expressed. We are currently in the process of using LCM to isolate phloem-specific cells from the lateral veins of mature source leaves to identify genes that are expressed in this tissue and that are central to sucrose flux in the maize plant. Progress toward our goal will be presented.

Funding acknowledgement: National Science Foundation (NSF)

P49

Maize eukaryotic translation initiation Factor 5A is highly expressed in maize endosperm and is associated with an actin-rich cytoskeletal fraction

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The eukaryotic translation initiation factor 5A (eIF5A) is a highly conserved protein among eukaryotes. Its functions in translation and cellular proliferation are poorly understood. Interestingly, this protein is the only protein known to contain the unusual amino acid hypusine, which is synthesized as a post-translational modification of a conserved lysine residue and is necessary for the activation of eIF5A. Maize eIF5A was observed to be one of several proteins cause a mobility shift of labeled RNA derived from the 3' UTR of the maize 27 kDa gamma-zein gene. There are 3 genes coding for eIF5A in the maize genome and eIF5A-1 is highly expressed in developing endosperm and expressed at lower levels in roots and shoots of seedlings. The expression of the other two genes was very low in in all tissues tested. Western blot analysis showed increased protein accumulation in later stages of endosperm development despite a reduction of mRNA expression. During these later stages cells begin to undergo programmed cell death in the central endosperm. Prior experiments in tomato fruit suggest eIF5A expression is correlated with programmed cell death in plants. Pulldown and immunoprecipitation analysis shows that eIF5A is associated with an actin and eEF1A rich protein fraction in maize endosperm. The association of eIF5A with this actin-rich fraction is hypusine independent, which is one of the first activities of eIF5A that has been demonstrated to not require hypusine. In contrast, the hypusine modification is required for association with ribosomes, consistent with prior experiments in yeast. Ongoing experiments are investigating the direct interaction of eIF5A with either actin or eEF1A.

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P50

Maize Sucrose Transporter Genes Display Distinct Circadian Expression Patterns

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Sugars are the key metabolites of photosynthesis and crucial to plant survival. For most plant species, sucrose is the primary sugar transported through the phloem, yet little is known about the genes responsible for the source-to-sink movement in the plant's vasculature system, a movement that is key to plant growth and crop yield. Several Sucrose transporters (Suts) have been characterized in dicots, but less is known about their function in monocots. Seven Sut genes, Sut1-Sut7, have been identified in maize (Zea mays L.). Past studies have shown diurnal changes in maize leaf sucrose levels, suggesting fluctuating transporter activity. In this study, we used qPCR to examine the diurnal expression of five maize Suts across five different tissue types representing source and sink tissues in the maize inbred B73 to gain a better understanding of the role these genes play within the plant.

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P51

Maize ZmTCX8.1 gene contributes to abscisic acid signal transduction in transgenic Arabidopsis

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The phytohormone abscisic acid (ABA) mediates many aspects of plant growth and development as well as tolerance to abiotic stresses. Based on the microarray expression analysis under drought stress, we have isolated and cloned a TSO1-like gene from maize. It is speculated to encode a transcription factor, and the deduced amino acid sequence showed that it contains two conserved CXC domains, and then we named it ZmTCX8.1. Quantitative Reverse transcription PCR revealed that the transcripts of ZmTCX8.1 were obviously induced by dehydration and ABA. Subcellular localization of the ZmTCX8.1-GFP confusion construct demonstrated that ZmTCX8.1 is distributed in the nucleus. The transgenic experiment showed that overexpression of ZmTCX8.1 in Arabidopsis could dramatically enhance the sensitivity to ABA at the germination and early seedling growth stages. We then detected the expression of ABA signaling pathway genes such as ABI1, ABI3, ABI4 and ABI5, and found that the expression levels of these genes were all significantly improved in transgenic lines compared to wild-type plants. Further examination showed that the endogenous ABA levels in the ZmTCX8.1 transgenic lines were significantly lower than that of wild-type. Subsequently we detected the expression of ABA biosynthesis genes such as ABA1, ABA3, NCED3 and AAO3. In agreement with the results of endogenous ABA level, the expression levels of these genes were significantly reduced in transgenic Arabidopsis lines compared to wild-type. Based on the results above, we proposed that over-expression of ZmTCX8.1 positively regulate the transcription of the genes in ABA signaling pathway, and then the enhanced ABA signaling may feed back to regulate the biosynthesis of ABA, which then lead to the decrease of ABA contains.

P52

Metabolic profiling of maize using DIESI-MS.

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Metabolomics is defined as the characterization, identification, and quantification of metabolites resulting from a wide range of biochemical processes in living systems. Sucrose and starch are the main components resulting from the dinamic photosynthetic process in corn plants. Compared to the exponential progress of DNA sequencing methods, the technical advances in metabolic profiling strategies have lagged behind, thus increasing the gap of knowledge between genes and phenotypes. Genetic improvement and selection in plant breeding depends on the abundante and reliability of quantitative phenotypic data. Due the shortage of water in the natural renewable cycle and the soil nitrogen loss, CIMMYT and CINVESTAV scientific efforts are focusing in finding new adaptation mecanisms in plants that allow them to select genotypes that yield more under stress. Later on, the designed breeding programs will include the results of phenotyping experimental work. Actually, mass spectrometry analysis has become a every day tool for identifying metabolites such as sugars, amino acids, nucleotides and lipids. We have therefore developed a biochemical profiling strategy particularly adapted to increase sample throughput, save reagents, reduce cost and simplify data análisis. Stem juice of fourteen drought tolerant genotypes (Water stress E's series), with its respective controls, were collected at Tlaltizapan experimental field, selecting the E-132 hybrid to be tested for metabolites identification, using the water DIESI-MS (direct infusión electrospray ionization) mass spectrometry method, and controlled by Mass Lynx V4.0 software program. The resulting files were saved as mzXML format using TOPPAS VIEW software to analyze and visualize the chromatograms. KEGG-compound metabolites data base was used for peaks identifying. Chromatograms showed an average of 324 different peaks registered from 20 to 200 (m/z) values. The following amino acids were identified: Arginine, 175.01; Asparagine, 133.0; Glutamine, 147.06; Proline, 116.06; Serine, 106.0; Tryptophan, 205.08; Threonine, 120.05; Tyrosine, 182.07; Leucine, 132.1. The Project is directed to reduce the breeding programs time through phenotyping, improving yield and maize crop quality.

Funding acknowledgement: Consejo Nacional de Ciencia y Tecnología (CONACYT). Centro de investigación y estudios de posgrado (CINVESTAV).

P53

Metabolomic characterization of iron biofortified maize grain

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Mineral nutrient deficiencies are a worldwide problem that is directly correlated with poverty and food insecurity. The most common of these is iron deficiency; more than one-third of the world's population suffers from iron deficiency-induced anemia, 80% of which are in developing countries. The consequences of iron deficiency include increased mortality and morbidity rates, diminished cognitive abilities in children, and reduced labor productivity, which in turn stagnates national development. The developed world has made tremendous success in alleviating nutrient deficiencies through dietary diversification, food product fortification, improved public health care, and supplementation. In developing countries, these strategies are often expensive and difficult to sustain. Poverty is the most common cause for dietary deficiency in developing countries, as consumers' dietary choices are limited as regards the quality, quantity, and diversity of foods consumed. The resource-poor typically consume what they grow and are dependent upon a small number of staple crops for the vast majority of their nutrition. Therefore, genetic improvement of staple crops (biofortification) is the most cost effective and sustainable solution to this global health problem. We have developed nearly isogenic line (NIL) derivatives of B73 and Mo17 with altered iron nutritional quality using a human cell culture bioassay as a phenotyping tool. Poultry feeding studies conducted over two years using field-grown maize clearly demonstrate the efficacy of our approach. Here we describe the characterization of lines divergently selected for iron nutritional quality using a non-targeted mass spectrometry based approach. We are also evaluating the Buckler Diversity Panel of inbred lines using the same separation/analysis protocols to estimate variation in compounds of particular interest from our B73 and Mo17 NILs and the boundaries of stakeholder acceptable chemical variation in maize grain.

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P54

MODULATING LIPID-DERIVED SIGNALING TO IMPROVE CORN TRAITS

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Integrated pest management is a valuable tool in breeding of crop plants for resistance/tolerance to abiotic and biotic stresses. The goal is to find diverse, novel, and natural alleles at loci associated with resistance/tolerance to stress. In plants, fatty acids are often oxidized to produce an estimated 400 distinct biologically active molecules known as oxylipins. Although the function of the majority of these metabolites is still poorly understood, some better studied oxylipins, such as jasmonic acid and green leafy volatiles, possess potent signaling activities that regulate plant development and response to biotic and abiotic stress. Here, we present an overview of our research program aimed at understanding the biological functions of oxylipins in diverse physiological processes in corn. The corn traits we study include resistance to mycotoxin contamination, resistance to pathogens and insects, as well as drought tolerance. For this purpose, we have generated near-isogenic knock-out mutants for the majority of the lipoxygenase (LOX) and oxo-phytodienoate (OPR) genes in corn genome. Functional studies of these mutants showed that shutting down specific LOX and OPR genes result in alteration of ear and tassel development, growth, reduction of aflatoxin and fumonisin contamination. Depending on the specific genes, we also show their involvement in defense against leaf, stalk and root-rot pathogens and insect herbivores. Specific examples of the studies that provide strong genetic evidence of the importance of lipid derivatives in the regulation of diverse processes are presented. Our research data, as well as the mutants generated, provide excellent resources to further our understanding of genes and metabolites that constitute the signaling networks in corn. Furthermore, we aim target these networks with associative genetics and marker-assisted selection approaches to improve important valuable agronomic traits.

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P55

Molecular genetic dissection of C4 malate transporter genes in maize

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C4 photosynthesis is a complex metabolic pathway responsible for carbon fixation in major feed, food and bioenergy crops. A key element of C4 photosynthesis is metabolic exchange between two distinct, specialized leaf cell types, mesophyll (ME) and bundle sheath (BS). These two cell types function to shuttle CO2 into the BS, effectively eliminating photorespiratory losses. Chloroplast membranal transporters play a key role in mediating high rates of metabolite transport. OMT1 and DCT2 are two maize dicarboxylate transporters that likely play critical roles in the movement of malate between BS and M cells in C4 plants. OMT1 is highly enriched in the mesophyll and DCT2 is highly enriched in the bundle sheath. The mechanisms that control their cell-specific expression in maize, however, are unknown.
We are utilizing bioinformatic and molecular methods to identify the mechanisms that control the cell-type specific expression of OMT1 and DCT2. To examine the function of DCT2, we performed Ac insertional mutagenesis to create mutant alleles and are characterizing the phenotypes of the mutant plants. We are using various experimental methods to identify putative cis-regulatory elements. We intend to characterize the network of trans-factors that may regulate the expression of OMT1 and DCT2, using cis-element::GUS transgenic lines of Setaria viridis, yeast one- hybrid assays and ChIP-seq. The precise dissection of the mechanisms that control the expression of transporter genes in C4 crops will aid in transferring these mechanisms into C3 crops to increase yield and feed a rapidly growing global population.

Funding acknowledgement: National Science Foundation (NSF)

P56

Molecular Mapping of a Carbon Partitioning Defective Mutant

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Through photosynthesis, plants assimilate carbon dioxide into sucrose, which is subsequently transported, via the phloem, from the leaves of the plant to non-photosynthetic sink tissues for utilization in growth and development. When the plant is unable to transport sugars out of the leaf, starch accumulation occurs within the chloroplasts. Phenotypically, this excess starch accumulation can lead to leaf chlorosis, anthocyanin accumulation, and/or necrosis. We identified several mutants manifesting these and other leaf phenotypes and have verified the occurrence of excess starch accumulation through potassium iodine staining. These mutants are collectively known as the *carbon partitioning defective* (*cpd*) mutants. One such mutant displaying significant anthocyanin and starch accumulation in the leaf is *cpd6*. Bulk segregate analysis has placed the gene near the centromeric region of chromosome five. To identify and clone the gene responsible for these phenotypes, we are using molecular mapping methods to fine map its genomic location. Identification of this gene should help elucidate the genetic pathways controlling sucrose flux throughout the plant.

Funding acknowledgement: National Science Foundation (NSF)

P57

Monsanto Biotech Efforts Toward Doubling Yield of Corn in the US by 2030

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The demands on world agriculture are greater than ever and continue to grow. At Monsanto, we are working to meet the world's growing food needs while protecting the environment and conserving natural resources. In June 2008, we issued this three-fold commitment that we call our Commitment to Sustainable Yield. We use breeding, biotechnology, and improved agronomic practices to work toward our pledge of doubling yield, reducing inputs, and improving farmer's lives. Specifically, we will help farmers with improved seeds and agronomics to double the yields of corn, soy, cotton, and canola crops while reducing the aggregate use of key resources (land, irrigation water, and energy) by 1/3 per unit of output over the 30 year interval from 2000-2030.

Achieving all three of these commitments is challenging. Monsanto's Research and Development (R&D) has developed a very strong biotechnology pipeline focusing on increasing crop stress tolerance and yield potential. For example, our Yield and Stress efforts on doubling yield of corn focus on improving nitrogen use efficiency, water use efficiency, light use efficiency, and general stress tolerance of corn through modification of gene expression and activity. Our goal is for our transgenic corn products to give high yield with less irrigation, less nitrogen application, and under high density planting conditions. Monsanto has implemented a high-throughput transgenic field screening program to evaluate Yield and Stress traits under broad acre conditions to measure yield. The quantitative nature of yield traits is highly complex; our field screening of transgenes will enable discovery of novel traits that bring value to our customers that can be observed in farmer's fields. Our gene discovery program combines collaborative efforts from Monsanto scientists, academic and industry collaborator innovations to ensure delivery of new products.

P58

Natural Diversity and Genetic Modifiers of Expression Pattern in Leaf Mutants

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Enhancer/suppressor screens have been a source of genetic modifiers for decades and genetic background effects on mutant phenotypes have been reported for even longer. We have taken advantage of the naturally occurring genetic diversity among maize inbreds to explore genetic modifiers of two leaf mutants of maize, *Sucrose transporter 1 (Sut1)*, which controls loading of photosynthate into the phloem, and *Ragged 1 (Rg1)*, which produces patterned formation of premature necrosis in the leaf blade. Variation in the extent, pattern and severity of these two mutant phenotypes is presented for crosses of these two mutants to the founders of the Nested Association Mapping (NAM) population. These crosses and the extensive genotyping of the NAM population and its founders now allow us to map and identify these genetic modifiers to identify genes that interact with and impact the expression patterns of *Sut1* and *Rg1*.

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P59

New releases from UniformMu in 2012: Seeds and sequences

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The latest UniformMu release includes approximately 9,000 new maize mutants providing insertions in a total of 14,156 genes. For investigators seeking mutations in genes of interest, there is now about a 35% chance that the UniformMu resource can provide at least one allele carrying a Mu insertion within or near the gene, and in over a third of these instances, two different alleles will be available. Of these insertions, over 80% are within the upper 1/3 of the coding sequence. Each of these mutations is available in one of 8,256 sustainable, F3 seed stocks distributed to the public without charge by the Maize Genetics Cooperation Stock Center. A searchable database of UniformMu insertions and mapping data can be accessed at MaizeGDB and via POPcorn, as well as downloadable forms of these data. The current resource contains over 41,000 independent germinal Mu insertions mapped precisely in the maize genome by NexGen sequencing. The resource is growing steadily. Tools for accessing mutants and tips for analyzing insertions in genes of interest will be presented.

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P60

Opaque7 Encodes an Acyl Activating Enzyme-like Protein that Affects Storage Protein Synthesis in Maize Endosperm

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In maize, a series of seed mutants with starchy endosperm could increase the lysine content by decreased amount of zeins, the main storage proteins in endosperm. Cloning and characterization of these mutants could reveal regulatory mechanisms for zeins accumulation in maize endosperm. Opaque7 (o7) is a classic maize starchy endosperm mutant with large effects on zeins accumulation and high lysine content. In this study, the O7 gene was cloned by map-based cloning and confirmed by transgenic functional complementation and RNAi. The o7-ref allele has a 12 bp in-frame deletion. The 4-amino-acid deletion caused low accumulation of o7 protein in vivo. O7 gene encodes an acyl activating enzyme with high similarity to AAE3. The opaque phenotype of o7 mutant was produced by the reduction of protein body size and number caused by a decrease in the α-zeins concentrations. Analysis of amino acids and metabolites suggested that O7 gene might affect amino acid biosynthesis by affecting α-ketoglutaric acid and oxaloacetic acid. Transgenic rice seeds containing RNAi constructs targeting the rice ortholog of maize O7 also produced lower amounts of seed proteins and displayed an opaque endosperm phenotype, indicating a conserved biological function of O7 in cereal crops. The cloning of O7 revealed a novel regulatory mechanism for storage protein synthesis and highlighted an effective target for the genetic manipulation of storage protein contents in cereal seeds.

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P61

Phylogenomic analysis of the Trihelix transcription factor family in grasses.

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The Trihelix (THX) family of transcription factors (TFs) has been described only in land plants, and may therefore be involved in plant-specific processes. There is experimental evidence from *Arabidopsis* and rice that these roles are mainly in flower, fruit, and seed development. This family has not been well investigated and most THX proteins are of unknown regulatory function. THX TFs exhibit one or two trihelix DNA-binding motifs that bind to GT *cis* elements to regulate transcription. We have taken advantage of the near complete maize genome to identify at least 27 trihelix family members in corn and have performed a phylogenomic comparison to those in rice, sorghum, and *Brachypodium*. We used the sequence of full length cDNAs and the maize genome to confirm gene models for these THXs. We report on the conservation of this family across multiple monocot and dicot species. We also find that the THX motif is present in lower land plants such as *Physcomitrella* but not in any algal species suggesting that family arose to regulate land plant specific processes. This project is part of the GRASS ORFeome project which aims to establish a collection of TF ORFs (www.grassius.org). These proteins will be used to raise antiserum to be employed in developing chromatin-immunoprecipiation (ChIP) techniques aimed at TF target genes in the maize genome. Thus far the DNA binding domain of one of these (*Zm*THX1) was cloned as a His-tag fusion protein in pDEST17 for study of it's preferred binding specificity. This project is funded in part by NSF grants DBI-0701405 and IOS-1125620.

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P62

Propiconazole is a specific and accessible brassinosteroid biosynthesis inhibitor for *Arabidopsis* and maize

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Brassinosteroids (BRs) are hormones that play pivotal roles during plant development. In addition to the characterization of BR deficient mutants, specific BR biosynthesis inhibitors played an essential role in the elucidation of BR function. However, high costs and limited availability of common BR inhibitors, such as brassinazole (BRZ), constrains their key advantage as a species-independent tool to study BR function (*1*).

We present our evaluation of the triazole fungicide, propiconazole (PCZ) (*2*), as an alternative BR inhibitor. *Arabidopsis* seedlings treated with PCZ phenocopied BR biosynthesis mutants. The steady state mRNA levels of BR-, but not GA-, regulated genes increased proportional to the concentrations of PCZ. Root inhibition and PCZ induction of BR biosynthesis genes was rescued by treatments with 24epi-BL, but not by GA co-applications. Maize seedlings treated with PCZ showed impaired elongation of mesocotyls, coleoptiles, and true leaves. The genetic background dramatically impacted the tissue-specific sensitivity towards PCZ. Although these varying responses may be due to differences in PCZ uptake, the evaluated inbred lines showed similar sensitivities to PCZ and BL, respectively. We also established an application method given PCZ's varied efficacy among media substrates. Based on these findings, PCZ is a potent and specific alternative to BRZ. The greatly reduced cost and increased availability of PCZ opens new possibilities to study BR function in crop species at a larger scale.

(1) Asami et al. (2000) *Plant Physiol.* 123, 93-99; (2) Asami et al. (2002) *J. Agric. Food Chem.* 50, 3486-3490

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P63

Quantitative proteomics of total and mitochondrial proteins reveal specific changes that are associated with higher level of heterosis in maize hybrids

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Maize hybrids usually show significant increases in yield and biomass; however the extent of vigor varies depending upon the genetic distance between the inbred parents used for crossing. We are testing the hypothesis that alterations in mitochondrial function partially account for the fitness increases in hybrids showing greater heterosis. Crossing B73 by three pollen parents selected from the NAM population gave hybrids with low, medium and high levels of heterosis, consistent across a variety of environments. To examine which changes are correlated with heterosis, the proteomes of the field-grown F1 hybrids were examined. Both total and mitochondria-enriched proteins extracted from ear shoots of medium and high heterosis hybrids were compared to those of the low heterosis hybrid. Proteins were resolved by high-resolution, large-format, two-dimensional difference gel electrophoresis (2-D DIGE), and spot abundances were quantified using DeCyder software. Protein spots of interest were identified using MALDI-TOF-TOF and QTOF LC-MS/MS. 2D DIGE revealed 19 differentially accumulated spots in the total-protein extracts and 27 spots in mitochondria-enriched fractions (above 1.5-fold, t-test p=0.05). Some components of glycolysis, the TCA cycle, the ETC, and several stress-responsive proteins were altered in the higher heterosis plants. Several differentially expressed proteins appeared as multiple spots suggesting either allelic variation or post-translational modification. Moreover, the combination of 1D SDS PAGE and LTQ Orbitrap LC-MSMS (geLC MS) was used to measure the overall changes in the amount of proteins that were differentially expressed in 2D DIGE analysis. Most of the proteins identified in multiple spots were not changed in their gross amount. Thus, our proteomics studies suggest that expression of specific alleles and/or post-translational modification of specific proteins correlate with higher levels of heterosis.

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P64

Regulation of the Nitrogen Sink in Maize Seed

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Healthy nutrition requires sufficient protein with balanced amino acid composition. Typical yellow dent maize contains around 10% protein, of which the essential amino acid lysine is only around 2% as opposed to 4%, whereas soybean has 35% protein with sufficient levels of lysine. To improve protein concentration in maize, a well-known long-term selection-experiment was initiated in 1896 by C. G. Hopkins at the University of Illinois and has lasted for more than a century, yielding four strains, i. e., Illinois High Protein (IHP), Illinois Low Protein (ILP), Reverse High protein (IRHP) and Reverse Low Protein (IRLP). By continuously accumulating quantitative trait loci (QTLs), IHP reached a protein level 2.5-fold higher than normal maize, with the most increased fraction being zein proteins. However, soon after the initiation of the Illinois long-term selection experiment, zeins were shown to contain no lysine by Osborne in 1914. Therefore, IHP is of little value for the nutrition of humans and monogastric animals. Although high-lysine lines of non-vitreous mutants were based on reduced zeins, the kernel soft texture precluded their practical use. Kernel hardness in *opaque 2* (*o2*) could be restored in quality protein maize (QPM) with QTLs called *o2* modifiers (*Mo2*s), but those did not increase total protein levels. It seems that the three critical traits of valuable maize germplasm, high protein, high lysine, and hard endosperm remained an ever-lasting breeding challenge that could not be overcome all at once. To achieve a combination of desired traits, we used RNA interference (RNAi) against α-zeins in IHP. The α-zeins were dramatically reduced, but the high total seed protein level remained unchanged by compensatory increase of non-zein proteins. Moreover, the residual zein levels still allowed for a vitreous hard seed. Such dramatic rebalancing of the nitrogen sink could have a major impact in world food supply.

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P65

Regulation of the Sulfur Sink in Maize Seed

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Crop seed is the major protein source for food in developing countries and feed for livestock in general. As such, a balanced composition of amino acids in seed flour is critical. However, seed proteins in cereals like maize, are deficient in lysine, tryptophan, and methionine. Whereas supplementation with legumes like soybean can compensate lysine deficiency, they are also relatively low in methionine. Therefore, studying methionine accumulation in the seed could be the basis for improved nutrition. In maize, the 22- and 19-kDa zeins are the most prominent storage proteins, nearly devoid of lysine and methionine. Although silencing synthesis of these proteins through RNA interference (RNAi) raises lysine levels in the seed, it fails to do so for methionine. Computational analysis of the B73 genome suggests that about 57% of all proteins exhibit a lysine content of >4%, whereas that number for methionine is only about 8%. On the other hand, the 15-kDa β-, 18-kDa and 10-kDa δ-zeins are three methionine-rich proteins expressed in the seed, but at variant levels. A654, an inbred with null δ-zein alleles, methionine levels are significantly lower than when the two intact δ-zein alleles are introgressed. Additional silencing of β-zein results in further reduction of methionine levels, indicating that β- and δ-zeins are the main sink for methionine in maize seed. Overexpression of the 10-kDa δ-zein can increase the methionine level, but the increased amount is shown to be at least in part at the expense of cysteines present in β- and γ-zeins. The reverse is true, when β- and γ-zein expression is silenced, then 10-kDa δ-zein accumulates to higher levels. Such rebalancing between the cysteine and methionine sinks in the seed suggests that insufficient sulfate reduction may be a critical limitation in methionine storage.

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P66

Role of plant specific N-terminal domain of maize CK2b1 subunit in CK2b functions and holoenzyme regulation.

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Plants CK2β regulatory subunits present striking features compared to their animals or yeast counterparts, notably, they present an additional specific N-terminal extension of about 90 aminoacids sharing no homology with any previously characterized functional domain. Here we show phylogenetic and functional data that highlights the importance of this domain in plants. Using maize CK2β1 and a deleted version dNCK2β1 lacking N-terminal domain as a model, we demonstrate that this plant specific N-terminal domain enhances CK2β1 stability against proteasome degradation and is involved in regulation of holoenzyme activity. By using bimolecular fluorescence complementation (BiFC) we show the in vivo localization CK2 holoenzyme in plant cells. Location of holoenzyme is different from those of the independent CK2αβ subunits. Even though the N-terminal domain of CK2β1 is not essential for export of CK2α subunits from nucleus/nucleolus to cytoplasm, the whole amount of data shown in this work suggest that this domain was acquired in plants for regulatory purposes.

P67

SnRK2 family in maize: regulation of a Zinc-Finger transcription factor

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Drought is an important environmental factor that limits agricultural production world wide. Maize production is very constrained by drought stress. Therefore, understanding the molecular basis of its tolerance response is necessary for the establishment of new strategies for the development of drought-resistant maize crops. Abscisid acid (ABA) is the major phytohormone that mediates drought responses in land plants. ABA triggers several signaling transduction pathways that lead to an altered gene expression with effects on stress response and acquisition of tolerance. Protein kinases and phosphatases are of dominant importance in ABA signal transduction and among these proteins, a subgroup of the SnRK2s, including OST1, has been shown to be a major hub on the regulation of this pathway. When ABA levels increase, SnRK2 becomes active affecting other proteins and cellular processes, such as the activation of ABF transcription factors by phosphorylation affecting gene expression. However, a full description of the ABA-induced transcriptional regulation may also include the identification of alternative transcriptional regulators SnRK2 dependent. We started this work cloning a maize SnRK2 gene aligned in the ABA activated group next to arabidopsis OST1 gene which is essential for the ABA dependent closure of stomata under drought stress. To identify new transcriptional targets affected by SnRK2 activity and involved in the regulation of drought inducible promoters we took a yeast two-hybrid approach using SnRK2 as bait against a library of drought stressed leaves. This technique yielded different potential targets and we started to further characterize a Zn-finger-type transcription factor with homology to an Arabidopsis protein, which is a regulator of proton responsive gene expression and ABA signaling components, such as the CIPK23 gene. We are currently characterizing the Zn-finger-type transcription factor interaction with SnRK2 and evaluating their function in ABA and in other abiotic /biotic stress responses as a potential candidate for crop yield improvement under sub-optimal environmental conditions.

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P68

Strength with Time: Biochemical and Physiological Basis for Adult Plant Resistance in the Maize-CCR1 Pathosystem

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The maize-*Cochliobolus carbonum* race 1 (CCR1) pathosystem is characterized by a lethal leaf blight disease, a key determinant of which is HC-toxin, a cyclic tetrapeptide of broad-spectrum histone deacetylase activity. To counter HC-toxin, maize has recruited the *Hm1* gene, which encodes HC-toxin reductase (HCTR), an NADPH-dependent enzyme that inactivates HC-toxin. Resistance conferred by *Hm1* is highly effective, operating in all parts of the plant at every stage of development. In contrast, resistance provided by two naturally-occurring variants of *Hm1* is developmentally regulated, becoming fully effective only at maturity. Cloning of these adult plant resistance (APR) genes has revealed that *Hm1A*, an allele of *Hm1*, encodes an HCTR with five amino acid substitutions, while *Hm2*, a homeolog of *Hm1*, encodes a truncated HCTR lacking the 52 C-terminal amino acids. Both of these changes are predicted to be mutagenic, capable of compromising enzymatic activity. Given that their transcriptional and translational levels remain unchanged during development, the APR phenotypes of *Hm1A* and *Hm2* are expected to be dictated post-translationally. Since NADPH is essential for HCTR's function, one possibility is that this cofactor underlies the APR nature of *Hm1A* and *Hm2*. In young maize plants with limited ability to produce and store photosynthates, it is likely that NADPH levels may not always stay above a threshold level needed by the mutant HCTRs to effectively degrade HC-toxin. As the plants mature, however, their increased photosynthesis may effect a rise in available NADPH, buffering any fluctuations in the NADPH pool. To validate this hypothesis, we are measuring temporal and developmental changes in NADPH levels in maize plants, as well as determining the enzyme kinetics of HM1, HM1A, and HM2 at varying levels of NADPH.

P69

Structural & Population analysis of Novel Teosinte genes

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The domestication of maize from teosinte involved a genetic bottleneck. It is widely appreciated that during such bottlenecks allelic diversity is lost. But our recent analyses have shown that in addition entire genes can be lost during domestication. Here, we report the sequencing, assembling and analysis of the transcriptomes of 11 teosinte lines. We identified hundreds of thousands of SNPs and small indels between these transcriptomes and the B73 reference genome. In addition, these analyses identified thousands of genes that are present and expressed in teosinte but absent from the B73 reference genome. To more fully characterize the structures of these novel genes we performed Exome Capture on two teosinte haplotypes. By applying array CGH & sequence-based analyses to maize and teosinte diversity panels we characterized the population genetics of these novel genes. By genetically mapped the novel genes in F1BC populations we revealed their non-random genomic distributions. The implications of these findings will be discussed.

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P70

The molecular genetic dissection of bundle sheath suberization in maize and *Setaria viridis*, model systems for C4 biology.

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C4 crops outperform C3 crops under hot, dry conditions; they have reduced photorespiration and greater water and nitrogen use efficiencies. These gains are achieved through intra- and intercellular compartmentalization of photosynthetic activities. It has been proposed that deposition of a suberin lamella in the bundle sheath (BS) cell wall reduces photorespiration by acting as a barrier to CO_2 escape and O_2 entry from surrounding mesophyll (M) cells. The biosynthesis of suberin monomers has been partially characterized in Arabidopsis roots and potato periderm. However, little is known about the biosynthesis and transcriptional regulation of BS suberization in C4 species.

We identified a subset of genes that are expressed during BS suberin deposition in maize and assembled a putative biosynthetic pathway based on functional characterizations from *A. thaliana* and *S. tuberosum*. We also identified a subset of candidate maize transcription factors (TFs) that are expressed during BS suberization based on homology to a set of Arabidopsis TFs expressed in the suberized root endodermis. Comparative analysis of transcript accumulation profiles in maize and rice leaves revealed that candidate suberin biosynthesis genes are differentially expressed, while candidate cutin biosynthesis genes are not. We are currently targeting these candidate biosynthetic genes and transcription factors in reverse genetic screens using the maize *Ac*/*Ds* transposons and through an RNAi-based approach using the new C4 model system *Setaria viridis* to elucidate the physiological function of BS suberization. The characterization of BS suberin deposition will be highly useful for the ongoing effort to develop C4 rice to meet the needs of growers in hot, arid environments.

Funding acknowledgement: National Science Foundation (NSF)

P71

The molecular mechanism of the Rp1-D21-regulated hypersensitive response in maize

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The maize Rp1-D21 gene confers a hypersensitive response (HR) in the absence of pathogen infection. Rp1-D21 came from a recombination event between Rp1-D, a NB-LRR protein, which confers rust resistance to corn, and Rp1-dp2, a homologue of Rp1-D. We have previously shown that hydrogen peroxide accumulation, the increased expression of PR1 and other defense marker genes (PRms and WIP1) were associated with Rp1-D21 expression. We also demonstrated that the Rp1-D21-associated HR phenotype is both temperature- and genetic background-dependent. Here we demonstrate a strong association between the strength of the Rp1-D21-associated HR in different genetic backgrounds and salicylic acid accumulation, defense gene expression and Rp1-D21 expression . In addition we demonstrate that the Rp1-D21-regulated HR is light-dependent. Transient expression of Rp1-D21 in N. benthamiana induced clear HR. We will report the results of experiments using this transient assay system to investigate the functional domains responsible for the HR phenotype of Rp1-D21.

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P72

The rate limiting step in the rate limiting enzyme, ADP-glucose pyrophosphorylase (AGPase).

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The allosterically-regulated enzyme, AGPase catalyzes the synthesis of the starch precursor, ADP-glucose from ATP and glucose-1-phosphate (G1P). That this enzyme plays a rate limiting role is documented by at least seven reports from five groups showing that over-expression, enhanced heat stability or altered allosteric properties increase starch synthesis in the agriculturally important crops, maize, potato, wheat and rice. In our continuing efforts to engineer superior AGPases we asked which step(s) in catalysis limits the speed or turnover number of the enzyme. Three steps in the reaction were studied: (i) substrate binding, (ii) chemical conversion of ATP and G1P to ADP-glucose and pyrophosphate (PPi) and (iii) product release.

Initial velocity studies of the maize endosperm and potato tuber enzymes in the presence of the activator, 3-phosphoglyceric acid (3PGA) revealed that ADP-glucose is competitive with respect to ATP and PPi is competitive with G1P. In all other circumstances the reactants were non-competitive. Additionally, G1P causes substrate inhibition when the ATP analog chromium ATP is included as a dead end inhibitor. These data are consistent with the following: (i) ATP is the first substrate to bind while ADP-glucose is the last product released and (ii) the enzymes exhibit a Theorell-Chance mechanism in that the intermediates [E-ATP-GIP] and [E-ADPglucose-PPi] do not accumulate. Hence, the rate limiting step in the reaction is most likely product release.

In the absence of 3PGA, the maize enzyme exhibits the same kinetic mechanism albeit the substrates do not bind at high affinity, whereas the conversion of substrates to products is now rate limiting for the potato enzyme. Further characterization showed this difference was caused by the fact that G1P and ADP-glucose function not only as substrates but also as activators for the maize endosperm. Activation occurs in both the synthesis as well as the degradation of ADP-glucose. Mapping of mosaic AGPases has identified regions responsible for this difference.

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P73

The RNA splicing machinery in chloroplasts: a window to the parallel RNA universe of plant organelles.

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Mitochondrial and chloroplast genomes in angiosperms each encode ~20 introns. Most of these are classified as group II introns based on their similarity to self-splicing group II intron ribozymes. However, the group II introns in plant organelles rely on nucleus-encoded proteins to splice in vivo. Because organellar introns interrupt genes that are essential for photosynthesis, respiration, or plant development, the identification of proteins that facilitate their splicing, and the elucidation of the roles of such proteins in gene regulation are important goals in plant biology. At the same time, these introns and their cognate splicing factors provide an opportunity to explore fundamental mechanisms by which proteins enhance ribozyme activity.
Through a combination of forward genetics, reverse-genetics, proteomics, and RIP-chip analyses, we have identified 16 nucleus-encoded proteins that are required for the splicing of group II introns in angiosperm chloroplasts. Whereas nuclear pre-mRNA splicing is catalyzed by a core spliceosome, the splicing of each chloroplast intron is accomplished by the combinatorial action of different subsets of splicing factors. This work led to the discovery of three plant-specific RNA binding domains, the CRM, PORR, and APO domains. Each of these domains is represented in a protein family whose members localize solely to mitochondria or chloroplasts. Several members of each family have been characterized, and they all participate in organellar group II intron splicing. These and other findings suggest that the expansion and diversification of the CRM, PORR, and APO protein families was spurred by the acquisition and subsequent degeneration of organellar group II introns in land plants.

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P74

The role of carbonic anhydrase in C_4 photosynthesis

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Current pressures on the global food supply have accelerated the urgency for a second green revolution using sustainable approaches to increase crop yield and efficiency. One such approach is to engineer C_4 photosynthesis traits into less productive C_3 crops such as rice and wheat. It has been estimated that C_4 rice would increase yield by as much as 50%. To accomplish this goal it will be necessary to achieve a deeper understanding of the biology driving C_4 photosynthesis, the method of carbon fixation utilized by the world's most productive food, feed and bioenergy crops. Carbonic anhydrase (CA) is involved in multiple cellular functions including nitrogen metabolism, water use efficiency, and photosynthesis. CA catalyzes the first dedicated step in C_4 photosynthesis, the hydration of CO_2 into bicarbonate, and is likely rate limiting in C_4 grasses. Our research is focused on understanding the role of CA in C_4 photosynthesis. Using *Ds* insertional mutagenesis, we generated mutants in several members of the maize CA gene family. Experiments are underway to characterize these mutants. Comparative transcriptomics of rice and maize RNA-seq data was used to identify the genes encoding the C_3 and C_4 isoforms of CA and experiments are also in progress to examine subcellular localization of the CA gene family members. A better understanding of C_4 biology will facilitate both engineering efforts, and breeding programs that could utilize natural genetic variation by selecting favorable alleles to increase photosynthetic efficiency and thereby increase food production and plant biomass.

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P75

The role of *ZmSUT4* facilitating carbohydrate partitioning

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Carbohydrate partitioning is the process by which carbohydrates, produced in photosynthetic tissues, are distributed through the phloem to source tissues, facilitating growth and development. Sucrose is transported into the phloem, requiring transport across the apoplastic cell wall. This transport is mediated by a group of sucrose transporter (SUT) proteins. We have investigated the role of SUT4 genes in facilitating carbohydrate partitioning. Previous studies reported that null mutations of the orthologous gene in Arabidopsis, *SUC3*, had no obvious phenotype, although *SUC3* is expressed mainly in sink tissues or is induced by wounding. In monocots, *OsSUT4* was found in all tissues examined in rice although, interestingly, *OsSUT4* was expressed at higher levels in sink tissues. More research is needed to understand the function of these *SUT4s* in both grasses and dicot plants.
Through iodine staining of accumulating starch, we have identified a *SUT4* phenotype in maize which was not previously reported. Future experiments will include expression and localizations studies identifying the role of *ZmSUT4* facilitating carbon partitioning.

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P76

The roles of ZmMYB31 and ZmMYB42 in the regulation of the maize lignin biosynthetic pathway

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Two R2R3-MYB type transcription factors (TFs) *Zm*MYB31 and *Zm*MYB42 have been linked to the negative regulation of the lignin biosynthetic pathway by overexpression in *Arabidopsis*. These TFs exhibit 92% identity but their regulatory role and the extent to which they have overlapping function had not been determined in maize. Using SELEX assays, we defined the consensus DNA-binding site of *Zm*MYB31 and showed that it corresponds to the canonical AC-II element (ACC $^{T}/_{A}$ ACC) recognized by R2R3-MYB factors. Mobility shift assays indicated that both proteins strongly interact with the AC-II element of the maize *COMT* gene promoter *in vitro*, and that *Zm*MYB42 can also bind to an ACIII element. Chromatin immunoprecipitation (ChIP)-PCR and transient expression assays demonstrated that they share a set of common target genes *in vivo*. Both directly repress and interact with the lignin *ZmCOMT* and flavonoid *ZmA*1 genes promoters *in vivo*. However *Zm*Myb31 requires both ACII elements for efficient repression of COMT, while *Zm*MYB42 only needs the second upstream ACII element. Further results show that both proteins also bind to different targets, *Zm*MYB31 interacts with the *ZmF5H* gene promoter while *Zm*MYB42 interacts with the maize *Zm4CL2* gene promoter *in vivo*. The combined information arising from the characterization of *Zm*MYB42 and *Zm*MYB31 shows that these two factors play non-redundant functions in the regulation of the phenylpropanoid pathway, even though they are phylogenetically closely related. Moreover, these studies highlight the complexity of the phenylpropanoid pathway regulation and can inform strategies to modify lignin content in biofuel grasses. Currently, we are investigating if this regulation is conserved in other grasses including sorghum, rice and switchgrass. This project was funded by grant NSF DBI-0701405.

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P77

The unique Inositol pyrophosphate phenotype of maize *lpa*1-1

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Inositol hexaphosphate (Ins P_6 or "phytic acid") represents a metabolic pool important to numerous functions including signal transduction, protein tertiary structure, and phosphorus and mineral storage in seeds, pollen and other plant tissues. In one pathway Ins P_6 serves as substrate for the synthesis of the more highly phosphorylated Ins pyrophosphates, Ins P_7 and Ins P_8. These compounds contain one or more di- or triphosphate moieties, in comparison to Ins P_6 which only contains monophosphate esters. These di- and triphosphates have "phosphate bond energies" similar to ATP, can serve as P-donors in ATP regeneration or in protein phosphorylation, and are believed to function as signals of cellular metabolic and phosphate status. Maize *lpa*1 encodes an Ins P_6-specific ABC transporter, termed Multiple Drug Resistance Protein 4 (MRP4). During seed development, this protein probably functions to sequester cytoplasmically-synthesized Ins P_6 into storage microvacuoles. Loss-of-function results in blocked transport/storage and subsequent breakdown of excess cytoplasmic Ins P_6. Here we describe how maize *lpa*1-1 provided the first clear proof of Ins pyrophosphates in plant cells and the first mutant plant Ins pyrophosphate phenotype. A survey of plant Ins phosphate pathway mutants revealed that in maize *lpa*1-1 seed, reductions in Ins P_6 reveals increased levels of Ins P_7 and Ins P_8. Three bands corresponding to relatively equimolar levels of Ins P_6, Ins P_7 and Ins P_8 are clearly observed in PAGE gel analysis. This unique phenotype is not observed in wild-type or other Ins phosphate pathway mutants, but is observed in seeds homozygous for mutations in the Arabidopsis homolog of maize *lpa*1. The structure of these compounds, the enzymology involved in their synthesis and a simple mechanism explaining their unique accumulation in maize *lpa*1-1 will be discussed. Ongoing studies of the role of Ins pyrophosphates in seeds and other plant tissues and organs will also be described.

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

P78

Toward a mechanistic understanding of the role of pentatricopeptide repeat proteins in organellar gene expression

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The pentatricopeptide repeat (PPR) family is the largest family of RNA binding proteins in plants. PPR proteins are characterized by tandem arrays of a 35 amino acid repeat that is related to the TPR motif. Genetic data have linked "pure" PPR proteins (those consisting primarily of canonical PPR motifs) to numerous RNA-related processes in chloroplasts and mitochondria. We are interested in understanding how proteins with a simple repeating architecture can mediate diverse functions and bind with specificity to RNAs of diverse sequence. An understanding of these issues will provide the basis for predicting the functions of the hundreds of uncharacterized PPR proteins in plants, and for exploiting PPR proteins to modulate organelle gene expression.
Detailed analysis of the chloroplast protein PPR10 showed that one simple activity- the sequestration of an RNA segment of ~20 nts – underlies PPR10's RNA processing, RNA stabilization and translational activation functions. When PPR10 binds to the atpH 5'UTR, it (i) presents a site-specific barrier to exonucleases, thereby stabilizing adjacent RNA segments, and (ii) promotes RNA remodeling, thereby exposing the atpH ribosome binding site. We have now obtained evidence that these are general themes of PPR function in chloroplasts. These inferences are based on (i) the finding that the in vivo footprints of some PPR proteins accumulate as small RNAs; (ii) large scale mapping of chloroplast RNA termini; and (iii) in vitro assays with the PPR protein HCF152 and the "PPR-like" protein HCF107. HCF107 consists of a distinct class of helical repeat- the HAT repeat- but has activities that are closely analogous to those of PPR10. Our results provide evidence that virtually all processed chloroplast 5' ends and many 3' ends are stabilized by PPR-like proteins, and that the binding of such proteins in proximity to a start codon often increases accessibility of the ribosome binding site. We are currently developing approaches to link "orphan" PPR footprints to cognate proteins, and to develop our understanding of the basis for sequence-specific RNA binding by PPR tracts.

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P79

Transcriptional profiling of tolerance gene under chilling stress in sweet corn using microarray

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Sweet corn is a popular vegetable crop and developed rapidly in China, especially in South China. Sweet corn can be grown in winter due to enough effective accumulation temperature in South China. But chilling stress is one important factor limiting winter grown sweet corn production. The aims of this study were to analyze the differential expression of genes under chilling stress in sweet corn and discover tolerance genes. The high-throughput detection of genes responding to chilling stress in seedlings of chilling-tolerant parent Richao-1, sensitive parent C5, and tolerant hybrid Yuetian13 was performed using Agilent 44K microarray. The primary results showed that early differentially expressed genes were much less than those expressed late, but including some important transcription factor-related genes. Among these differentially expressed genes, 52 genes showed opposite direction of expression between tolerant and sensitive lines. Gene ontology analysis showed that some of these differentially expressed genes responding to oxidative stress, auxin stimulus, or related to calmodulin or calcium ion. Independent qRT-PCR validated well the microarray data. The further study will perform to identify gene related to chilling tolerance and reveal the molecular genetic mechanisms of chilling tolerance in sweet corn.

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P80

Transcriptomics of maize silks: Understanding hydrocarbon accumulation patterns on the cuticle of developing silks

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The cuticle of maize silks functions as a water barrier and as a protective layer against abiotic and biotic stresses. This cuticle is composed primarily of simple hydrocarbons (alkanes and alkenes), which are produced via elongation of fatty acids with subsequent conversion to hydrocarbons. However, the biochemical and genetic mechanisms involved in hydrocarbon synthesis and accumulation are unknown.

We have demonstrated in the inbred B73 that cuticular hydrocarbons accumulate ~5-fold more on silks that have emerged as compared to those still encased within ear husks. Moreover, accumulation continues to increase between 3 and 6 days after silks emerge from the husk, suggesting that the genes involved in the synthesis and accumulation of hydrocarbons are actively transcribed at 3-days post-emergence. The transcriptomes of emerged and encased silks from this time point (2 biological replicates) were sequenced via the Illumina RNA-seq technology. The resulting ~36,000,000 short paired-end reads were mapped to the B73 genome and ~25,000 genes were identified to be expressed in both emerged and encased silks. Several maize homologs of genes known to be involved in fatty acid synthesis and elongation were expressed highly in emerged silks, including FAR1 fatty-acid acyl-CoA reductase (~4-fold higher) and acyl-transferase (~5 fold higher). In contrast, FAE2 fatty-acid elongase2 was expressed ~2 fold higher in encased silks. This work is being combined with quantitative genetic analyses of intermated B73 x Mo17 populations to identify gene candidates involved in hydrocarbon accumulation.

Furthermore, this set of experiments provides broader information about genetic regulation of diverse pathways during silk development. For example, gene ontology and pathway analysis of differentially expressed genes revealed enrichment of metabolism- and development-related activities in encased silks and stress and defense-related activities in emerged silks.

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P81

Transition Metal Ion Homeostasis in Maize

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Strategy II is an iron accumulation mechanism specific to the grasses that chelates and transports ferric iron from the rhizosphere. The maize Yellow Stripe 1 (YS1) transporter was the first transporter shown to transport phytosiderophore-Fe(III) compounds from the soil into maize roots. Subsequent work in plant species including and beyond the grasses has linked yellow stripe-like family members with the internal transport of nicotianamine and metal bound nicotianamine complexes. With the sequencing of the maize genome, returning to maize to conduct a thorough investigation and characterization of the yellow stripe-like transporter family has been initiated. We will present a gene expression study conducted to examine the relative expression of ZmYS1 and ZmYSL family members across a developmental time course (seedlings, root, leaves, stems, tassel and ear) and after nutrient deficiency and resupply treatments (for the essential metal nutrients- copper, iron, manganese and zinc). The importance of the YSL family for both developmental processes and the transport and movement of metals throughout plant tissue and organs and supply to seeds and other edible plant portions has been demonstrated in a variety of plant species. Through the systematic characterization of gene expression across the maize *YSL* family, we can further identify those family members essential for the transport of iron and other metal nutrients for proper plant nutrition.

Funding acknowledgement: United States Department of Agriculture (USDA)

P82

Understanding the disrupted division plane orientation during stomata formation in maize cyclin D4 mutant, asc1.

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In plant cells, the plane of division during cytokinesis is determined by the arrangement of microtubules and microfilaments that forms the preprophase band (PPB). The PPB region is later recognized by the phragmoplast which coordinates the formation of the new cell membrane and wall that seperates the daughter cells. Asceapen1 (asc1) encodes a D4-type cyclin whose elimination results in a multiple phenotypes including division plane orientation defects in the maize leaf (Brooks et al. 2009). D4-type cyclins are thought to regulate the G1 to S transition during the cell cycle, but the asc1 phenotype suggests a specific role in orienting the division plane.
Loss of asc1 results in abnormally shaped subsidiary cells in the maize leaf epidermis. The abnormal shaped subsidiary cells point to a direct or indirect role in division plane orientation for asc1. Our investigation into the cause of the asc1 mutant phenotype will begin with an analysis of cell cycle progression in asc1 mutants expressing YFP-TUBULIN. We will extend our studies by localizing molecular markers and structures needed for division plane orientation in plant cells in asc1 mutants. Localization of the ASC1 protein both spatially and temporally within dividing cells will help determine how it functions in the cell.
The proposed analysis of the asc1 phenotype is easily done in maize due to the wide variety of cell biology tools available. This research objective will help us understand how loss of a cyclin D4 affects division plane orientation in plants. This will lead to an expansion of understanding about the proteins involved in division plane orientation.

Funding acknowledgement: University of North Texas; Denton TX

P83

Using a Candidate Approach to identify a Restorer-of-Fertility Gene for CMS-C in Maize

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Cytoplasmic male sterility (CMS) is a maternally inherited mitochondrial defect that results in the failure of a plant to produce functional pollen. In maize three types of CMS (T, S, and C) exist. In CMS-C, pollen is aborted at the tetrad stage of pollen development (Lee et al., 1979). Not much is known about why this pollen abortion occurs. The other two types of CMS in maize are sterile due to expression of chimeric mitochondrial genes that are toxic during pollen development, but no such novel chimeric open reading frames have been identified as a possible cause of CMS-C. Pollen function can be restored in sterile CMS-C in the presence of a dominant allele of the nuclear *restorer-of-fertility 4 (Rf4)* gene. *Rf4* was previously mapped to the end of the short arm of chromosome 8, within a 410-kb region. Most of the known restorer genes code for pentatricopeptide repeat (PPR) proteins. However, no PPR protein genes have been identified within the 410 kb region. Therefore, *Rf4* might represent a new type of fertility restorer gene. The goal of this project is to identify *Rf4* using a candidate gene approach. Primers have been designed for ten genes in the region and four have been successfully amplified and sequenced from lines that carry either *Rf4* or *rf4*. Thus far the sequencing results have not identified differences that would change the amino acid sequence between the alleles. The next step of the project will be sequencing the remaining genes and analyzing them for possible differences.

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P84

Using genome wise association to unravel the genetic architecture of provitamin A and vitamin E biosynthesis in maize and Arabidopsis seeds

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Micronutrient deficiency is a widespread problem affecting both developed and developing countries. To alleviate such deficiencies, breeding programs that enhance crop nutrition levels have been developed in several species. Maize as the dominant staple food in much of Africa and America has been a prominent target for enhancing traits that range from minerals to vitamins. Vitamin A (carotenoids) and vitamin E (tocochromanols) as essential components of human diet, carry major health implications when in deficit, and as such the enhancement of their levels in crop species is of paramount importance. As the key model plant for crop genetics and due to its extensive phenotypic diversity, maize lends itself as a powerful tool to identify and study favorable alleles that can be ultimately used for biofortification programs.
To achieve such goal, a high pressure liquid chromatography (HPLC) method was established and validated that allows the separation and resolution of several carotenoid and tocopherol compounds at a high-throughput level. Using a genome wide association mapping approach, the genetic architecture of carotenoid and tocopherols traits was interrogated in maize (NAM population and an association panel) and Arabidopsis (association panel) seeds. Several carotenoid and tocopherol core biosynthetic genes were identified as contributing to variation i.e. lycopene cyclase, beta hydroxylase, zeta carotene desaturase and gamma-tocopherol methyltransferase for multiple traits in both maize and Arabidopsis. This leads us to believe that there is shared genetic architecture of such traits between maize and Arabidopsis.

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P85

Using RNAseq to Discover Novel Regulators and Genes in Nitrogen Utilization Pathways

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The ability of plants to remobilize nitrogen accumulated in vegetative tissues to promote reproductive development is an important physiological process contributing to nitrogen (N) utilization. Although a key target for improving maize yield response to nitrogen, relatively little is known about the gene regulatory systems that modulate N remobilization. We have employed Illumina RNASeq to profile changes in gene expression in both leaf and developing ear tissues of plants grown in the field with different levels of soil N supply, during the period of active N remobilization. RNA profiles were obtained from three genotypes that differ in N utilization: Illinois High Protein (IHP), Illinois Low Protein (ILP) and B73. IHP and ILP are inbred strains developed from the Illinois long term selection experiment for seed nitrogen concentration. Relative to the reference genotype B73, IHP is more efficient at absorbing and translocating nitrogen, and ILP exhibits a reduced ability to absorb and remobilize nitrogen. Prior studies in our lab have shown that changes in the regulation of both the synthesis and degradation of asparagine are associated with the different N remobilization phenotypes of IHP and ILP. Thus, of particular interest are genes which share expression patterns with either asparagine synthetase (*AS*, increased in IHP) or asparaginase (*ASNase*, increased in ILP). Such transcripts could function in either the regulation of asparagine cycling, or as downstream responses to asparagine signaling. Progress towards identifying these and other genes that function in N remobilization will be presented.

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P86

Variable lobes, a polarity-affected anther mutant in maize

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Maize anthers serve as ideal developmental models for the study of cell fate acquisition in floral meristems and differentiation of germinal cells from somatic progenitors. Mature wild type anthers consist of four lobes containing five concentric cell layers: the epidermis, endothecium, middle layer, tapetum and, at the center of each lobe, pollen mother cells. Here we present initial characterization and genetic mapping of a polarity-affected anther development mutant, *variable lobes* (*vlo*), for which there are two alleles. Phenotypes in the *vlo* mutant range from curly two and three lobed anthers to aborted stamen primordia, which yield antherless spikelets. Most commonly a two lobed anther is seen containing pollen in the abaxial lobes, which fail to dehisce. Ear development occurs almost normally producing a fertile ear with two superfluous glume-like structures adjacent to each kernel. Through SNP and SSR-based mapping we have narrowed the position of this mutant to 25 Mbp on chromosome 3L.

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P87

ZmNlr1: A function and structure analysis

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The flat leaf blade of higher plants is an important adaptation for efficient photosynthesis. We recovered a maize mutant that impacts development in multiple tissues including distinct narrow leaf and rough endosperm (nlr1) phenotypes. We identified a Robertson's Mutator (Mu) transposon insertion tightly linked to nlr1. The transposon disrupts the coding sequence of a J-domain containing protein leading to reduced accumulation of the full length transcript. J-domains activate Hsp70 ATPase and proteins containing J-domains have diverse functions in the cell. In addition to the J-domain, the protein contains two nuclear localization signals (NLS) and an Arginine/Serine (RS)-rich domain at the N-terminus. RS domains are found in pre-mRNA splicing factors and presence of this domain suggests NLR1 is associated spliceosomal complexes. Transient expression of N and C terminal fusions with GFP shows subnuclear localization consistent with nuclear speckles. pre-mRNA splicing factors are frequently localized to nuclear speckles. Domain deletion assays revealed that the N-terminal RS domain is required for the speckling pattern, while deletion of either the C-terminal NLS or J-Domain has no affect on the localization pattern. A yeast-two-hybrid (Y2H) screen using NLR1 as bait, retrieved FK506-binding protein 12 (FKBP12). Members of the FKBP family are immunophilins and possess peptidyl-prolyl cis/trans isomerase activity. FKBPs are ubiquitous and serve in protein folding, cell stress, signal transduction, transcription and cell cycle regulation. Arabidopsis FKBP12 has roles throughout plant development, especially during endosperm development and embryogenesis. Interestingly, AtFKBP12 is known to interact with AtFIP37, which is homologous to HsWTAP and DmFL, two metazoan proteins involved in splicing. These data support a model in which NLR1 is involved in transcriptional regulation through interaction with spliceosomal complexes.

P88

Competing dynamics of two tandem repeats in maize: TR-1 neutralizes the knob 180 meiotic drive system

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Heterochromatic knobs have been observed across *Zea* at 31 distinct loci on chromosomal arms. These knobs are composed of two repeat units (knob180 and TR-1) that form long tandem arrays. The profusion of knobs in *Zea* has been attributed to maize abnormal chromosome 10 (Ab10). When Ab10 is present, the typically inert knobs are transformed into neocentromeres. Neocentromeres move poleward laterally along meiotic spindles ahead of true centromeres, and TR-1 moves ahead of knob180. This striking movement allows the knobbed chromatids to reach the spindle poles quickly, thus establishing a strong outward orientation that is maintained through the end of female meiosis. Due to the outward orientation, all knobbed chromatids end in the outermost (top and bottom) cells of the naturally linear tetrad. As the bottom cell goes on to form the egg, this allows Ab10 to transmit itself and all other knobbed chromatids in a non-Mendelian fashion. We recently characterized Ab10-L2, finding that it exhibits exclusively TR-1 neocentromere activity and does not exhibit preferential transmission. The characterization of Ab10-L2 definitively shows that knob 180 and TR-1 have independent neocentromere activities and that TR-1 neocentromere do not lead to preferential transmission. Moreover, our data show that Ab10-L2 is able to suppress the preferential segregation of other Ab10 types, and the overall abundance of the 180bp repeat. TR-1 is believed to have evolved from the knob180 repeat, and we propose it evolved in direct competition with the 180bp repeat. The combined data on drive ability, knob repeat abundance, and motility point to a competitive and neutralizing role of Ab10-L2 on the original Ab10 meiotic drive system.

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P89

Environmentally produced polyploid cells in primary root tips

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Haploid-inducing line, RWS, generates maternal haploid progeny when used as a male parent. The mechanism by which this elimination occurs, however, is not understood. In the process of studying this line, cells with increased ploidy were discovered. When germinated in vermiculite over-saturated with water, root tips with obvious distal bulges appear. Collected three days after germination, cells within these bulbous root tips are larger in size and invariably show abnormally high numbers of chromosomes. Using Fluorescence *in situ* Hybridization (FISH), we karyotyped chromosome spreads of these roots and found that most cells are typically tetraploid (4n=40), yet octaploid (8n=80) and diploid (2n=20) cells are also observed at a lower frequency. In addition, cells with aneuploid variations of these three euploidies have been observed. Affected individuals typically show all of these characteristics. While abnormal ploidy variations in a whole plant ordinarily lead to altered phenotypic effects, this phenomenon only lasts for a short period in development. Secondary roots collected at least seven days after germination do not show any further patterns of irregular ploidy, and the plants grow and develop as normal, fertile diploids. Further testing and screening will aim to determine what specific environmental conditions maximize the induction of polyploidy cells.

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P90

Genomic evaluation of immortal maize cell cultures: Black Mexican Sweet - 30 years and 10,000 somatic generations of selection and mutation

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Plant tissue culture is a mutagenic environment, characterized by frequent cytological events including chromosomal rearrangements and aneuploidy, as well as changes in DNA sequence. Tissue culture also acts as a selective environment, where a dynamic population of cells can change in constitution over time due to selection using specific agents, or simply due to characteristics such as growth rate. We are analyzing a unique maize cell line originally initiated from stem segments of seedlings from the cultivar Black Mexican Sweet (BMS). This now immortal cell culture has been maintained *in vitro* for over 30 years, representing nearly 10,000 cell cycles. The goal of this study was to characterize chromosomal and sequence variation within this cell line. Comparative genome hybridization was used to assess copy number variation in BMS relative to non-cultured control plants from the same cultivar. Substantial genome fragmentation was observed in contrast to a recently established culture of A188, which showed little variation. We evaluated the hypothesis that sequences necessary for rapid growth might be amplified in BMS, but no instances of large-scale amplification of single sequences was observed. We searched for evidence of aneuploidy and polyploidy using flow cytometry. In general, our flow cytometric data indicate that the cells are primarily euploid with the predominance being diploid and a small proportion tetraploid. Our conclusion to date is that mutational forces are balanced by the selective environment of tissue culture, maintaining a population of cells with DNA composition relatively similar to non-cultured controls. This conclusion will be explored further by cytological assessment of chromosome composition of individual cells and by isolation of single cell sublines by selection with a transgene.

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P91

Heritable Loss of Replication Control of a Minichromosome Derived From the B Chromosome of Maize

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A small telomere truncated B chromosome was increased to multiple copies using the B chromosome's accumulation mechanism, namely nondisjunction at the second pollen mitosis and preferential fertilization of the egg. During the process of this accumulation regime, instances were found in which this chromosome had amplified in copy number without separation, resembling endoreduplication. This chromosome structure has been inherited over seven generations. To characterize the chromosome we applied CentC, CRM, Stark repeat, and the WY76 transgene probes, all of which were present. We used immunocytochemistry to identify centromeres with phosphorylated H2A and phosphorylated H3S10, which are associated with the maintenance of sister chromatid cohesion. We characterized the mitotic and meiotic behavior of this chromosome and observed an increasing likelihood of nondisjunction that correlates with an increasing chromosome size. This autonomously endoreduplicating chromosome could multiply by failing to separate sister chromatids, but this would dilute the observed frequency of large endoreduplicated chromosomes. Another possible mechanism for the single chromosome endoreduplication is an error of replication or an epigenetic modification resulting in multiplicative DNA synthesis in a single cell cycle.

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P92

In vivo modification of a maize engineered minichromosome

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Engineered minichromosomes will provide efficient platforms for stacking transgenes in crop plants. Methods for modifying genes *in vivo* would be essential to the development of customizable minichromosome systems, such that selection genes and unwanted DNA could be removed and novel genes could be added. Previous studies had demonstrated that the site-specific recombinase Cre could induce recombination at *lox* sites on transgenes contained on maize minichromosomes; however, these studies only demonstrated somatic recombination, and modified minichromosomes were not recovered in progeny plants. In this study we describe the recovery of an engineered chromosome composed of little more than a centromere and transgene, which was derived by telomere truncation. Fiber FISH found that the transgene was inserted among stretches of CentC centromere repeats, and that the transgene locus was approximately 10.5 kb, suggesting a tandem insertion. The bar selection gene was deleted from the chromosome by crosses with a Cre-recombinase expressing plant, leaving behind a single *loxP* site in the transgene locus of the modified minichromosome. This study demonstrates that engineered chromosomes can be modified *in vivo* using site-specific recombinases, a demonstration essential to the development of amendable chromosome platforms in plants.

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P93

Maize Chromosomal Knob Locations and their Influence on Genome Evolution, Structure, and Function

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Almost eight percent of maize genome is composed of large blocks of repetitive DNA arrays called knobs. The genome of most lines contain between four and eight knobs, with 34 defined cytological positions in *Zea* spp. variable for their occurrence. The existence of knobs may result from the actions of a rare form of chromosome 10 (called Abnormal Chromosome 10 or Ab10) that increases recombination rates and causes knobs to preferentially segregate to gametes during the meiotic events leading up to oogenesis. Conversely, some knobs have been reported to reduce recombination in the absence of Ab10. Given this information, it seems likely that knobs have significantly affected maize diversity and evolution. However, previous to the work described here the genetic positions of knobs were effectively unknown, making this hypothesis difficult to test. Here we used FISH and two recombinant inbred populations (Ki3 x B73 and CML277 x B73) to genetically map seven knobs and to accurately place all five B73- specific knobs on the physical map and reference genome. Surprisingly, our data show that knobs tend to lie in very gene-dense regions of the maize genome. Analysis of recombination in the Ki3 and CML277 RIL populations and comparison to 23 other RIL populations segregating for knobs at the same sites show that knobs have a surprisingly small effect on recombination overall. Nevertheless, knobs do show minor but significant effects when individual lines or knobs are taken into account, as observed by Rhodes and Dempsey (1957) and Kikudome (1959).

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P94

Phenotypic Effects of Varying the Dosage of A Chromosome Segments Utilizing Maize B-A Translocations

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This investigation aims at understanding the effects of altering the dosage of large segments of chromosomes on maize inbred plant phenotype. A set of B-A translocation stocks bearing dominant genetic markers on the A chromosome segment in an inbred W22 background for 14 of the 20 maize chromosome arms were used. The effects of each of the 14 chromosome segments on plant phenotype were studied by growing groups (families) of plants where the plants contain either one dose (hypoploid), two doses (euploid- no B-A translocation present), two doses(euploid – B-A translocation present) or three doses (hyperploid) of the A chromosome segment borne on the B-A chromosome. The phenotypes examined were leaf length, leaf width, plant height, ear height, internode length, primary ear node circumference, tassel-branch number, days to anthesis, days to silk, and days between anthesis and silking.

Statistical analysis revealed that the two euploid groups were not significantly different from each other in any of the chromosome arms for any of the phenotypes, except for plant height and internode length inTB-5Sc. Compared to the euploid plants, significant decreases in morphological traits or increases in flowering time traits were seen in all of the hypoploid plants for each B-A translocation, although not all traits were affected in each family. Increases were seen in leaf width for TB-3Sb hypoploid plants. Hyperploid plants showed a decrease in measurements or an increases in flowering traits in 12 of the 14 B-A translocations, although with fewer phenotypes being affected, and those that were affected were not as severely affected as their hypoploid counterparts. An increase was observed in the primary ear height for TB-9Sd hyperploid plants. The phenotypic effects displayed by hypoploid plants are greater than those displayed by hyperploid plants indicating that the maize plant is more tolerant of increases in chromosome dosages.

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P95

The characterization of maize null mutants of SPO11-1 reveals its role in the conformation of chromosome axis during meiosis

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Meiosis is a highly conserved process in eukaryotes that is essential for producing haploid gametes with genetic variation. During meiotic prophase, homologous chromosomes find each other, pair and undergo homologous recombination and synapsis. During synapsis, an axial element assembles between the two sister chromatids of each homolog and the central element assembles between the axial/lateral elements of the two homologous chromosomes to form a tripartite synaptonemal complex (SC). Here, we present the cytogenetical characterization of two mutants (mtm99-14 and mtm00-03) with meiotic defects that lead to univalent formation at metaphase I.
By TEM, spread prophase chromosomes of mtm99-14 and mtm00-03 display irregular and thicker axial elements than wild-type chromosomes. AFD1 and ASY1, axial element components, are assembled onto chromosomes with the same timing as in wild-type but ASY1 present abnormal coiling. ZYP1, a component of the central element, starts loading onto the chromosomes during zygotene, but fails to complete assembly resulting in the formation of foci and limited elongated synapsis structures.
Both mutations are allelic. Using map based cloning and high throughput sequencing methods, we have cloned the mtm99-14 and mtm00-03 mutation that completely delete the SPO11-1 gene. SPO11 is a conserved protein responsible for meiotic DNA double strand breaks that initiate meiotic recombination. Maize has three SPO11 genes as in Arabidopsis but other plants such as rice have five SPO11 genes. Interestingly, the maize gene SPO11-1 shows differently spliced variants as in human and mouse [Bellani et al. 2010]. We are interested to analyze the function of this regulation and are developing antibodies against the two maize SPO11-1 predicted isoforms. These results suggest that SPO11-1 is required for the initiation of recombination as suggested by the absence of the RAD51 recombinase in both mutants but also underline a previously unobserved link between axial element conformation and the initiation of recombination.

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P96

A New *barren stalk* Gene Required for Axillary Meristem Development in Maize

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Axillary meristems (AMs), which are small groups of stem cells that form in the axils of leaf primordia, generate shoot branches (eg., in maize; tillers) and inflorescences (eg., in maize; tassels and ears), and thus are important in determining the architecture of higher plants. Previous studies revealed several critical genes that function in auxin biosynthesis, transport or downstream of auxin in regulation of AM production. We have identified a new mutant *ba*3112* which produces no ear shoots and maps to chromosome 2.04. Based on the map position and phenotype, we propose to name the mutant *ba2*, which was first described by Hofmeyer in 1930, but was subsequently lost. In hybrids, *ba2* mutants have a normal tassel but when introgressed into the B73 background the mutants have fewer tassel branches and spikelets and a higher percentage of single spikelets than the normal plants. SEM (Scanning Electron Microscope) analysis shows that the *ba2* mutants have defects in reproductive AM formation. The *ba2* mutation suppresses tiller growth in the *teosinte branched1* mutant, suggesting an essential role in vegetative AM formation. The *ba2* gene was positionally cloned and the *ba2-3112* mutant allele has a nonsense mutation at the 3' coding region. Another *ba2* allele isolated from an independent EMS mutagenesis has a nonsense mutation in the 5' coding region. Phylogenetic analysis shows that the *ba2* gene is grass-specific and encodes a protein with a plant-specific conserved domain. Characterization of the genetic interaction between *ba2* and other genes involved in AM production demonstrates that the *barren stalk1* (*ba1*) gene, which encodes a basic helix-loop-helix transcription factor functioning in AM formation, is epistatic to *ba2* and shows a dosage effect in *ba2* mutants, suggesting that *ba1* and *ba2* act in the same pathway.

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P97

A TCP transcription factor, *BRANCH ANGLE DEFECTIVE 1* (*BAD1*), is required for normal tassel branch angle formation in Maize

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In grass inflorescences a structure called the pulvinus is found between the inflorescence main stem and lateral branches. The size of the pulvinus affects the angle of the lateral branches that emerge from the main axis, and therefore has a large impact on inflorescence architecture. Through EMS mutagenesis we have identified three complementation groups of recessive mutants in maize having defects in pulvinus formation. All mutants showed extremely acute tassel branch angles accompanied by a significant reduction in the size of the pulvinus compared to normal plants. Two of the complementation groups correspond to mutations in the previously identified genes, *RAMOSA2* (*RA2*) and *LIGULESS1* (*LG1*). Mutants corresponding to a third group were cloned using mapped based approaches and found to encode a new member of the plant-specific TCP family of DNA binding proteins, *BRANCH ANGLE DEFECTIVE 1* (*BAD1*). *BAD1* is expressed in the developing pulvinus as well as in other developing tissues including the tassels and juvenile leaves. Both molecular and genetics studies show that *RA2* is upstream of *BAD1*, while *LG1* may function downstream of *BAD1*. Our findings demonstrate that *BAD1* is a TCP class II gene that functions to promote cell proliferation in a lateral organ, the pulvinus, and influences inflorescence architecture by changing lateral branch angle.

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P98

Analysis of inbred-specific effects on the maize ta-siRNA pathway

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Leaf patterning along the dorsiventral (adaxial-abaxial) axis relies upon the coordinated regulation of many genes and small RNAs. The ta-siRNA pathway, which generates trans-acting small RNAs, termed ta-siRNAs, plays an important role in this regulation from very early on in the development of the maize plant. Mutants in the ta-siRNA pathway disrupt this process and display abnormal leaf characteristics including the presence of sectors of abaxial leaf identity on the adaxial surface, reduced leaf margins and/or radialization of the leaf blade. Interestingly, the severity of these phenotypes is greatly dependent on the inbred background. Ta-siRNA pathway mutants, as well as other small RNA biogenesis mutants, typically exhibit weaker phenotypes when introgressed into B73 as opposed to other inbred backgrounds, i.e. Mo17, A619 and W22. Very severe mutant phenotypes are frequently observed in W22, where severe alleles of many of the ta-siRNA mutants fail to produce a shoot apical meristem. To understand how inbred background influences mutant phenotype severity with respect to the ta-siRNA pathway, we are using the *ragged1* allele of the *leafbladeless1* (*lbl1-rgd1*) mutant. By taking advantage of the very different *lbl1-rgd1* phenotypes in B73 and W22, we are using an F2 mapping population to fine map modifiers of the pathway that either enhance the phenotype in W22 or suppress it in B73. Additionally, we have performed a transcriptome analysis of *lbl1-rgd1* mutant embryos and non-mutant siblings in B73 and W22 backgrounds that condition a moderate leaf phenotype or a severe embryo lethal phenotype, respectively. By combining these datasets we hope to dissect the early genetic requirements that predetermine the morphological and developmental fates of the *lbl1-rgd1* mutant in these two different inbred backgrounds.

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P99

Analysis of the maize *fuzzy tassel* mutant reveals broad roles for microRNAs in vegetative and inflorescence development

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microRNAs (miRNAS) are small non-coding RNAs that repress gene expression in all multicellular organisms and function in diverse developmental and physiological processes in both plants and animals. We have isolated a maize mutant, *fuzzy tassel (fzt)*, that contains a mutation in DICER-LIKE1, a key enzyme required for miRNA biogenesis in plants. *fzt* plants have striking vegetative and reproductive defects, implicating miRNAs in a wide range of developmental processes. *fzt* plants are shorter in stature and make fewer narrower leaves than normal siblings. In addition, *fzt* plants have subtle defects in leaf polarity and phase change, processes known to be regulated by miRNAs. *fzt* also dramatically affects inflorescence development. All inflorescence meristem types are abnormal in *fzt* mutants and less determinate than normal. *fzt* mutant tassels and ears do not make the normal complement of floral organ and *fzt* is both male and female sterile. To determine how the *fzt* mutation affects miRNA populations in vivo, we analyzed the small RNA populations from fzt and normal seedlings and found that there is a slight reduction of most miRNAs in *fzt* compared to normal seedlings. We are currently examining gene expression in *fzt* and normal seedlings using RNA seq to determine which genes are misregulated in *fzt* plants and likely responsible for *fzt* mutant phenotypes.

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P100

B class genes and sex determination in the tassel florets of maize

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B class MADS-box genes play a crucial role in floral organogenesis. Loss of function in either of *Arabidopsis'* two B class genes results in homeotic conversion of 2nd whorl organs (petals) into sepals and 3rd whorl organs (stamens) into carpels. To assess conservation of B class function across the angiosperms knockout mutations have been and are being created in each of maize's four B class genes: *Silky1 (Si1), Sterile tassel silky ear1 (Sts1), Zea maize mads 18 (Zmm18),* and *Zea mays mads 29 (Zmm29)*.

Although tassel florets of many of the maize B class mutants generated thus far show homeotic conversion of 2nd and 3rd whorl organs, stamens of these florets do not show the expected conversion to carpels. Rather, stamens of these mutants are converted to lemma or palea-like organs. To investigate these unexpected results further we have crossed one of our B class mutants, *sts1*, to *tassel seed1 (ts1)*, a mutant that lacks carpel abortion in tassel florets. Resulting double mutants show homeotic conversion of stamen primordia into carpels. This suggests that the carpel abortion pathway in male florets may be responsible for the non-canonical B class mutant phenotype of maize.

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P101

Characterization of a New Developmental Mutant of Maize: *raggedseedling-378*

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The trans-acting small interfering RNA (ta-siRNA) pathway is involved in the establishment of abaxial/adaxial polarity in maize leaves. Mutations that disrupt this pathway, such as in *raggedseedling2* (*rgd2*) and *leafbladeless1* (*lbl1*) plants, lead to dramatic alterations in leaf shape and potentially to defects in leaf polarity. A new developmental maize mutant, *rgd-378*, with phenotypic characteristics similar to *rgd2* and *lbl1* has been identified. *rgd-378* mutants are small plants with variable leaf abnormalities including split leaves, filamentous leaves and leaves that develop only one half of the blade. Histological analysis of *rdg-378* leaves suggests that these plants do not display internal polarity defects; however, the epidermis displays patterning defects. Epidermal impressions of the abaxial and adaxial surfaces of juvenile leaves exhibit altered stomata patterning, smaller pavement cells and mild alterations in subsidiary cell shape in *rgd-378* plants when compared to wild-type siblings. These epidermal abnormalities are also exhibited on leaves that do not display grossly altered morphology. We used RT-PCR to qualitatively characterize the expression of genes in the ta-siRNA pathway in both leaf and shoot apical meristem-enriched tissue. We found differences in expression of some of these genes, namely *rgd-2*, *tas3*, and *arf3a*, in the *rgd-378* tissue compared to wild-type siblings, indicating that expression of some genes in the ta-siRNA pathway are misregulated in the mutant.

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P102

Characterization of *gcn2* mutations in maize

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General control non-derepressible-2 (GCN2), first discovered in yeast as a regulatory protein kinase, plays an important role in cellular responses to amino acid availability. It phosphorylates the α subunit of the trimeric eukaryotic translation initiation factor-2 (eIF2), which in turn decreases the general rate of protein synthesis in response to nutrient starvation or stresses. The phosphorylation of eIF2A enhances the translation of the transcription factor GCN4, resulting in increased expression of many amino acid synthesis genes. The GCN2-like kinases are highly conserved among eukaryotes, including fungi, animals and plants. *Opaque2* (*O2*) is believed to be the maize homologue of GCN4 and we were interested to investigate if O2 is a target of GCN2 regulation in maize endosperm. To date no mutations that affect GCN2 have been reported in maize.

Three *Mutator* insertion mutations of *gcn2* were identified from the Pioneer TUSC populations and backcrossed to the inbred line B73. Analysis of gene expression by qRT-PCR showed that the *gcn2-1* transcript is reduced but still present in developing endosperm, although at lower levels than B73. Several O2 regulated genes such as ribosome inactivating protein and alpha-zeins are indistinguishable between B73 and *gcn2-1* homozygous mutants. Additionally, there were no clear differences in the O2 protein accumulation observed on western blots of either genotype in developing kernels. Analysis of tissue specific gene expression indicated that in the *gcn2-1* mutant the transcript level was much lower in maize seedling leaves than in any other tissue tested. The *gcn2-1* mutant is more sensitive to herbicides that affect amino acid biosynthesis, which is similar to the phenotype of *gcn2* knockouts in Arabidopsis. We are working to clarify the regulation of O2 by GCN2 by characterizing additional alleles that may provide a more thorough disruption of GCN2 function.

Funding acknowledgement: United States Department of Agriculture (USDA)

P103

Characterization of inflorescence architecture in transgenic Setaria viridis expressing the maize or Sorghum bicolor ra1 locus

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Mutations in maize ramosa genes result in branching architectures often reminiscent of the morphological diversity observed across grass inflorescences. ramosa1 (ra1) encodes a C2H2, EPF-subclass zinc-finger transcription factor with two EAR-repression motifs and is hypothesized to be associated with inflorescence diversity in the grasses. Our previous studies support our hypothesis: ra1 expression in maize and in sorghum specifies spikelet pair meristem (SPM) determinacy and temporal regulation of ra1 expression and of RA1 activity levels influence degree of branching. A long-term goal of our work is to elucidate the involvement of ra1 and the ramosa pathway in the natural diversity observed in grass inflorescences. To this end, we have transferred ra1 along with its native promoter among Panicoid members maize, sorghum and Setaria (S. viridis) and have begun testing how function of the ra1 locus has changed throughout evolution. With a recently sequenced genome and highly branched inflorescence, Setaria is an attractive model organism for a component of this work. An interesting feature unique to the Setaria clade is that some inflorescence branch meristems are converted to an undifferentiated, indeterminate branch, called a bristle. Bristles have some aspects of spikelet identity and some branch identity; we are interested in testing if bristle length correlates with degree of determinacy, and hence ra1 expression. Here we report the characterization of transgenic Setaria containing either 5.8 kb of the maize ra1 locus or the 6.0 kb of the sorghum ra1 locus. Sorghum ra1 consists of a tandem duplication, where a natural frameshift mutation disrupts the upstream copy. Our data indicate the ra1 transgene has an effect on inflorescence elongation and branching pattern relative to non-transgenic siblings. Data on spikelet and bristle density, bristle length as well as expression patterns of endogenous Setaria ra1 and maize and sorghum ra1 transgenes will be presented.

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P104

Characterization of *ms32*, a bHLH gene required for tapetum development in maize

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An early stage anther consists of three layers (L1, L2 and L3) that undergo regulated cell divisions to produce a mature anther with five distinct cell layers: the epidermis, endothecium, middle layer, tapetal layer, and meiocytes. Proper regulation of cell differentiation in the anther is crucial since the process is tightly connected with meiosis. Mutants with defects in this process cause male sterility. *ms32* is a male sterile mutant that has excess cell division in the tapetal layer. The defect in *ms32-R* initiates after the middle layer and tapetal layer formation, which results from a single periclinal division of the secondary parietal cells. Additional periclinal divisions in the tapetal layer lead to excess tapetal cells and meiocytes collapse. This phenotype suggests that *ms32* is required for preventing additional divisions in the tapetal layer. Using positional cloning, the *ms32* gene was mapped to a 0.75Mb interval on chromosome 2. Among the candidate genes within this interval, a deletion was found in a basic helix loop helix (bHLH) transcription factor. Lesions within this gene were also confirmed in *ms6066* and *ms6066-Mu*, which were found to be allelic to *ms32-R* by complementation test.

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P105

Characterization of the pollen-specific *stk1* and *stk2* genes in maize

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stk1 lies adjacent to *bz* in *9S*. It is predicted to encode a serine/threonine protein kinase, hence its name. Deletions of this *bz* region have been known to transmit poorly in heterozygote. A closely related gene, *stk2*, is found located in *4L*. *stk1* and *stk2* are also found in sorghum and rice, and phylogenetic analysis shows that they are paralogous genes, in other word, the gene duplication happened before the maize-rice speciation event dated around 50 MYA (million years ago). Both paralogs are only highly expressed in pollen and in the mature tassel, but not in other tissues. Transgenic plants producing STK1/2 fluorescent fusion proteins showed that they both are located on the pollen membrane, and likely only do so upon hydration, as indicated by temporal *in vitro* germination observation. Though not highly expressed in the pollen tube (PT), the *stk* genes function in PT growth since mutations of *stk1* show reduced pollen transmission only when competing with wild type pollen, explaining the reduced pollen transmission of the *bz* region deletions. We have obtained mutants of both genes: 4 *Ac* insertions for *stk1* and 3 *Mu* insertions for *stk2*. Mutations of *stk2* have a smaller pollen transmission effect, suggesting that *stk1* may play a more important role in pollen development. We have introgressed the *stk* mutations into a common genetic background and generated double mutants. The double mutant combination is essentially pollen lethal, as the double mutant pollen germinates at a very low rate *in vitro*, leading to rare recovery of the double mutant. We conclude that the *stk* paralogs play an essential, though slightly unequal, role in pollen development.

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P106

Characterization of the role of *tassel-less1* in maize development

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The *tassel-less1(tls1)* mutant has severe defects in vegetative and reproductive meristem development. *tls1* mutants produce either small tassels with reduced or absent main spike or fail to produce any tassel, and ears are small and ball-shaped. SEM analysis of immature tassels indicates that *tls1* mutants have reduced apical inflorescence meristems. Along with irregularities in reproductive development, *tls1* mutants also display developmental abnormalities consistent with defects in the vegetative meristem, including narrower and shorter leaves. In addition, *tls1* mutants generate extra tillers, indicative of a failure to maintain the shoot apical meristem. An apical meristem defect is further supported by the fact that in the most severe cases, *tls1*-null mutants die three weeks after germination, when the apical meristem is undergoing the transition to reproductive growth. Positional cloning revealed that *tls1* encodes a major intrinsic protein in the aquaporin family. Our analysis indicates that *tls1* plays a critical role in meristem function, and our current work is focused on determining whether *tls1* plays a role specific to meristem maintenance or whether failure to maintain the meristem is a secondary effect of defects in critical cellular processes.

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P107

Closing the gap: the maize PIN gene family of auxin transporters

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Auxin is a key regulator of plant development and its differential distribution in plant tissues is established by either a polar cell-to-cell transport or a long distance movement from source tissues to the roots. However, the polar cell-to-cell transport, with the resulting auxin concentration gradients, is the main mechanism involved in the wide spectrum of auxin-related developmental processes. Multiple transporters are involved in the establishment and maintenance of this polar-auxin-transport (PAT): a better understanding of auxin transporter role in PAT is of outstanding importance for basic plant research and for crop improvement as well, given that shoot and root architectures, two of the main issues in determining crop yield, are strictly regulated by directional auxin flows. In Arabidopsis, three families of auxin transporters were identified: AUXIN RESISTANT 1 (AUX1)/LIKE AUX1 (LAX) uptake symporters, P-GLYCOPROTEIN (ABCB/MDR/PGP) and PIN-FORMED (PIN) efflux carriers. In maize a few members of the two families of auxin efflux transporters, PIN and ABCB, have so far been characterized.

Here we present the characterization of the *Zea mays* auxin efflux carriers PIN family members: four *PIN1* homologs (*ZmPIN1a–d*), one ortholog to *AtPIN2* (*ZmPIN2*), three orthologs of *PIN5* (*ZmPIN5a–c*), one gene paired with *AtPIN8* (*ZmPIN8*), three monocot-specific *PINs* (*ZmPIN9, ZmPIN10a-b*) and two *PIN-like* genes (*ZmPINX-Y*) were identified and analyzed. ZmPIN phylogenetic analysis and expression-localization patterns during pre- and post-embryonic development indicate that subfunctionalization of some maize PINs can be associated to the differentiation/development of monocot-specific organs and tissues and might have occurred after the divergence between dicots and monocots. The expression of ZmPIN genes was also analyzed in *br2* mutant roots and after NPA and NAA treatments. Finally, the transcriptional regulation of the monocot-specific member *ZmPIN9* (expressed in root pericycle and endodermis cells) and its activity of auxin transporter are currently under investigation.

P108

Deep sequencing of maize seedling transcriptomes to understand the impact of the ROUGH ENDOSPERM3 splicing factor

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The *maize rough endosperm3* (*rgh3*) mutant encodes the U2AF^{35}-related protein (ZmURP). In humans, URP is associated with the minor and major spliceosomes, but its biological role is unknown in maize. Prior characterization of the *rgh3* mutant indicates that the gene is required to repress cell proliferation and promote cell differentiation. The *rgh3* mutants are highly proliferative in endosperm cell culture when compared to wild-type endosperm culture that undergoes endoreduplication. Our data indicate that *rgh3* endosperm cells remain frozen in this early, undifferentiated stage throughout kernel development. Fewer than 50% of *rgh3* kernels germinate, and *rgh3* seedlings have adherent leaves and stunted growth, typically dying 15-18 days after planting. We hypothesize that *rgh3* has an effect on RNA splicing and gene expression. To test this, we compared *rgh3* and normal sibling transcriptomes in an RNA-seq experiment. Twelve libraries were constructed with mRNA extracted from the roots and shoots of three seedlings of each genotype. The libraries were multiplexed and sequenced on one lane of the HiSeq 2000 platform. The run produced 149 million paired-end 100 bp reads that mapped to 35,028 genes. The number of reads mapping across splice junctions in the *rgh3* and wild-type transcriptomes were quantified in order to detect potential differences in splice site usage.

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P109

*Defective endosperm*18 (*De*18) encodes a seed-specific YUCCA1 protein essential for IAA biosynthesis, normal endosperm development and seed mass in maize.

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Auxin (IAA) plays a fundamental role in vegetative and reproductive plant development. Developing seeds synthesize and accumulate the highest levels of IAA of all tissues in a plant; however, nothing is known about IAA biosynthesis genes / enzymes critical to endosperm development, nor is there any seed mutant known to be caused by IAA-deficiency. We analyzed a previously described (Theor. Appl. Genet., 1986, 72: 602) seed-specific viable mutant *de*18 that accumulates 10- to 15- fold less IAA and ~40% less dry mass compared to *De*18. Gene expression analyses of seed-specific Tryptophan-dependent IAA pathway genes, *ZmYucca*1 and two Trp-aminotransferases (*Tar*) co-orthologs (Molecular Plant, 2010, 3: 1026), were done using q-PCR to understand the molecular basis of IAA-deficiency in the mutant. Temporally, all three genes showed high expression coincident with high IAA levels; however, only the *ZmYuc*1 correlated with the reduced IAA levels in the mutant throughout endosperm development. Sequence analyses of *ZmYuc*1 cDNA and genomic clones revealed many changes specific to the mutant, including a 2-bp insertion that generated a premature stop codon and a truncated YUCCA1 protein of 212 amino acids, compared to the 400 amino acids in the *De*18. The putative ~1.5 kb *ZmYuc*1 promoter region also showed many rearrangements, including a 151 bp deletion in the mutant. Further, our molecular mapping and annotation studies of chromosome 10, contigs 393 - 397, showed that the *De*18 locus mapped to the gene *ZmYuc*1. In conclusion: (1) The loss of *De*18-encoded YUCCA1 protein is the causal basis of IAA-deficient *de*18 mutant. (2) The lack of compensation of the mutant *yuc*1 function despite the high transcript abundance of the two *ZmTar* genes suggests that YUCCA1 constituted a rate-limiting step in a single pathway of multiple steps of IAA biosynthesis, consistent with the very recently reported studies (in the last two months) in Arabidopsis.

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P110

Deficient boron transport affects maize inflorescence development

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Although the metalloid boron has a relatively low natural abundance, it is an essential plant micronutrient. Deficiency of this element is common in several parts of the world and is responsible for major crop losses. Oftentimes, crops grown on soil with low amounts of boron show no symptoms during vegetative growth, but as plants mature, reductions in yield and fruit/seed quality can be observed. We recently identified a novel recessive mutant, called *rotten ear* (*rte*) impaired in the development of both tassel and ear. *rte* mutants are sterile and fail to develop fully fertile inflorescences. Mutant ears appear to arrest during development and show a characteristic brown or "rotten" appearance. We cloned the *Rte* gene by a map based cloning approach and showed that it encodes a boron transporter similar to the Arabidopsis thaliana *BOR1* gene, responsible for boron loading in the xylem. Overexpression of *Rte* is capable of rescuing the developmental defects of the Arabidopsis *bor1* mutant. The *Rte* gene is expressed in both vegetative and reproductive tissue, and in situ hybridizations show localized expression surrounding vascular tissue. Transient expression assays show that the RTE protein is membrane localized. Phylogenetic analysis identified a close paralog of *Rte*, called *Rte-like*, that is also expressed in identical domains. Our goal is to investigate the molecular mechanisms regulating the transport of this element to the inflorescences, and how it affects inflorescence development and fertility.

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P111

discordia2 is needed for correctly oriented asymmetric cell divisions in the maize epidermis

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In plants, cell wall placement during cytokinesis is determined by the position of the preprophase band (PPB) and the subsequent expansion of the phragmoplast, which deposits the new cell wall at the cortical division site delineated by the PPB. New cell walls are often incorrectly orientated during asymmetric cell divisions in the leaf epidermis of the *discordia2 (dcd2)* maize mutants. To aid in our understanding of the role of *dcd2* in division plane orientation, we are using map-based cloning to identify the gene affected by the *dcd2* mutation. Since the *dcd2* genetic background is not defined, two different mapping populations were generated. Useful mapping markers were identified from public sources as well as through our own sequencing efforts. Currently, *dcd2* is localized to a 2.14 MB interval on chromosome 4.

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P112

Division plane orientation in plant cells

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Proper orientation of cell division planes is important for asymmetric and symmetric divisions, which in turn are essential for development and differentiation. Misorientation of the division plane occurs during metastasis in human cancers, therefore, understanding the mechanism by which cells orient their division planes may provide insight into the process of metastasis. Plants are an excellent model system for studying division plane orientation because their cells are fixed in place by rigid cell walls, preventing migration. The cell walls can be stained, and the "history" of division plane orientation can be inferred by the orientation of the cell walls. Unlike animal cells, plant cells establish their division plane before the onset of mitosis, but the mechanisms by which it is maintained is unknown. The preprophase band (PPB), a plant specific microtubule and microfilament structure, establishes the future division plane before the cell enters mitosis (1). But as the cell enters metaphase, the PPB is disassembled to form the spindle. How does the PPB set the division plane? A longstanding hypothesis is that the PPB places a static division site marker that is maintained throughout mitosis to recruit the cytokinetic machinery back to the division site. Recent work has identified several potential landmark proteins that localize to the division site when the PPB is formed and stay throughout mitosis and cytokinesis (5, 6). The first protein identified with this characteristic is TANGLED, a protein with weak similarity to the microtubule binding domain of the adenomapolyposis coli (APC) tumor suppressor (5). We have identified two separate domains of TANGLED that are required for temporally distinct localization to the division site. In addition, these separate domains not only interact with different proteins, but are also differentially regulated by phosphatases. Our model suggests that the plant division site is not static, but is continually modified throughout mitosis and cytokinesis.

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P113

Duplicate *naked endosperm* genes encode ID domain transcription factors required for maize aleurone differentiation

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The outermost layer of the endosperm is a specific cell type called the aleurone which remains alive during seed maturation and produces digestive enzymes during germination. Aleurone also has health benefits such as anti-cancer and anti-obesity activity. The aleurone layer is an attractive system to study cell fate determination because of the simplicity and amenability to genetic analysis. Here we report the identification of *naked endosperm (nkd)* genes which are involved in aleurone differentiation. The *nkd* mutant shows defects in aleurone cell identity and has two or more outer cell layers instead of the single layer in wild types. However these cells do not contain dense granular cytoplasm typical of normal aleurone and have sporadic expression of a *Vp1* promoter GUS transgene, which is an aleurone identity marker. The *nkd* mutant phenotype shows 15:1 segregation ratio in F2 populations suggesting two recessive genes are involved in this phenotype. We performed map-based cloning and found two homologous genes in syntenic regions. The INDETERMINATE1 domain containing transcription factors *ZmIDDveg9* and *ZmIDD9* correspond to the *nkd1* and *nkd2* mutant genes on chromosome 2 and 10, respectively. An independent *Ds* transposon insertion *nkd1* allele, *nkd1-Ds*, failed to complement the original nkd mutant. The *nkd2*-RNAi lines, in which both of *nkd* genes were knocked down, also showed a *nkd* mutant phenotype. The *nkd* transcripts were most abundant in developing kernels around 15 days after pollination. The NKD proteins have putative nuclear localization signals as other IDD genes and GFP fusion proteins showed nuclear localization. NKD protein was detected by immunolocalization in nuclei of aleurone and subaleurone in developing kernels. The results suggest NKD functions as a transcriptional regulator required for aleurone cell fate acquisition and differentiation.

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P114

EA1-box peptides, grass-specific extracellular hydrophobic signalling ligands

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Small secreted peptides mediate numerous cell-cell-communication events in reproductive and developmental processes in plants [1]. Although computational analyses of plant genomes have revealed the existence of many genes encoding for putative secreted peptide ligands, only little is known about their possible roles in cell signalling [2, 3]. By searching for homology to the signalling peptide ZmEA1 (*Zea mays* Egg Apparatus 1), the sole female gametophyte derived pollen tube attractant identified in maize [4], we have discovered a novel class of hydrophobic and polymorphic small proteins in grasses, named EA1-box peptides. Besides maize, ZmEA1 homologues proteins were also found in other grasses such as rice, *Sorghum bicolor* and *Brachypodium distachyon*. As a common feature they all share a C-terminal EA1-box, short P-and A-boxes as well as N-terminal located signal sequences [5]. We will present a molecular and cellular survey of several EA1-box containing proteins. While EA1-box peptides enter the secretory pathway, larger EA1-box proteins locate to the nucleus. Particularly, we will present ZmEAL2 (*Zea mays* EA1-like 2) that is very closely related to ZmEA1. *In silico* analysis shows a strong expression of ZmEAL2 in the embryo at later stages providing a hint that the peptide may play a role in the development of embryonic organs.

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P115

Ectopic expression of *Zmm19* gene causes the maize mutant Tunicate1 in a dosage-dependent manner

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Pod corn was once regarded as the ancestral form of cultivated corn due to its characteristic of enclosing the kernel in glumes and was prized by pre-columbian peoples for its magical properties. The Tunicate1 (Tu1) mutant of maize is a naturally-occurring dominant mutation, resulting in a striking pod corn phenotype. In a dosage-dependent manner, Tu1 results in not only the elongation of outer glumes, but also sex determination and branching defects in tassel and ear. We cloned *Tu1* and found that it encodes the maize MADS-box gene *Zmm19* whose 5'UTR is interrupted by a novel *mutator-like* DNA transposon. Interestingly, our genetic mapping analysis suggests that the insertion of the *mutator-like* element is the cause of a 1.8Mb chromosomal inversion immediately upstream of *zmm19*. This inversion may have prevented fine-mapping of this candidate gene in previous studies (G. Thiessen and H. Saedler) because rearrangement prohibits recombination, and likely accompanied the origin of this ancient gene. Here we show that YFP and RFP-tagged Tu1 transgenic maize plants phenocopy Tu1. YFP and RFP-fused Tu1 proteins are co-localized to the nucleus in male and female reproductive organ initials, suggesting *Tu1* participates in specifying floral organ identity. Especially, YFP and RFP-fused Tu1 proteins are expressed in discrete cup-shaped domains at the base of spikelet pair meristems in young ear primordia. Genetic and molecular data indicate that misregulation of *Tu1* gives rise to pleiotropic pod corn phenotypes.

P116

FASCIATED EAR 4 encodes a transcription factor required for maize meristem size homeostasis

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Plant architecture is dictated by the precise control of meristematic activity. An imbalance in positive or negative maintenance signals can result in a fasciated meristem phenotype. *fea4* is a semi-dwarfed mutant with fasciated ears and tassel due to greatly enlarged inflorescence meristems. Vegetative meristems are also increased in size, accounting for the reduced stature, wider leaves, and other vegetative phase defects of the mutant.

We mapped *fea4* to a 2.7 Mbp region of chromosome 6 containing approximately 30 genes. A transcription factor in this interval contained an EMS-induced early stop codon in the reference allele. A second allele, originally identified as a modifier of *ramosa2*, also contained a premature stop codon, confirming the identity of the gene.

We have constructed a YFP-FEA4 translational fusion under the control of the native promoter in order to assay the expression of the protein, and are also carrying out *in situ* hybridization. We are profiling transcriptional changes in the mutant relative to wildtype by RNA-seq. Genetic analysis suggests that FEA4 functions in parallel to the FEA2-TD1 (CLAVATA) pathway, suggesting that it defines a novel pathway in meristem size regulation.

Funding acknowledgement: National Science Foundation (NSF), DuPont Crop Genetics, NSERC

P117

Genetic Analysis of Maize Leaf Development: Characterizing *Hoja loca*

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Plant leaves are generated from the shoot apical meristem (SAM), a group of totipotent cells at the shoot apex. Control of organogenesis requires the coordinated action of plant hormones and the downregulation of *knox* genes required for meristem maintenance. Mutations affecting the auxin signaling pathway often produce mutant phenotypes with defects in leaf organogenesis and patterning. We have identified a novel developmental mutation in *Zea mays* called *Hoja loca*, a dominant mutation exhibiting defects in both leaf and inflorescence initiation and development. Total leaf number in mutant pants is reduced to approximately 5 leaves, many of which lack midribs and often have fused margins to form a tube leaf. Initiating organ primordia often take on a ring-like appearance that can completely envelop the SAM. Mutant meristems appear enlarged indicating that leaves often fail to initiate, which is consistent with later internode elongation that clearly shows the absence of leaves at many nodes. Immunolocalization shows that *knotted* is downregulated as expected in initiating primordia in mutant SAMs. Confocal imaging of *Hoja loca* crossed with the PIN1a:YFP reporter line shows dramatic misexpression of the auxin efflux transporter PIN1a in mutant SAMs. We are in the process of mapping and cloning the mutant locus, which lies along the long arm of Chromosome 8. Future studies seek to elucidate *Hoja loca's* effect on auxin signaling and its function in organogenesis.

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

P118

Genome-wide expression map of functional domains within the shoot apex

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The shoot apical meristem (SAM) is responsible for the production of all aerial parts of the plant. The SAM contains a population of stem cells at the tip that divide to maintain the meristem and to produce new cells for lateral organ formation. The gene regulatory networks controlling meristem indeterminacy and organogenesis remain largely obscure. To gain insight into the gene networks involved in these distinct aspects of meristem function, we generated a high-resolution gene expression map for the shoot apex. Functional domains of the meristem were isolated by laser microdissection and analyzed by RNA deep sequencing. These tissue samples include: the whole meristem; the stem cell containing SAM tip; the newly initiating leaf (P0); the epidermal layer of the SAM (L1); the subepidermal region (L2) and vasculature within the stem. Because gene networks controlling meristem maintenance and leaf development are largely interconnected, we further compared expression profiles in an ontogenic series of leaf primordia, P1, P2, and P3, as well as between the adaxial and abaxial sides of developing leaves. We identified 2942 genes that are differentially expressed between the stem cell containing SAM tip and the P0, and 1282 genes were found to be differentially regulated between the L1 and the L2. Using unsupervised k-means clustering, we were able to identify genes that specifically mark the meristem, its stem cell domain, the P0 or developing leaf primordia. This comprehensive data set allows us to precisely predict genes involved in meristem maintenance, leaf initiation, and/or leaf patterning, and to assess the distinct contributions of the L1 and L2 to these processes. *In situ* hybridization experiments are ongoing to verify the predicted tissue-specificity of selected genes. Finally, this rich resource will be used to guide functional genomics experiments to address the role of new candidate genes in meristem function.

Funding acknowledgement: National Science Foundation (NSF)

P119

Genomics of the Maternal to Zygotic Transition in Rice

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Fertilization in plants results in a single cell totipotent zygote which gives rise to the entire adult plant. The growth of the zygote and the early embryo is initially dependent upon maternal factors provided by the egg cell, and later becomes independent of these maternal factors and relies only upon embryonically synthesized RNAs and proteins. The transition from reliance on maternal gene products to independent growth controlled by embryonic genes and gene products is called the Maternal to Zygotic transition (MZT). During this transition, extensive changes occur to the zygotic genome: the DNA in the chromosomes is re-packaged, genes are activated in the zygotic nucleus, and the maternally-supplied RNA is removed. Due to the small size and inaccessibility of the zygote, very little is currently known about this fundamental transition in plants. Using rice zygotes collected at specific time points after fertilization, we are studying the changes in gene expression as the zygote progresses from fertilized egg to the first embryonic division. Preliminary RT-PCR data demonstrates the loss of several maternal transcripts while other genes appear to be activated during these time points, implying that the zygotic transition is initiated as early as the first cell cycle of the zygote. Future experiments will include a complete transcriptome analysis of specific stages to gain insights into gene networks that underlie the zygotic transition, as well as investigate possible parent-of-origin effects during early zygotic development.

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P120

Global transcriptome analysis of two *ameiotic1* alleles in maize anthers: defining steps in meiotic entry and progression through prophase I

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Developmental cues to start meiosis occur late in plants. *Ameiotic1* (*Am1*) encodes a plant-specific nuclear protein (AM1) required for meiotic entry and progression through early prophase I. Pollen mother cells (PMCs) remain mitotic in most *am1* mutants including *am1-489*, while *am1-praI* permits meiotic entry but PMCs arrest at the leptotene/zygotene (L/Z) transition, defining the roles of AM1 protein in two distinct steps of meiosis. To gain more insights into the roles of AM1 in the transcriptional pre-meiotic and meiotic programs, we report here an in depth analysis of gene expression alterations in carefully staged anthers at 1 mm (meiotic entry) and 1.5 mm (L/Z) caused by each of these *am1* alleles. 1.0 mm and 1.5 mm anthers of *am1-489* and *am1-praI* were profiled in comparison to fertile siblings on Agilent® 4 × 44 K microarrays. Both *am1-489* and *am1-praI* anthers are cytologically normal at 1.0 mm and show moderate transcriptome alterations. At the 1.5-mm stage both mutants are aberrant cytologically, and show more drastic transcriptome changes. There are substantially more absolute On/Off and twice as many differentially expressed genes (sterile versus fertile) in *am1-489* than in *am1-praI*. At 1.5 mm a total of 4,418 genes are up- or down-regulated in either *am1-489* or *am1-praI* anthers. These are predominantly stage-specific transcripts. Many putative meiosis-related genes were found among them including a small subset of allele-specific, mis-regulated genes specific to the PMCs. Nearly 60% of transcriptome changes in the set of transcripts mis-regulated in both mutants (N = 530) are enriched in PMCs, and only 1% are enriched in the tapetal cell transcriptome. All array data reported herein will be deposited and accessible at MaizeGDB http://www.maizegdb.org/. Our analysis of anther transcriptome modulations by two distinct *am1* alleles, *am1-489* and *am1-praI*, redefines the role of AM1 as a modulator of expression of a subset of meiotic genes, important for meiotic progression and provided stage-specific insights into the genetic networks associated with meiotic entry and early prophase I progression

Funding acknowledgement: National Science Foundation (NSF)

P121

Histological and Molecular Characterization of Maize Mutant *rld*5409*

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In maize, the *rolled leaf1* (*rld1*) and *rolled leaf2* (*rld2*) genes encode HD-ZIPIII proteins that accumulate on the adaxial side of developing maize leaves. *rld1* and *rld2* are involved in establishing abaxial/adaxial leaf polarity and in regulating *yabby* gene expression. The expression domain of *rld1* and *rld2* is controlled by *microRNA166* (*miR166*), which targets *HD-ZIPIII* transcripts for degradation and thereby limits their expression domain to cells on the adaxial side of the leaf. Misregulation of *rld1* results in mutant plants that exhibit an upward rolling of leaves along their margin, as well as ectopic ligules on the abaxial leaf surface. A new, recessive *rld*-like mutant, designated *rld*5409*, displays a similar phenotype, which first becomes evident on transition stage leaves. We have begun characterizing this mutant. Transition and adult leaves were examined using various histological techniques. We observed a loss of leaf hairs and bulliform cells on the adaxial surface of the mutant leaf as well as altered stomatal patterning on both adaxial and abaxial surfaces. Vascular tissue polarity appeared normal. These observations suggest that the *rld*5409* mutant has some adaxial/abaxial patterning defects. Expression of genes in the *miR166/HD-ZIPIII* pathway was performed on *rld*5409* tissue using RT-PCR and compared to wild-type tissue. For these studies cDNA was prepared from leaf tissue taken at two stages of leaf development (transition and adult), as well as from the developing female inflorescence. Expression of *rld1*, *yabby* genes and *miR166* precursor were found to be altered in *rld*5409* tissue as compared to wild-type, indicating that *miR166/HD-ZIPIII* pathway is not functioning properly in this mutant.

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P122

Identification and characterization of *indeterminate gametophyte1 (ig1)* interactions in female gametophyte development

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The *indeterminate gametophyte1 (ig1)* gene of maize *(Zea mays)* restricts the proliferative phase of female gametophyte development. *ig1* mutant female gametophytes undergo extra free nuclear divisions leading to a variety of embryo sac abnormalities, including extra egg cells, extra polar nuclei, and extra synergids. *ig1* encodes a LATERAL ORGAN BOUNDARIES domain protein with high similarity to ASYMMETRIC LEAVES2 of Arabidopsis thaliana. To understand the mechanism by which *ig1* regulates differentiation in the embryo sac, we have performed experiments to identify interacting proteins and downstream genes. Analysis of IG1 protein reveals it acts as a transcriptional activator in yeast. An ovule-specific yeast two-hybrid library was screened with the IG1 LOB domain as bait to identify protein partners important for embryo sac functions of IG1. Genes acting downstream of *ig1* in embryo sac development are being identified using RNA-seq analysis. Transcript abundance in *ig1* mutant embryo sacs and ovules is being compared to wild-type to identify genes misexpressed specifically in mutant embryo sacs. We expect the combination of transcriptome analysis and protein interaction screens will provide insights into the development of female gametophyte in plants.

Funding acknowledgement: National Science Foundation (NSF)

P123

Identifying Proteins that Interact with DCD1, a PP2A Phosphatase Regulatory Subunit Needed for Cell Division Plane Orientation in Maize

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During plant cell division, a cortical ring of cytoskeletal filaments called the preprophase band (PPB) establishes the plane of division. The PPB disappears as the mitotic spindle forms. As it disappears, its zone of localization becomes the cortical division site (CDS). The phragmoplast is a structure specific to plant cells that builds the new cell wall during cytokinesis. The phragmoplast expands towards the CDS and separates the two daughter cells. DCD1 and ADD1, closely related B" regulatory subunits of the PP2A phosphatase complex in maize, play a role in the PPB establishment. Plants that lack DCD1 and ADD1 fail to make PPBs disrupting all cell divisions. It has been shown that ADD1 and DCD1 co-localize with the PPB and CDS during metaphase, but not during telophase/cytokinesis. Thus, DCD1 and ADD1 are involved in PPB formation and CDS establishment. Our goal is to identify proteins that interact with DCD1 in order to understand how DCD1 and ADD1 regulate these processes.
To identify DCD1 interacting proteins, a Matchmaker gold yeast two-hybrid screen will be performed by fusing DCD1 to the GAL4 transcription factor binding domain (BD) and screening it against a library of maize proteins fused to the GAL4 activation domain (AD). When bait and library fusion proteins interact in the yeast two-hybrid system, the DNA-BD and AD are brought into proximity and activate transcription of four independent reporter genes. Positive interactors will be re-tested, isolated and sequenced to determine the identity of the interacting protein. Finally, the interaction will be confirmed via in vivo. We will report our current progress.

Funding acknowledgement: University of North Texas

P124

Influence of sodium chloride on maize callusogenesis

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The resistance to salinity in maize is the important agricultural characteristics. Sodium chloride is one of the basic components of saline soils. The application of in vitro tissue culture method for creation of genotypes resistant to chloride salinity is a perspective direction of biotechnology investigations. We investigated the callusogenesis under salinity in maize inbred DK212 which belonged to subplasm Oh43 of Lancaster germplasm. Immature zygotic embryos, 1-1.5 mm in length, were harvested on the 11th day after self-pollination from field donor plants and cultivated scutellum up on modified inductive N6 medium (medium Ind) in the darkness. Calli derived in 60 days in culture were transplanted to next modified MS media: control medium (C), control medium + 0.1 Mol/l sodium chloride (1C) and control medium + 0.5 Mol/l sodium chloride (2C) and cultivated at the light. Medium Ind as compared to media C, 1C and 2C had contained less sucrose for delaying the osmotic load because the osmotic pressure was created later with sodium chloride. 50.00 and 20.45 percent of green calli were observed respectively on media C and 1C at the 30th day of cultivation at the light. Green coloration was disappearing through 30 days after its appearance. Visually changes in calli sizes depending on sodium chloride contents were noted only to the 60th day of cultivation at the light. For estimation of callus cultivation specific diameters and specific raw weights of calli were measured. Specific dry weights and humidity of calli were determined at the end cultivation. Maximal value of specific raw weight of calli was observed on the 120th day of cultivation at the light in all variants of the experiment. The specific diameter values achieved to the 150th day were preserved to the 210th day of cultivation at the light. The tendency of the decrease of specific dry callus weight and humidity under the increase of sodium chloride concentration was observed. Thus, inhibitory effect of sodium chloride on callus growth depends on the age of culture and the sodium chloride concentration. 0.5 M sodium chloride is non-lethal concentration for tissue culture of maize inbred DK212 and can be further examined as a selective one.

P125

Investigating how cell fate acquisition is regulated during maize leaf development

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We aim to understand how novel cell types are differentiated along the length of the leaf. A mature maize leaf exhibits four distinct cell types: blade, auricle, ligule and sheath. Ligule cells differentiate at the boundary between distal blade and proximal sheath cells; auricle cells arise on the distal side of this boundary after ligule initiation. My work aims to investigate interactions between the few factors known to play a role in this process as well as to uncover novel regulators controlling leaf cell fate acquisition. Specifically, I aim to elucidate potential genetic and physical interactions between a set of transcription factors (*Liguleless1, Liguleless2* and *Wavy auricle in blade*) and a pair of receptor like kinases (*liguleless narrow* and *liguleless narrow-like*) implicated in the control of this developmental process. Complementary goals include determining the subcellular localization of these factors using immunofluorescence and tagging approaches. Additionally, we are using previously characterized fluorescent markers to investigate the mutant phenotypes associated with lesions in these genes, looking for altered expression patterns compared to wild type. Lastly, I have begun characterizing novel alleles of *liguleless1* for use in future genetic screens with the aim of identifying novel factors in the *lg1* regulatory pathway.

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P126

Is calcium needed for the localization and functioning of FASS/TONNEAU2 in *Arabidopsis thaliana*?

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Plants possess unique features in many aspects of development. One of these is that cell wall placement during cytokinesis is determined by the position of the preprophase band (PPB) and the subsequent expansion of the phragmoplast, which deposits the new cell wall. During phragmoplast expansion, the phragmoplast tracks to the cortical division site, which was delineated by the PPB. Thus the position of the PPB determines the orientation of the division plane. *Arabidopsis thaliana* FASS/TONNEAU2 is required for preprophase band (PPB) formation and has been shown to interact with a type A subunit of the PP2A phosphatase in the yeast two-hybrid system. In Arabidopsis *fass* mutants, abnormalities of the cortical microtubule cytoskeleton, such as disorganization of the interphase microtubule array and lack of PPB formation before mitosis markedly affects cell shape and arrangement as well as overall plant morphology. Loss of *dcd1/add1*, the maize *fass* homologues gives rise to a similar phenotype in *Zea mays*. FASS has a calmodulin (CaM) and two calcium-binding sites, which raises the question: “Does calcium binding contribute to the localization and function of FASS at the PPB?” To address this question, the Ca2+ binding sites will be mutated on constructs containing the FASS gene fused to GFP. These constructs will be transformed into the *fass* mutants and the localization of FASS fusion protein and whether the construct is capable of rescuing the mutant phenotype will be observed. The results will help us determine if Ca2+ is a key player in FASS/TONNEAU2 localization and function.

Funding acknowledgement: University of North Texas

P127

Lesion Target Spots: a Model for Cell Death Signalling

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As a group, the disease lesion (*Les*) mimics are the most frequently occurring dominant mutants from EMS mutagenesis in maize. From the screening of over 50,000 M1 plants for all variations of the lesion phenotype, we have been able to identify 51 separate dominant cases. A much smaller, though probably comparable number of recessive *les* mutants, have also been seen.

The mutants range widely in expression, but have the common phenotype of leaf lesions that are strikingly similar to those caused by various leaf blight diseases. In all cases tested, the phenotypes have been found to occur in the absence of a pathogen. The lesions are initiated by sunlight and certain chemicals, and can be chlorotic, necrotic, or sequentially both. The lesions of different mutants vary in size, shape, color, frequency, distribution, time of onset, position, rate of expansion, sharpness of their boundaries, *etc*. In some mutants, the lesions expand to cover the leaf, resulting in its senescence. It appears that particular cells on the leaf surface are, at specific developmental stages and within a certain temperature range, highly susceptible to damage by sunlight.

We hypothesize that there are two signals involved. The first arises from the dissolution of the cell membrane, which releases highly active cell contents that cause lethal damage to neighboring cells. This damage spreads continuously outward, forming a necrotic lesion that stops growing only when conditions change. The second signal is revealed by the "Target Spot" oscillatory phenotype --- a central spot of dead tissue surrounded by alternating rings of healthy and dead tissue. This phenotype suggests there is signaling between dead and living cells, across living tissue, that causes lesion formation. This signal proceeds more rapidly than the first, through several ranks of normal cells without damaging them, during a diurnal cycle of conditions that do not favor lesion formation.

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P128

Maize Cell Genomics: Developing a two component transactivation system

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Analysis of maize at a systems level is now possible with advances in functional genomics tools. New methods are still needed to interrogate protein function experimentally and at the subcellular level. To this end, we have generated over 100 stable, natively expressed, fluorescent protein (FP) fusion lines that mark all common subcellular compartments. These lines thus far have provided new views of maize subcellular organization, identified novel promoters, and provided a useful molecular resource for the maize community http://maize.jcvi.org/cellgenomics/index.shtml. We are currently developing a 2-component transactivation expression system to drive cell, tissue and organ-specific expression. Selected promoters are used to activate expression of the maize-codon optimized LhG4 transcription factor, which in turn will transactivate genes of interest driven by the pOp promoter. Drivers currently being constructed include meristem-specific promoters and upstream regulatory regions from *WUSCHEL*, and *RAMOSA3* and leaf-specific promoters from *LIGULELESS1*, and *WARTY2*. Others currently in the pipeline include a *YABBY* promoter, *MALATE* DEHYDROGENASE, *ZmOCL6* and *ZmRAB2A*. Once experimentally tested and confirmed, our project will deliver to the research community stable transformants of 50 promoter/driver lines, as well as 20 new FP tagged lines and new methods for live cell imaging of meristems and leaves. Experimental results, images and metadata will be available and processed using Bisque, an imaging database management and analysis system and accessible to the maize community via our project website. We welcome new requests for genes to be tagged or suggestions of tissue specific promoters for making driver constructs from the maize community through our website. Contact Dave Jackson or Anne Sylvester for more information.

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P129

Mapping and phenotypic analysis of *Vestigial glume1*

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Vestigial glume1 (*Vg1*) is maize mutant with shortened glumes and underdevelopment of all floral organs, first described in a population received from the Belgian Congo (1931). *Vg1* is semi-dominant, and homozygous *Vg1/Vg1* have shortened internodes and wide dark-green leaves. *Vg1* in some genetic backgrounds are male sterile and show a tassel phenotype similar to drought stressed wild types. *Vg1* phenotype is not apparent until the later stages of development. It is known to be located on chromosome 1, Here we report the high resolution mapping and progress towards positional cloning of the *Vg1* mutant. We are also investigating leaf and tassel morphological effects of *Vg1* in a developmental series for both homozygous *Vg1/Vg1* and heterozygous *Vg1/+* plants in order to determine onset of *Vg1* expression as well dosage effects.

P130

Metabolic characterization during the seed ontogeny of *Brachypodium distachyon* (L.) P. Beav. (Poaceae).

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Brachypodium distachyon (L.) P. Beauv.] is a member of the Pooideae subfamily in the Poaceae, which is a wild annual grass native to the Mediterranean and Middle East region. This species is emerging as a new plant model for temperate grasses and can be used to study comparative evolution, reproductive biology, and seed metabolism. Any insights gained in *Brachypodium* might be useful to understand and enhance the production of its close relatives oat, barley, and wheat, which together with other grass crops (maize and rice) are the most important sources of human nutrition. Recently, the first comparative reports on protein storage and endosperm development with other domesticated cereals have been published. In order to gain knowledge regarding the biochemical composition of *Brachypodium* seed and the dynamical changes of carbon metabolism during development, we sampled various stages from a non-fertilized flower to a physiologically mature seed. We performed metabolic profiling analysis on the embryo and the endosperm separately. The quantitative changes during caryopses development of soluble sugars including glucose, fructose and sucrose, total amino acids, and starch were determined. Reserve carbon in *Brachypodium* seeds is stored mainly as starch and sucrose, which varied in concentration and proportion according the developmental stage and the tissue evaluated. Sucrose content in the embryo is higher compared to the endosperm. During the ontogeny, embryo sucrose levels reached the highest values (200 umol/g DW) at the final stage of maturation before seed dry. In contrast, the endosperm sucrose levels reached the highest values just after fertilization (75 umol/g DW), and then, gradually decreased during seed ripening (0.5 umol/g DW). In the mature non-pollinated embryo sac, low concentrations of starch were observed before fertilization (15 umol/g DW). Embryo starch increased as the seed became mature showing the highest values before drying (200 umol/g DW). In the endosperm ocurred just the opposite with high values (450 umol/g DW) before pollination and fertilization. Unexpectedly, endosperm starch went continuously down till maturation (20 umol/g DW). We conclude that there are important differences of seed storage metabolism across different grass species and more research should be performed in comparative metabolic studies in seeds of *Brachypodium* and other plant species.

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P131

Molecular isolation of the *gametophyte factor1* (*ga1*) locus from *Zea mays*

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Very little is known about the molecular determinants that mediate pollen-pistil communication in an outcrossing, self compatible species. *Zea mays* is a self compatible species whose varieties are generally cross compatible, although loci that govern cross incompatibility have been identified. The best genetically described of these loci is the *gametophyte factor1* (*ga1*) locus which controls non-reciprocal (unidirectional) cross incompatibility. This locus was first identified in certain popcorn varieties that could only be successfully pollinated with their own pollen and are now known to carry the dominate allele *Ga1*. Pollen from dent, sweet or flint varieties all carry *ga1* alleles and either do not pollinate or are inefficient at pollinating *Ga1* silks. Work by Kermicle and Evans indicates *Ga1* silks do not actively reject *ga1* pollen but *ga1* pollen is incongruent with *Ga1* silks and is thus outcompeted by *Ga1* pollen. Two dominant *Ga1* alleles have been described; the *Ga1-s* strong allele and a weaker allele named *Ga1-m* for *Ga1-"male"*. Pollen carrying *Ga1-m* is accepted by *Ga1-s* silks but *Ga1-m* silks do not reject *ga1* pollen. Thus, *Ga1-m* only has the male function but not the female function of *Ga1-s* cross incompatibility. In order to understand the molecular mechanisms underlying cross incompatibility, we used map-based cloning to delineate the *ga1* locus to a region of 550 kb that contains six predicted gene models. Through sequence analysis of these predicted gene models in the *ga1*, *Ga1-s* and *Ga1-m* genotypes, we identified a candidate gene with significant sequence differences that have the potential to produce changes in both the expression and structure of the candidate *ga1* gene in these three genotypes. In our poster, we will present our results on the analysis of the candidate genes and a model predicting how these sequence changes could change gene function to mediate unilateral cross incompatibility.

P132

Mutations in an AP2-Like Transcription Factor Affect Internode Length and Leaf Shape in Maize

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Plant height is an important agronomic trait that affects yield and tolerance to certain abiotic stresses. Understanding the genetic control of plant height is important for elucidating the regulation of maize development and has practical implications for trait improvement in plant breeding. In this study, two independent, semi-dwarf maize EMS mutants, referred to as dwarf & irregular leaf (dil), were isolated and confirmed to be allelic. In comparison to wild type plants, the mutant plants have shorter internodes, shorter, wider and wrinkled leaves, as well as smaller leaf angles. Cytological analysis indicated that the leaf epidermal cells and internode parenchyma cells are irregular in shape and are arranged in a more random fashion, and the mutants have disrupted leaf epidermal patterning. In addition, parenchyma cells in the dil mutants are significantly smaller than those in wild type plants. The dil mutation was mapped on the long arm of chromosome 6 and a candidate gene, annotated as an AP2-like transcription factor, was identified through positional cloning. Point mutations near exon-intron junctions were identified in both dil alleles, resulting in mis-spliced variants.

P133

NARROW SHEATH and auxin function in initiation of the sheathing leaf base in maize

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Leaves are initiated from the periphery of the shoot apical meristem (SAM) via the recruitment of leaf founder cells, a process that requires auxin transport. Unlike eudicots, in which the recruitment of founder cells is localized to near the site of leaf insertion at the node, monocot leaves are recruited from the entire circumference of the SAM, a process that gives rise to the sheathing leaf bases observed in maize leaves. Plants that have mutations in the duplicate *wuschel*-like transcription factors *ns1* and *ns2* are characterized by narrow leaves that lack a lateral domain that includes the sheathing leaf base. Previous investigations show that NS functions from two lateral foci to send a non cell-autonomous signal to recruit founder cells for leaf lateral domains. To further elucidate the role of NS, we conducted a transcriptomic analysis of laser-microdissected *ns* and wild-type SAMs, and investigated the dynamics of auxin transport and perception in maize leaf initiation. Localization of PIN1 auxin transport proteins indicates that auxin is transported basipetally in the outer (L1) cell layer to the site of midrib initiation, and from the inner cell layers toward the L1 at the leaf margins. Intriguingly, expression of the DR5 auxin reporter was detected at the midrib side of the initiating primordium, but not in the leaf margin domain, suggesting that auxin perception or response differs in the midrib and margin domains. We show that ns expression is induced in response to auxin and repressed by inhibition of auxin transport. Transcriptomic data indicate that auxin response and cytokinin signaling pathways function downstream of NS. We present *in situ* hybridization analyses of candidate genes identified in this analysis. Our results support a model in which NS functions downstream of auxin transport and mediates auxin and cytokinin signaling pathways that are required to form the sheathing bases of monocot leaves.

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P134

Natural Variation in Maize Shoot Apical Meristem Architecture and its Genetic Regulation

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The shoot apical meristem (SAM) contains a pool of undifferentiated cells, and generates all of the above-ground organs of the plant. Groups of cells branch off from the meristem to initiate leaves, yet the SAM preserves its pool of meristematic cells. In addition, overall shoot architecture is determined in part by regulatory mechanisms acting in the SAM. Here, we investigated the genetic control of maize SAM architecture and whole plant traits. We examined the extent of natural variation for SAM architecture in the nested association mapping (NAM) parents, we mapped quantitative trait loci for SAM architecture and whole plant traits in a subset of the intermated B73xMo17 recombinant inbred line (IBMRIL) population, we mapped expression-QTL (eQTL) from the shoot apex in the same IBMRIL individuals, and we identified coincident eQTL with phenotypic QTL. Our results showed that in the NAM parental lines, B73 and Mo17 represent the majority of natural variation in maize for SAM height. QTL analysis on SAM height, width, midpoint-width, and arc length to the cleft of the first incipient leaf primordia (P1), cell counts along the arc length in the L1, and P1 height identified 6-9 QTL per trait, each contributing between 5-33% of the trait variation. The relationship between SAM architecture and plant morphology showed that two-thirds of QTL associated with one or more SAM traits were found to be coincident with other morphological QTL. In some cases, these coincident QTL reflected correlations among these traits. eQTL mapping identified 11,504 cis and 19,270 trans eQTL, as well as 96 trans eQTL hotspots. Coincidence of the two mapping analyses (phenotypic and expression) and possible methods to clone QTL controlling SAM architecture will be discussed as a means of identification of potential master regulators of maize morphology.

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P135

necrotic upper tips1 is a sheath specific transcription factor that promotes proper water movement during the floral phase

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During the floral transition special mechanisms exist to ensure sufficient water transport to the growing floral organs. If water is limiting during the floral phase it can result in severe developmental defects such as leaf wilting, tassel browning, and sterility, which together comprise a condition known as “tassel blasting.” In order to understand the genetic mechanisms underlying this process, we have identified a mutant from an Activator (Ac) transposon screen, necrotic upper tips1 (nut1), which mimics tassel blasting. The nut1 mutant phenotype is evident only after the floral transition, while early vegetative development is normal. nut1 upper stems and leaves have difficulty moving water as shown by dye uptake and movement assays. Plastic sections and TEM of nut1 vasculature show defects in xylem vessel integrity, which could provide the basis for its mutant phenotype. The nut1 mutant is caused by an Ac insertion into the transcription unit of a NAM-like transcription factor. Wildtype revertants were isolated with restored open reading frames caused by Ac excision, thus proving that loss of this transcription factor is responsible for the nut1 phenotype. Immunolocalization experiments using a NUT1 specific antibody showed that the protein localizes to developing vasculature within the sheath portion of leaves. These results show that unique transcription factors function within specific leaf compartments to maintain xylem vessel integrity during periods of high water movement.

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P136

New *tassel-less* mutants with defects in vegetative and reproductive development in maize

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Tassel-less mutants are characterized by an absent or reduced tassel and ear and vegetative defects. At least eight *tls* loci have been identified and mapped and two have been cloned. Here we present the mapping of four *tls* mutants and present the mapping and characterization of the *tls5* mutant. *tls5* mutants have defects in both vegetative and reproductive development. *tls5* tassels have a decrease or complete lack of reproductive organs. Tassel branch and spikelet quantification demonstrates that the mutants produce fewer branches and spikelet pairs (92.5% less), with more single spikelets (85% more) than normal siblings. *tls5* mutants produce narrower leaves and are shorter than normal due to the production of shorter internodes. Through BSA mapping, the mutant has been mapped to bins 9.01-9.02 on chromosome 9. Fine mapping is ongoing to identifying the *tls5* gene. We propose that the *tls5* gene plays a fundamental role in vegetative and reproductive development in maize.

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P137

Phenotypic Analysis: Pleiotropy

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Phenotype is the message we get that tells us that there has been a change in a gene controlling some biological function. What we learn from the phenotype actually depends on our ability to recognize and properly interpret what we observe. In the simplest case it is the presence or absence of a measurable product (anthocyanin) or structure (ligule). In actual fact there are many functions involved in production of a particular product or activity and these are all a part of a complicated choreography leading to a certain display. Maize is an exceptionally well suited organism for demonstrating this point. We have produced and have access to a unique large collection of several thousand mutants induced by EMS, by transposons, by radiation and of spontaneous unknown origin. All are currently kept in an extensive "Mutants Data Base" with high resolution photo images and pertinent information. A duplicate copy of most of these files with images and data is also kept at MaizeGDB. This presentation will use photo images of two mutant gene systems to demonstrate the intricate relationships involved in going to and from a gene and a recognizable phenotype.

(1) **clf1** (**dek1**); EMS induced recessive mutant; **Ac Ds-1S2,4 Clf1** transposon analysis.
(2) **PgD**; EMS induced dominant chimera case.

In reviewing this material it is quite clear that there are many important genetically controlled activities that regulate expression of a phenotype without being in the biochemical pathway that leads to the observed phenotype.

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P138

Phenotypic characterization of vegetative development in the maize *fuzzy tassel* mutant

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microRNAs (miRNAS) are short, non-coding RNAs that regulate gene expression post-transcriptionally in all multicellular organisms. In plants, miRNAs are processed from dsRNA hairpin precursors by the RNase III-containing enzyme, DICER-LIKE1 (DCL1). We have isolated the maize *fuzzy tassel (fzt)* mutant, which contains a missense mutation in the first RNase III domain of DCL1. *fzt* mutants have striking vegetative and reproductive phenotypes, implicating miRNAs in a broad range of developmental processes. This project focuses specifically on the role of miRNAs during vegetative development and here we report characterization of the vegetative *fzt* phenotype. *fzt* mutant plants are shorter in stature with shorter and narrower leaves than normal siblings. miRNAs have well established roles in plant development, including establishing leaf polarity and phase change, however both these processes appear grossly normal in *fzt* mutants. To ask if *fzt* plants have subtle leaf polarity defects, we examined epidermal cell types by scanning electron microscopy and vasculature polarity in hand sections. Indeed, we found that *fzt* leaves have adaxial-specific cell types on the abaxial surface and vascular organization suggests leaf polarity is perturbed. We also analyzed phase change in *fzt* mutant plants using Toluidine Blue O staining, which differentially stains juvenile and adult leaf waxes. *fzt* mutant plants transitioned to the adult phase ~ 1 leaf early, consistent with defects in miRNA levels. We are currently analyzing cell size and cell number in *fzt* and normal sibling leaves to determine why leaves are smaller in *fzt* mutants than normal siblings.

P139

PINs lost and PINs gained: Auxin-transport mediated patterning in the grasses

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Auxin transport mediated by the PINFORMED (PIN) family of efflux carriers helps create auxin gradients on which many developmental processes depend. Current models suggest that *Arabidopsis* PIN1 has two concurrent functions during leaf initiation: 1) concentrating auxin "up-the-gradient" to create local maxima in the meristem epidermis, and 2) transporting auxin "with-the-flux" away from the epidermal maxima and into the internal tissues. The resulting auxin gradient is required for leaf initiation and vein patterning. Here we identify an uncharacterized angiosperm PIN clade placed sister to PIN1, termed Sister-of-PIN1 (SoPIN1), that has likely been lost within the *Brassicas*, including in *Arabidopsis*, but remains in all other angiosperms sampled. Using live-cell imaging and immuno-localization we characterized the expression and localization of SoPIN1 members in both maize and *Brachypodium*. SoPIN1 was highest in the epidermis and was consistently oriented toward auxin maxima, suggesting that SoPIN1 functions in the "up-the-gradient" creation of auxin maxima. We also characterized the localization of the maize and *Brachypodium* PIN1a and PIN1b proteins, which are the result of ancient duplication event within the PIN1 clade. PIN1a and PIN1b localization, largely absent from the epidermis and oriented rootward in the internal tissues, suggests these PIN proteins likely transport auxin "with-the-flux" after maxima formation during the canalization of leaf and stem veins. These data support the functional division of PIN proteins into "up-the-gradient" and "with-the-flux" modes. In addition, the loss of SoPIN1 within the *Brassicas* suggests that PIN1 in this group may be unique amongst the angiosperms in its ability to dynamically switch between these two functional modes. Finally, we hypothesize that the PIN1a/PIN1b duplication in the lineage leading to the grasses may relate to the novel morphological and anatomical characteristics found in monocot plants.

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

P140

Polar cell growth and speciation? A cellular and molecular perspective to understand the barrier to cross-pollination between maize and teosinte

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Even with the increasingly available genomic, genetic, cellular and molecular tools, mechanisms of how a crossing barrier forms between two populations that were previously interbreeding is still illusive. This study aims to understand the crossing barrier between maize and some strains of teosinte, which is governed by a single locus *tcb1* (teosinte cross barrier1). The *Tcb1-s* haplotype present in these strains encodes a female factor that blocks maize pollen (haplotype *tcb1*) and a male factor that overcomes that block. *In vivo* assays showed that maize pollen tubes are arrested in *Tcb1-s* silks. The first step in understanding this crossing barrier is to clone the *Tcb1-s* genes. Out of a mapping population of 16,451 chromosomes, the male factor *Tcb1-m* and female factor *Tcb1-f* have been separated by two recombination events. *Tcb1-m* and *Tcb1-f* are within an interval flanked by markers that are 43,691 base pairs apart on the B73 reference genome. Only one protein-coding gene and a portion of the promoter of a second gene are in this region in the maize B73 reference genome. To determine whether or not there are additional genes in the *Tcb1-s* haplotype that could encode *Tcb1-s* function, we have constructed a BAC library from plants of a *Tcb1-s* containing W22 subline. BACs that cover this region are being fished out and sequenced. Candidate genes will be cloned and transformed to maize to confirm their function. Identification of the male and female genes that produce a reproductive barrier will provide novel insights into the processes of speciation, polar cell growth and cell-cell communication. More broadly, this understanding may lead to novel applications for the control of cross-pollination and the restriction of gene flow.

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P141

PPR2263, a DYW-subgroup pentatricopeptide repeat protein, is required for mitochondrial *nad5* and *cob* transcript editing, mitochondrion biogenesis and maize growth

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RNA editing plays an important role in organelle gene expression in various organisms including flowering plants, changing the nucleotide information at precise sites. Here, we present evidence that the maize (*Zea mays*) nuclear gene *Pentatricopeptide repeat 2263* (*PPR2263*) encoding a DYW domain-containing PPR protein is required for RNA editing at the *nad5*-1550 and *cob*-908 sites in the mitochondrial *NADH Dehydrogenase5* (*nad5*) and *cytochrome b* (*cob*) transcripts, respectively. Its putative ortholog MITOCHONDRIAL EDITING FACTOR 29 (MEF29) fulfills the same role in *Arabidopsis thaliana*. Both the maize and the Arabidopsis protein show preferential localization to mitochondria but are also detected in chloroplasts. In maize the corresponding *ppr2263* mutation causes growth defects in kernels and seedlings. Embryo and endosperm growth are reduced leading to the production of small but viable kernels. Mutant plants have narrower and shorter leaves, exhibit a strong delay in flowering time and generally do not reach sexual maturity. Whereas mutant chloroplasts do not have major defects, mutant mitochondria lack complex III and are characterized by a compromised ultrastructure, increased transcript levels and the induction of alternative oxidase. The results suggest that mitochondrial RNA editing at the *cob*-908 site is necessary for mitochondrion biogenesis, cell division and plant growth in maize.

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P142

Shoot meristem allometric variation in the genus *Zea*: implications for adult plant traits

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Domesticated maize (*Zea mays mays*) has accumulated many traits not found in its wild relatives. Many traits that distinguish domesticated maize from its wild progenitors result from changes in shoot meristem activity. Because the entire plant shoot is derived from pluripotent stem cells found in the SAM, modifications to SAM allometry (size and shape) and function have important effects on adult plant agricultural characteristics. This study will exploit natural variation within the genus *Zea* in quantitative and qualitative genetic analyses of SAM allometry and its correlation to adult plant traits. 3D data from nano-scale computed tomography (nano-CT) is used to examine the SAM allometric space within *Zea*. SAM allometric space will be populated by nano-CT analysis of the five species of *Zea*, including four subspecies of *Zea mays* (domesticated maize *Zea mays mays* and the teosintes *Zea mays parviglumis*, *Zea mays mexicana*, and *Zea mays huehuetenangensis*) and four teosinte species (tetraploid annual *Zea perennis*, diploid annual *Zea diploperennis*, *Zea nicaraguensis* and *Zea luxurians*. To closely explore SAM allometric diversity in the most recent wild progenitor of domesticated maize, we will analyze twenty accessions of *parviglumis*. We are pairing nano-CT analysis with QTL mapping in bi-parental recombinant inbred populations of domesticated maize and its wild progenitor (*Zea mays mays* B73 x *Zea mays parviglumis*, BC2S3) to identify QTL contributing to variation in SAM allometric space in domesticated maize and its wild progenitor. At the same time, we are using laser microdissection/RNA seq of domesticated maize and its wild ancestor, *Zea mays parviglumis* to identify genes and genetic pathways that regulate SAM architectural variation in *Zea*. By taking advantage of high-throughput technologies already in place for the analysis of maize and its wild relatives, we hope to utilize maize SAM allometry as a predictive trait impacting agronomically important adult plant traits.

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P143

Sos2-tls Functions in Maize Reproductive Development

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Maize, and all other species in the Andropogoneae, produce spikelets (short branches that bear the florets) in pairs while other grasses, such as rice, wheat and barley produce spikelets singly. We have identified several mutants in maize, which produce single instead of paired spikelets indicating that these genes may be of importance in the evolution of the paired spikelet. A previously characterized semi-dominant mutant, *Suppressor of sessile spikelets1* (*Sos1*) produces fewer branches and spikelets due to defects in the size of the apical meristem and the production of single instead of paired spikelets. The dominant *Suppressor of sessile spikelets2* - *tasselless* (*Sos2-tls*) mutant also produces fewer branches and spikelets in the tassel and fewer kernels in the ear. *Sos2-tls* mutants often have an additional phenotype with no tassel and small, round ears, similar to *tassel-less* mutants, suggesting that the *sos2-tls* gene also plays a role in the development of the apical meristem. To determine the developmental basis of the defect, immature tassels from wild type and *Sos2-tls* mutant plants were dissected after approximately 4-5 weeks of growth, and viewed using Scanning Electron Microscopy (SEM). These experiments indicate that *Sos2-tls* mutants, like *Sos1*, produces single instead of paired spikelets, but in addition have defects in apical meristem determinacy. We have mapped *Sos2-tls* to the short arm of chromosome 10, and fine mapping of the gene is on-going. We propose that the *sos2-tls* gene plays a critical role in apical and axillary meristem development in maize.

P144

SWEET-based pathogen susceptibility: from sugar transport in Arabidopsis to pathogen resistance in the field.

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Sucrose is a major product of photosynthesis in plants and serves as the principal long-distance translocation compound for plant growth and development. We hypothesize that plants keep apoplasmic levels of sucrose low as a protective strategy against fungal and bacterial infections, by limitating efflux to sites inside the phloem tissue. SWEET sugar effluxers are responsible for providing sucrose to the SUT sucrose transporters for phloem loading. Pathogens hijack SWEETs efflux activity to redirect sugar efflux at the site of infection. In Arabidopsis leaves, the bacterium *P. syringae*, and fungi Powdery Mildew and Gray Mold induce a different set of SWEET mRNAs. In rice, OsSWEET11 and 14 are co-opted during infection by *X. oryzae*: pathovar-specific effectors secreted by this bacterium activate the transcription of the SWEET genes, while mutations in the effector binding sites in SWEET promoters lead to *X. oryzae* resistance. Our preliminary data show that also in maize a subset of SWEET genes is transcriptionally recruited during the fungus *U. maydis* infection. This pathogen annually causes hundreds millions of dollars in crop damage and food spoilage. Thus, this study will elucidate the SWEETs recruitment process in corn, possibly describing a model useful to engineer plant resistance.

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P145

Tasselless 1 (tls1) encodes a boron channel protein in maize

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Tasselless 1 (tls1) is a naturally occurring mutant first described almost two decades ago. Mutant plants have no or severely truncated tassels, reduced ear size, as well as leaf defects. Identifying the gene and mutation responsible for the mutant phenotype may enhance our understanding of the genetic and molecular regulation of tassel and ear development in maize. A NOD26-like gene has been identified as TLS1 by positional cloning. BAC sequencing from homozygous tls1 plants demonstrated the tls1 allele is a complete deletion of the NOD26-like gene. Mutator-insertional alleles (TUSC) were identified, and the phenotypes of these lines, as well as allelism tests with the tls1 reference allele, confirmed that the NOD26-like gene is TLS1. Further sequence analysis suggested that the NOD26-like protein is a boron channel. Functional characterization and potential applications of the NOD26-like protein will be presented.

P146

Testing the function of Brassinosteroid signaling in maize shoot architecture

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Brassinosteroids (BRs) are a type of phytohormone that has important roles in plant development such as sex determination, internode elongation, and leaf development. Research on Arabidopsis and rice revealed a general conservation in the key components of BR signaling. However, BR biosynthesis and signaling in maize are not well characterized. Understanding BR's role in maize, which is an important crop worldwide, might help manipulate this crop for production of biofuels, biomass, and grain yield. To understand some of the signaling components of BRs in maize, we took a transgenic approach to generate maize plants altered for different BR related gene functions. For this purpose, *BRI1*, which encodes the receptor of the BR signaling pathway, and *BIN2*, which encodes a negative regulator of the pathway, were targeted by an RNAi approach. Individuals from the transgenic events were recovered in greenhouse and planted in the field. BIN2RNAi plants in the greenhouse and the field showed reduced stature, elongated leaves with crenulated margins, and reduced anther production. In the field, BIN2RNAi plants also showed an unusual tassel morphology with geniculate (zig zag) tassel branches, suggesting there might be environmental effects. BRI1RNAi plants showed dwarf stature, and dark green, upright, and twisted leaves in both greenhouse and field conditions. Both BRI1RNAi and BIN2RNAi also showed altered auricle morphology.
In addition, we seek to develop marker genes and lines which can be used to report BR activity in maize. A BES1-YFP fusion shows BR-responsive nuclear accumulation. RNAseq was used to identify several genes that show dose-dependent expression. Such markers will facilitate characterizing transgenic lines, screening maize dwarf mutants potentially related to BRs, and analyzing BR activity in maize tissues.

Funding acknowledgement: Plant Sciences Institute at Iowa State University

P147

The convergent evolution of obligate B-class protein heterodimerization in the Poales

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Parallel molecular evolution has been described a handful of times, most often in response to herbicide treatment of plants, or antibiotic treatment of microbes. We have uncovered an intriguing instance of parallel evolution in protein-protein interaction between the B class MADS box genes, *APETALA3* (*AP3*) and *PISTILLATA* (*PI*). *AP3*-like and *PI*-like genes are transcription factors that control floral organogenesis in both monocots and the eudicots. In all eudicots investigated thus far, B class proteins bind DNA as obligate heterodimers: AP3-like proteins cannot bind DNA without PI-like proteins and vice-versa. Maize is distantly related to the eudicots, yet two of the homologs of AP3 and PI also bind DNA as obligate heterodimers. In a number of other monocots, including the grass relative *Joinvillea*, PI proteins can bind DNA as homodimers. These data imply that the AP3/PI obligate heterodimer relationship, rare in the large MADS box gene family, evolved in parallel in the order containing the grasses (the Poales) and in the lineage leading to the core eudicots. We are working towards a detailed dissection of the evolution and function of this interaction. Why did this very particular protein-protein interaction evolve at least twice? How and when in monocot history was PI- like protein homodimerization lost and when did obligate heterodimerization evolve? Here we present results of electrophoretic mobility shift experiments aimed at pinpointing when in the evolutionary history of the Poales PI-like homodimerization was lost, and when obligate AP3-like/PI-like heterodimerization was gained. Through domain swapping experiments and site-directed mutagenesis, we are also investigating which amino acid residues are responsible for PI-like homodimerization and AP3-like/PI-like heterodimerization.

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P148

The effect of genetic relatedness of endosperm to its compatriot embryo on maize seed development

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As one of two sexual products resulting from double fertilization in angiosperms, the endosperm nourishes its compatriot embryo during seed development and/or germination and ultimately dies. Based on the theory of kin cooperation, previous theoretical studies suggest that the coefficient of relatedness (genetic relatedness) of an endosperm to an embryo in either the same or other seeds might determine the amount of nutrients an endosperm will garner from the maternal sporophyte (plant) and ultimately make available for its compatriot embryo. In this study, we took advantage of the phenomenon of heterofertilization in maize to empirically test, for the first time, whether genetic relatedness between a diploid embryo and its triploid nourishing endosperm within a seed impacts the process of resource allocation between these two sexual reproductive entities. We used five genetically distinct maize inbred lines to perform two crossing experiments in the greenhouse and in the field. Dry mass of dissected embryos and endosperms of mature heterofertilized and adjacent homofertilized kernels were compared. Our results show that the fertilization type (homofertilization vs. heterofertilization) has a significant effect on embryo weight, but surprisingly, not on endosperm weight. Embryo weight in heterofertilized kernels is significantly less than that of embryos of homofertilized kernels, while there is no significant difference in endosperm weight between the two types of kernels. Our results are consistent with the prediction that the degree of genetic relatedness of an endosperm to its compatriot embryo affects seed development, and specifically the amount of nutrients reserved in an endosperm that are turned over to an embryo within a kernel. Thus, the endosperm of heterofertilized kernels appears to behave less cooperatively with respect to nutrient transfer toward its less-closely-related embryo compared to those in homofertilized kernels.

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P149

The *FASCIATED EAR3* gene encodes a receptor-related protein that regulates stem cell proliferation in maize in a pathway distinct from the known CLAVATA pathway.

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Stem cell proliferation in the shoot apical meristem is regulated by the CLAVATA (CLV)-WUSCHEL signal pathway. Originally, it seemed as though the *CLAVATA* pathway for regulation of meristem size was simple, but now it is evident that it is more complicated. In fact, the separate action of three major receptor complexes (CLV1-BAM1 (BARELY ANY MERISTEM 1), CLV2-CRN (CORYNE), and RPK2/TOAD2 (RECEPTOR-LIKE PROTEIN KINASE2/TOADTOOL 2)) is necessary for proper meristem size control in Arabidopsis. The CLAVATA pathway appears to be conserved in maize, since orthologs of *CLV1* and *CLV2*, *THICK TASSEL DWARF1* (*TD1*) and *FASCIATED EAR2* (*FEA2*) have similar fasciation phenotypes. Here we present an additional mutant with fasciated phenotypes, *fasciated ear 3* (*fea3*) derived from a radiation mutagenesis screen in Russia. *fea3* shows an over-proliferation of the inflorescence meristem. We cloned the *fea3* gene using map-based cloning, and the mutant results from the insertion of a partial retrotransposon into an exon of the *FEA3* locus. We confirmed this identity by isolation of new alleles of *fea3* from an EMS targeted mutagenesis. *FEA3* encodes a predicted receptor protein. Double mutants of *fea2* (a mutant in an LRR receptor like protein) and *fea3* have an additive fasciated phenotype in ear and tassel, indicating that they act in independent pathways. These results suggest that the function of *FEA3* as a predicted receptor protein is in a new pathway distinct from that of *FEA2*.

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P150

The interaction of *fasciated ear2* and *compact plant2* in controlling meristem size

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One important aspect of maize inflorescence architecture is the number of kernel rows per ear. This number, which also has a large impact on yield, is determined by the size of the inflorescence meristem (IM) located at the tip of developing ear primordia. Our analysis of the *thick-tassel dwarf1* and *fasciated ear2* mutants, the maize orthologues of the leucine-rich repeat proteins CLV1 and CLV2, has shown that IM-size is controlled via the CLAVATA signaling pathway. To further understand the regulation of IM-size in maize, we continued to characterize mutations affecting IM-size such as *compact plant2 (ct2)* . We discovered that *ct2* encodes the alpha subunit of a heterotrimeric GTP-binding protein, a membrane-associated protein involved in the transduction of extra cellular signals to induce specific cellular responses by activating downstream effectors. To identify CT2 interacting proteins we pursued immunochemistry approaches using transgenic maize lines expressing a translational fusion of CT2 and YFP under control of the endogenous CT2 promoter. We present co-immunoprecipitation results showing an interaction between FEA2 and CT2. This result is further supported by a phenotypic *ct2/fea2* double mutant analysis, where the double mutant phenotype resembles the *ct2* single mutant phenotype.
Taken together these data implicate a novel interaction between a leucine-rich repeat protein and the alpha subunit of a heterotrimeric GTP-binding protein, and therefore defines a new receptor module for G-protein mediated signaling in plants.

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P151

The Maize Pentatricopeptide Repeat 6 (MPPR6) protein facilitates 5' maturation and translation initiation of rps3 mRNA in maize mitochondria

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A collection of 300 maize kernel mutants was analyzed by a new cDNA-based forward genetic approach to identify genes involved in maize kernel development. In several mutant lines the mutation of a PPR protein gene, named mppr6, was identified as causal for the mutant phenotype. Pentatricopeptide repeat (PPR) proteins are members of one of the largest nucleus-encoded gene families and are involved in posttranscriptional processes within mitochondria and chloroplasts. A co-segregation analysis and the identification of a second mutant allele confirmed the correlation with the mutant phenotype. The analysis of the mutant phenotype revealed that the seed mutation coincides with abnormities in the transfer cell layer, retardation of embryo development and a considerable reduction of starch level. Since homozygous mutated seeds failed to germinate in soil, immature embryos at 18 DAP were recovered on MS- medium. In contrast to wild-type plants, rescued mppr6 mutants exhibited an obvious growth delay. T-DNA tagged Arabidopsis mutants of the putative orthologous gene in Arabidopsis showed a phenotype similar to that of the mppr6 mutation in maize. We were able to complement the appr6 mutation in Arabidopsis by the heterologous expression of the maize mppr6 gene. GFP/dsRed fusion studies confirmed a predicted subcellular localization of the PPR6 protein within mitochondria. To identify the target mtRNA associated with the MPPR6 protein a co-immunoprecipitation coupled with RT-PCR was carried out. This analysis revealed the ribosomal protein S3 (rps3) mRNA as potential target of MPPR6. Binding of MPPR6 to the 5'UTR of the rps3 mRNA was confirmed by electrophoretic mobility shift assays. The analysis of the rps3 mRNA in the mutants showed that there is no effect on RNA editing, -splicing and -stability, but that the RPS3 protein in the mutant is truncated at the N-terminus. Our results suggest that MPPR6 is involved in the 5'maturation and translational initiation of rps3 mRNA.

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P152

The mapping and cloning of the *discordia3* mutations

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Correctly oriented cell divisions are important during plant development since plant cells lack the ability to migrate during tissue and organ formation. In plants, cell wall placement is determined by the location of the preprophase band (PPB). Prior to mitosis, the PPB marks the future cell division site and disappears once the mitotic spindle forms. Subsequently, the phragmoplast, a plant specific cytoskeletal structure, deposits the new cell wall as it expands towards the site previously labeled by the PPB. In the maize *discordia3* mutants (*dcd3*) the subsidiary cells, which are part of the stomatal complex, are abnormally shaped suggesting defects in division plane orientation. Additionally, *dcd3* mutants are shorter than wild-type plants. Segregation observations suggest that two independent mutations are required for the strongest *dcd3* phenotype. We are using a map based cloning approach to identify the genes mutated in the *dcd3* mutant. Three mapping populations were generated by crossing *dcd3* to the W23, B73, and Mol17 wild-type inbred lines. Sites of genetic recombination are being identified using mapping markers and our results so far have localized the two *dcd3* mutations to chromosomes 3 (*dcd3a*) and 8 (*dcd3b*). We are in the process of identifying more markers to determine the exact positions of *dcd3a* and *dcd3b*, as well as analyzing segregating populations to evaluate the contribution of each mutation to the observed phenotype. The chromosomal locations of *dcd3a* and *dcd3b* are syntenous and upon further refinement of the map position, this information will be used to identify paralogous gene pairs that are candidates to be *dcd3a* and *dcd3b*.

P153

The Polycomb Group Gene *EMF2B* regulates floral organ development in rice

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The Polycomb Group (PcG) complex, conserved between animals and plants, represses the transcriptional activity of target genes through trimethylation of Lys 27 of histone 3. In Arabidopsis, the PcG complex has been shown to regulate several developmental pathways from vernalization to flower and seed development, but nothing is known about specific functions of the PcG in grasses. We studied the function of *EMF2B*, a putative rice ortholog of the Arabidopsis PcG gene *EMBRYONIC FLOWER2* (*EMF2*), which represses flowering, and is a homolog of Drosophila *Su(z)12*. Loss of *EMF2B* in rice does not affect flowering time, but results in flowers that lack stamens, possess multiple carpels and palea/lemma, and lodicules are transformed into leafy, sepal-like structures. *emf2b* flowers also display an indeterminate phenotype. By transcriptome analysis of mutant panicles, we were able to identify 6 MADS-box transcription factors that are mis-regulated in the *emf2b* mutant. Five of these MADS-box genes have previously been implicated in the regulation of floral organ identity and meristem determinacy in rice. We propose a model in which the PcG complex directly targets some of these MADS-box genes in order to control floral development and determinacy in rice. Chromatin Immunoprecipitation (ChIP) experiments are underway to determine precisely which of these MADS-box genes are directly regulated by the PcG complex. This study represents the first detailed characterization of PcG function in a monocot species and establishes a new role for the PcG complex in grass flower development distinct from that in Arabidopsis.

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P154

The quest for apomixis in *Zea mays*. Episode II: The egg cell of doom

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Apomixis is defined as asexual reproduction through seed (Nogler 1984). This phenomenon was reported in over 400 species but is absent in major crops. The production of seeds that are genetically identical to their mother is of tremendous agricultural potential to maintain desired genotypes indefinitely. The application of apomixis to seed production would allow e.g. the maintenance of heterozygosity in hybrids, thereby facilitating the production and use of hybrid seeds in agriculture.

Gametophytic apomixis deviates from sexual development in three major steps; (i) apomeiosis (abortion/omission of meiosis leading to the formation of unreduced, unrecombined egg cells), (ii) parthenogenesis (activation of embryogenesis without fertilization of the egg cell), and (iii) functional endosperm formation. Our approach is to search for maize mutants displaying these individual elements of apomixis. Combining such mutants should result in the synthesis of apomixis and, thus, in clonal offspring (Grossniklaus 2001).

The work presented here focuses on the search for mutants displaying the second element of apomixis: parthenogenesis which results in the formation of embryos without paternal genetic contribution. The screen was based on the pollination of *bz1*^{mum9} (Brown 1989) lines with 2n^*R-nj* (Kumar 1986). The absence of *R-nj* pigmentation in the embryo from such a cross is an indication for possible parthenogenetic development of the embryo. The kernels with *r* embryos were germinated and tested for their ploidy by flow cytometry. Confirmed haploids, which are usually male sterile, but have residual female fertility (Chalyk 2001), were transplanted and will be pollinated by 2n^*R-nj* to confirm the genetic basis of the observed phenotype. Promising parthenogenetic mutants will be analyzed by molecular and cytological means.

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P155

Transcriptome and cytological profiling of maize anthers in the male sterile mutants *ms32* and *ms23* and other newly characterized middle layer and tapetal defective mutants

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Plants lack a germ layer and hence must transition from a somatic to germinal cell fate. This change in cell fate specification is poorly understood. Using the five cell layers of anthers, our project has endeavored to identify and characterize the genes responsible for the transition to meiotic cells. Using male sterile mutants with defects in these cell layers, particularly the middle layer, tapetum, and meiotic cells, we have classified many mutants cytologically along with cloning and transcriptional profiling of a few significant developmental candidates. In particular, a pair of tapetal mutants, *ms32* and *ms23*, are analyzed in depth both transcriptionally and cytologically using microarrays and confocal microscopy, respectively. The *ms32* mutant has been cloned. Many similarities in gene expression exist between these two phenotypically similar mutants, although the defective, two-layer tapetum, termed t1 and t2, proves to be caused at different points in development. Candidates for tapetal markers have been identified from the *ms32* and *ms23* datasets. A subset of middle layer and tapetal mutants has also begun to be analyzed cytologically and will be presented as well. This study is funded by a grant from the United States National Science Foundation (07-01880).

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P156

Using Natural Variation to Identify Gene Modifiers of Three Developmental Mutations in Maize

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Mutant phenotypes can vary when combined with different genetic backgrounds, mainly because of gene modifiers. We are crossing three dominant mutations, *Liguleless3* (*Lg3-O*), *Few branched1* (*Fbr1*) and *Clumped tassel1* (*Clt1*), both in hybrid and in B73 inbred backgrounds, to the twenty seven Nested Association Mapping (NAM) founder lines and five ex-PVP lines to uncover gene modifiers. Segregation distortion has identified lines that may contain gene modifiers. For example, *Lg3-O* X M37w F2 families show 9:7 segregation of *Lg3*:nonmutants, suggesting that M37w has a recessive suppressor of the *Lg3-O* phenotype. For *Fbr1*, the F1 of *Fbr1* X IL14H have no individuals with less than 6 primary tassel branches, but plants with 1-5 branches segregate 3:13 in the F2, indicating a possible, dominant suppressor of *Fbr1* in IL14H. Finally, a selfed F2 family of PHJ40 X *Clt1* segregate 3:13 for mutants:nonmutants, suggesting that PHJ40 contains a dominant suppressor of *Clt1*. This cross also produced very small plants with small but fertile tassels and ears, potentially indicating an enhancer of *Clt1*. We are confirming the presence of the mutant alleles in the suppressed lines using molecular markers and mapping the modifiers to identify potentially novel genes.

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P157

Viviparous8 and *Big embryo1* regulate development of embryo and lateral organs in maize.

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Plant architecture is regulated by complex genetic interactions that integrate cell division, growth and differentiation. Leaf number is a critical component of canopy area. Branching of the root system affects water and nutrient uptake. In maize the *Viviparous8* (*Vp8*) and *Big embryo1* (*Be1*) genes have key roles in determining the number of leaves and adventitious roots produced by the plant. In contrast to other genes implicated in lateral organ formation, these two loci also regulate embryo development. The embryo phenotype is mediated in part by regulation of abscisic acid (ABA) accumulation and sensitivity, concomitant with modulation of expression of the genes directly involved in accumulation and signaling of this hormone. Whereas loss of function of *vp8* show severe seed phenotypes in maize, mutations in *AMP1*, the Arabidopsis orthologous of *Vp8*, does not have a discernible phenotype in the matured seed. Moreover, orthologous mutants of *be1* have not been described in any plant species including Arabidopsis. In order to compare the function of orthologous Arabidopsis *Be1* genes with the maize *Be1*, we first characterized phenotypes of the Arabidopsis mutants. Interestingly, Arabidopsis genome potentially has two *Be1* orthologs, *ATBE1A* and *ATBE1B*. Neither single mutants nor the double mutant showed any evident seed phenotypes. In contrast, the *atbe1a* single mutant, as well as the double mutant, developed additional rosette leaves, similar to the maize *be1* plant phenotype. These results reinforce the potential of the cereal system for identifying novel genes involved in seed development. Because scutellum tissues of the *vp8* and *be1* mutant embryos show particularly striking phenotypes, we suspect that scutellum development involves novel pathways that are unique to cereals or grasses.

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P158

Near-Isogenic Lines of Tropical Inbred Hi27

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A series of >170 NILs (near-isogenic lines) of tropical inbred Hi27 are available from Hawaii Foundation Seeds (www.ctahr.hawaii.edu/hfs). these have been bred in the background of a tropical flint and include most familiar mutants from Coe & Neuffer's classical publication. Many newly identified and essentially unmapped loci also occur, the result of our "mining" of tropical germplasm that is less-well known by temperate geneticists. Hi27 traces to India's CM104 that was based on a Colombian flint. Unlike e.g. B73 or P39, which are susceptible to "most everything" in the tropics, Hi27 is resistant to "most everything" (biotic, abiotic stresses). Hi27 is a prolific, hard orange flint with high beta-carotenoids. Its wide tropical adaptability has been validated in many tropical countries since its inclusion in these conversions in 1967 at Thailand's Kasetsart U. Most NILs represent 6 or more backcrosses. Many types of linkage drag have been observed. Almost all NILs show heterosis when intercrossed, as one example. The NILs are reviewed with accompanying photos in forthcoming issue of Maize Genetics Newsletter.

Funding acknowledgement: United States Department of Agriculture (USDA)

P159

Using the maize TFome project to *F*oster the *I*ntegration of *R*esearch with Undergraduate *E*ducation (FIRE).

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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. Whereas this goal is widely recognized it is difficult to implement by letting students experiment in traditional research laboratories simply because of the numbers involved. At the University of Toledo we have initiated a program to *F* oster the *I* ntegration of *R* esearch with undergraduate *E* ducation (F.I.R.E.). The first implementation of this is to incorporate aspects of an NSF PGRP project into an existing Molecular Genetics Laboratory taken mostly by second and third year undergraduates. As part of the PGRP project to investigate and understand grass regulatory networks, we had initiated The Grass Transcription Factor ORFeome Project (TFome). The long term goal is to generate a complete maize TFome (~3500 TFs) that will be immensely useful in studies aimed at finding TFs and their partners for any gene of interest. This type of project lends itself very well to incorporation into undergraduate education as the collection is built up over several years. Here we report on a teaching module that was developed and integrated into a class and can be readily adopted into similar classes at other institutions. In this class student pairs were each assigned to identify and clone a novel transcription factor from corn in the “Keys of Corn Project”. A set of five laboratories were designed that introduced students to database mining, the polymerase chain reaction, gene cloning, bacterial transformation, and the use of bioinformatics to characterize a novel gene. To date over 250 students have participated in this project and cloned over 100 TFs while learning database mining and gene cloning skills. This project is funded by NSF grants DBI-0701405 and IOS-1125620.

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P160

3-dimensional imaging and high-throughput phenotyping of living root systems reveals dozens of QTL controlling cereal root architecture

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Plant phenotyping methods lag behind sequencing technologies and hinder our progress in understanding the genetic basis of agriculturally beneficial traits. This genotype-to-phenotype gulf is particularly wide for root system architecture (RSA), due to its developmental plasticity and recalcitrance to imaging. The critical role that root systems play in plant adaptation to challenging environmental conditions emphasizes the need for high-throughput, accurate measurements of RSA. We describe such a method here, and demonstrate its ability to query the quantitative genetic basis of RSA in cereals. We collected >56,000 high-resolution images of the root systems of a Bala x Azucena rice RIL population grown in a transparent gellan gum over 5 days of development. Images were processed in an automatic phenotyping pipeline that quantified 36 traits representing key components of root growth and network topology, using either a rotational set of 2D images that capture 3D-RSA, or directly from reconstructed 3D models. From these data, we identified 106 significant univariate QTL by Composite Interval Mapping. QTL for many traits co-localized, marking genomic hotspots and suggesting the possibility of genetic trade-offs. To refine our analysis, we applied a multivariate mapping technique using a core subset of the 36 traits. This approach pinpointed regions of the genome that were most influential to root architecture as a whole. Despite the artificial growth conditions of our study, many QTL co-localized with root-trait and drought-resistance hotspots that were previously identified in this mapping population when grown under various field and greenhouse conditions. We have adapted these methods to survey RSA in the maize NAM parental lines, as well as capturing the response of living roots to either nutrient stress or planting density in high-resolution time series. We outline our approach to combining this phenotyping method with sequencing to identify and characterize the genes underlying RSA QTL.

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P161

A genome-wide association analysis identifies loci that modulate hypersensitive response caused by a maize auto-active resistance gene

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Rp1-D21 is a maize auto-active resistance gene that confers a spontaneous hypersensitive response (HR). Depending on the genetic background in which it operates; variable levels of HR are observed. This offers a convenient system to identify alleles that modulate HR and genes involved in disease response. We report an association mapping strategy based on the MAGIC (Mutant Assisted Gene Identification and Characterization) approach to identify naturally occurring genetic variations associated with variation in HR in population of diverse maize lines. A collection of 232 diverse inbred lines of maize constituting a high-resolution association mapping panel were crossed to an Rp1-D21 heterozygous parent stock and the segregating F1 generation was evaluated for phenotypes associated with lesion severity for two years at two locations. Using a linear mixed model that controlled for spurious associations due to population structure, a genome-wide scan for associations with HR was conducted with 51,150 SNPs. Three of the five highly associated SNP markers identified were localized within exons and a promoter region of candidate genes (CGs). For the two other associated SNP markers, one was localized 29 kb upstream of a CG (HSP70), a gene that lacked markers in our SNP marker panel, while the other was localized within a non-coding sequence flanked by a transposase gene. In each case, the candidate genes were predicted to play significant roles in the control of programmed cell death.

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P162

A Sequential QTL Fine-Mapping Strategy and its Application in Discovery of Genes that Underlie the Resistance QTLs in Maize

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Most agronomically important traits, controlled by multiple quantitative trait loci (QTLs), are severely affected by genetic backgrounds and environmental conditions. Although many agronomically important QTLs have been mapped, cloning of QTLs remains a substantial challenge in crops. Here, we proposed a sequential QTL fine-mapping strategy. The strategy provides a very powerful approach for fine-mapping QTLs, particularly those minor-effect QTLs. The progeny derived from recombinants are divided into heterozygous or homozygous genotype subgroups, with or without the donor segment in the QTL region, respectively. Statistically significant differences in mean values of the trait between heterozygous and homozygous genotypes are determined using a paired-sample t-test. No significant difference ($P \geq 0.05$) and significant differences ($P < 0.05$) between the two subgroups indicate the absence or presence of the QTL in the donor segment, respectively. Comparison of the donor regions to phenotypes for all recombinants enables the fine mapping of QTL.

This QTL fine-mapping strategy has many advantages as compared with other methods. First, all progeny are grown in the same plot and this should maximally reduce environmental error. Second, the number of progeny can be adjusted based on R^2 values to reveal the true phenotypic difference between homozygous and heterozygous genotypes. Third, new recombinants could be obtained in each mapping generation, and this guarantees the sequential fine mapping until the candidate genes that underlie the QTL are detected. In our laboratory, a minor resistance QTL-*qRfg2* against Gibberella stalk rot in maize, which could only explain 8.9% of the total phenotypic variation, was fine-mapped to a genomic interval of ~6 kb, covering a single gene. Similarly, a major QTL (*qRfg1*) for resistance to Gibberella stalk rot and a QTL for head smut resistance (*qHSR1*) have also been fine-mapped using this mapping method, and the candidate genes that underlie these resistance QTLs were identified.

P163

A transcriptome-based approach to predict heterosis and to reveal its genetic basis - Identifying QTL underlying genes in breeding populations.

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Heterosis has been intensively used in plant breeding since the early 20th century and has since then resulted in a steady linear increase in crop trait performance. However, despite of its great agricultural importance the molecular and genetic mechanisms underlying the phenomenon are yet not fully understood. To characterize heterosis and to develop transcriptome-based methods for its prediction, we analyzed the transcriptome of 7 day old seedlings from 21 maize parental inbred lines from an extensive breeding program. Certain differentially expressed genes were identified as promising genetic markers for prediction and were shown to be directly associated to heterosis. By identifying the genes underlying heterosis we were able to look for important heterosis-associated genomic regions and to identify genes potentially underlying QTLs from other studies. Furthermore the role of additive expression in the formation of heterosis was examined leading to a model in which heterosis is positively influenced by a shift of absolute expression levels of the identified, mostly additive expressed genes in hybrids. Thus, as an accumulative transcriptome wide effect, additive expression might compensate fixed detrimental expression levels of the inbred parents.

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P164

Combining Ability for Grain Yield and Provitamin-A Carotenoid Concentrations in Tropical Maize

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Developing biofortified maize cultivars is one significant approach to overcome the widespread problem of vitamin-A deficiency in the developing world, especially in sub-Saharan Africa. The objective of this study was to study gene action (general and specific combining ability, GCA and SCA) for grain yield and provitamin-A concentrations of hybrids among 21 tropical maize inbred lines from the CIMMYT/HarvestPlus provitamin A biofortification project. These lines were assigned to three putative heterotic groups based on maximizing genetic distance (calculated based on 402 SNPs) while maintaining pedigree distinctness. The lines were crossed to each other following a North Carolina Design II. Six sets of crosses were produced, where sets 1 - 3 contained crosses between putative heterotic groups (1x2, 1x3, and 2x3), and sets 4 - 6 were crosses within groups (1x1, 2x2, and 3x3). This resulted in a total of 152 hybrids, after bulking reciprocals. The hybrids were evaluated agronomically at four environments in Mexico: Agua Fria (AF) during winter 2010-2011, and Tlaltizapan conventional tillage (TL), Tlaltizapan conservation agriculture (TLCA), and Celaya (CE) during summer 2011. The experimental design was an alpha-lattice with two replications of one-row plots. The first plant in each plot was self-pollinated for provitamin-A analysis at AF 2010 and TL 2011 (data is pending for TLCA 2011). The traits measured included grain yield, anthesis date, plant height, and provitamin-A concentrations in grain. The results showed that GCA and SCA were significant for all traits, except SCA for provitamin-A concentrations. This suggests that provitamin-A concentrations were controlled by additive gene action. In addition, yield comparison of between and within heterotic group crosses reveals that there was difference among these (P=0.04), but not large (0.37 t/ha). Grain yield was correlated with beta-cryptoxanthin (r=0.26**), whereas anthesis date was correlated with lutein (r=0.50**), zeaxanthin (-0.52**), b-carotene (r=-0.35**), and provitamin-A (r=-0.36**). Significant but small yield advantage of among versus within putative heterotic group crosses confirms that genetic distance is not a panacea, but suggests that further breeding work may be effective in developing useful heterotic groups from those putatively identified by maximizing genetic distances.

Funding acknowledgement: HarvestPlus

P165

Creation of a Nearly Isogenic Line Allelic Series (NILAS) for photoperiod responsive loci in maize

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Increasing the resiliency and sustainability of crop production requires effective approaches that harness genetic diversity to adapt plants to new and existing environmental challenges. Tropical maize possesses significantly greater genetic variation than US maize. It is known to harbor favorable alleles for numerous traits that are absent from US maize but will be important for adapting to climate change. The search for favorable alleles for maize productivity under abiotic stress and emerging pathogens will require meaningful phenotypic evaluation of tropical germplasm in the temperate target environments where maize is produced. The latitudinal cline in daylength and temperature extending from Central America—the center of maize diversity—to the Northern US represents the most significant barrier to the introduction of tropical genetic diversity for corn improvement. Our goal is to deepen our understanding of the genetic factors preventing access and use of novel genetic variability harbored by tropical germplasm. Here we report our progress on using marker assisted selection to develop a NILAS at each of four major photoperiod-responsive loci in the maize genome. Each NILAS comprises a large set of inbred lines harboring introgression segments at a single specific genomic locus across a range of functional alleles. In this case, we have begun incorporating alleles from seven tropical donor lines into both stiff stalk and non-stiff stalk temperate genetic backgrounds such that locus-specific introgression libraries are made for each of the 14 tropical X temperate contrasts. We discuss the technical details of this marker-assisted breeding effort as well as the range of questions that can be addressed using a NILAS.

Funding acknowledgement: United States Department of Agriculture (USDA)

P166

Density Response of Flowering Time and Plant Stature QTL in IBM Populations of Maize

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Because step-wise changes in maize grain yield on U.S. farms are likely going to occur as a result of increased planting density, breeders are considering strategies for developing density-tolerant hybrids. High density plantings result in greater competition for resources, as well as morphological changes to the plant, that can present agronomic and yield challenges to traditional hybrids bred in lower density conditions. Each grower has interpreted the yield benefits and agronomic challenges of high density plantings differently, and as a result, planting densities have been increasing incrementally on some farms (up to 110,000 plants/ha), while remaining stable on others. This poses difficulties for breeders targeting a market which includes growers using both higher and more typical planting densities. The breeder could run two parallel breeding programs, both a traditional program and another program using a high density approach from the start, including densely planted nurseries and yield tests, as well as marker-trait associations derived from high density experiments. Alternatively, the breeder could run a traditional program, and only test the best subset of advanced stage hybrids in high density. If the second strategy was employed, it would be beneficial to use MAS targets with effects consistent in both high and more typical density plantings. Barren-ness, plant height, ear height, and growing degree units until pollen shed and silk extrusion were measured in high and low density plantings of the IBMRILs and IBMSYN10 doubled haploids in a wide variety of environments. QTL searches for each trait were conducted using composite interval mapping with a set of ~2000 markers on a high density, ~8,000 cM map. Approximately half of the QTL detected were observed with density dependence, and effect size differences were observed at the two densities for the remaining QTL. QTL of interest were further refined using linear regression analysis of an ultra-high density set of ~13,500 markers. Functional hypotheses are proposed for the QTL identified.

P167

Development of a statistical framework for association mapping in recurrently selected populations

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The genetic architecture underlying response to selection has not been widely characterized so far. A new experimental framework called response to artificial selection (RAS) mapping that combines association mapping with selection mapping (analysis of allele frequency change) is being developed to characterize the genetic basis of crop improvement in populations subjected to recurrent selection. Association mapping allows for the identification of quantitative trait loci based on correlations between sequence and trait variation, provided genetic structure is appropriately accounted for. In recurrently selected populations, there is an evident basis for genetic structure that is influenced by genetic factors such as recombination, chromosomal assortment, selection, and drift. Controlling for genetic structure in association analysis helps limit false positive associations and minimizes bias in the estimation of allele effects. However, currently available estimators of coancestry used to control for genetic structure assume Hardy-Weinberg equilibrium and are thereby not warranted for selected populations. A new quantitative identity-by-state (QIBS) estimator is proposed for calculating coefficients of coancestry or probabilities of identity-by-descent, specifically in closed breeding populations under recurrent selection. Allele-specific weights based on identity-by-state information of the founders are used to arrive at probabilities of identity-by-descent for the derived population. We have developed a whole genome simulator in R to mimic any real-world plant breeding program and correspondingly generate high-density genotypic and phenotypic data. This is being applied to examine the effects of the aforementioned genetic factors on shaping genetic structure in recurrently selected populations and to compare QIBS to commonly applied estimators of coancestry in terms of accuracy, precision, and power of the association mapping framework.

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P168

Discovering and dissecting natural variation underlying R gene immunity in maize by MAGIC

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The main objective of this project is to unravel the genetic architecture of the hypersensitive response (HR), the most common immune response of the plant world. To this end, we are making use of a constitutively active R gene in a MAGIC (for Mutant-Assisted Gene Identification and Characterization) screen to sift through HR-impacting variation present naturally in the germplasm. This autoimmune R gene inherits partially dominantly, and is derived from the Rp1 disease resistant locus that confers gene-for-gene resistance to the common rust pathogen, Puccinia sorghi. Using this MAGIC trick on the maize diversity panel, we have shown that a remarkable amount of variation exists in maize germplasm capable of dramatically impacting HR. When conducted on the IBM RIL population, this approach led to the identification of a large QTL, which we have named Hrml-1 (for HR modulating locus-1). Encouraged by the success of this study, we have started to integrate MAGIC with the NAM (Nested Association Mapping) resource. NAM is a collection of 5,000 RILs derived from a cross of B73 with 25 diverse maize lines (called NAM founders), with each cross yielding 200 RILs. RILs from 24 of these NAM populations were crossed with the R gene mutant and the resulting testcross progenies were assessed for changes in the HR phenotype at two locations, Indiana and North Carolina. Composite interval mapping analysis for individual sub-populations identified a total of 33 unique QTL, all with a relatively large effect on the HR phenotype. Genes underlying these QTL will be fine mapped and eventually cloned using various approaches, such as association mapping, transposon tagging, and targeted EMS-mutagenesis. These studies indicate that MAGIC is a very effective approach in mining and harnessing natural variation underlying the HR response in maize.
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P169

Dissecting maize genome complementation effects on hybrid vigor

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Since 1930, maize breeding programs made it possible to highly increase yield by first using double-cross hybrids and then single-cross hybrids. Hybrid vigor or heterosis, which is the increased performance of the hybrid progenies compared to their homozygote parents, reaches high levels in maize with certain combinations of complementary heterotic groups. Three genetic mechanisms have been proposed to explain heterosis: dominance, overdominance and positive epistatic interactions.

In order to better understand these mechanisms, a diallel crossing scheme was designed using 12 inbred lines, representative of the current U.S. commercial germplasm base. The resulting hybrids were phenotyped and the inbred parents were sequenced, making it possible to directly link heterosis to genetic differences between inbred lines.

Preliminary results of some genomics regions of interest are presented here, based on the above data set. Through this project, we aim to explore how better understanding of the basis of heterosis can contribute to more accurate estimation of breeding value and prediction of performance, for increased efficiency in genetic improvement of maize.

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P170

Dissecting the impact of population structure on GWAS of yield traits in Syngenta's North American Elite Inbred maize lines

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Syngenta relies heavily on association mapping to develop native-trait products, including the water-optimized Agrisure Artesian™ hybrid maize product line. Successful association mapping of any quantitative trait begins with successful dissection of population structure. Structure in natural populations has made it notably difficult to dissect life-history traits such as flowering time with classical association mapping approaches. Syngenta scientists have found that analysis of highly selected-for commercial traits such as yield is also significantly complicated by population structure in elite germplasm pools.

Although maize (*Zea mays mays*) is naturally monoecious, a half-century of commercial breeding has selected for dioecious behavior in elite breeding pools, and commercial breeding programs maintain reproductively isolated, genetically diverse pools of conventional females (Stiff Stalks) and conventional males (Non-Stiff Stalks). These pools exhibit population structure that cannot be adequately accounted for by Bayesian clustering on genetic markers, pedigree analysis, and other conventional methods.

We present a new approach to GWAS that exploits historical information by applying network analysis to pedigrees. For yield and yield component traits, our approach reduced false discovery due to population structure several fold compared to the equivalent classical unified mixed model (Q+K model).

P171

Drought and genotype affect phyllosphere communities

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Plants have numerous microbial organisms on their leaf surfaces (the phyllosphere). These epiphytic organisms can be beneficial; beneficial bacteria on maize leaves may allow for a higher tolerance of drought conditions. In this study, variation in phyllosphere bacteria among maize plants of differing genotypes and growth conditions was observed. Efficiency of various sampling techniques was also examined. The leaf epiphyte community in the various genotypes and stress treatments was measured with DNA-based species identification and analyzed using multivariate methods.

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P172

eQTL mapping of the maize shoot apex reveals complex gene regulation

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The genetic architecture of transcriptional regulation is still largely unknown. Here, we report the genome-wide transcript variation in shoot apices among 105 individuals from the intermated B73 × Mo17 recombinant inbred line (IBM RIL) population. We conducted RNA-Seq on RNA isolated from shoot apices and detected the expression of 28,603 genes. Our results revealed that for 23,136 (81%) genes, the population mean, the coefficient of variation, and the distribution patterns could be predicted by the parental expression patterns, which implies that additive effects play an important role in the regulation of transcriptional variation in the RIL population. We also identified 598 (2%) genes that exhibited traditional transgressive segregation, and three novel types of aberrant inheritance patterns (extraordinary transgressive patterns) in the other 4,869 genes (17%). These aberrant inheritance patterns included: genes expressed in both B73 and Mo17, but not in at least 10% of the RILs (Type I); genes expressed in neither B73 nor Mo17, but expressed in at least 10% of the RILs (Type II); and genes expressed in either B73 or Mo17, but that deviated from a 1:1 Mendelian segregation ratio in the RILs. Global eQTL mapping of the 22,242 genes, which were expressed in more than 90% of the RILs, identified 30,774 eQTLs, of which ~37% are cis-eQTLs and ~63% are trans-eQTLs. For the 3,340 genes exhibiting aberrant inheritance patterns that were expressed in at least 40% of the RILs, we detected 5,642 eQTLs with a cis-eQTLs:trans-eQTLs ratio of 22%:78%. The ratio of trans-eQTLs underlying aberrantly expressed genes is significantly higher than that of genes with parental expression patterns and traditional transgressive segregation, indicating that trans-eQTLs play an important role in the regulation of the genes exhibiting aberrant inheritance patterns. Moreover, 96 trans-eQTL hotspots were identified, of which 20 were coincident with trans-eQTL hotspots associated with the aberrant inheritance patterns. Our results help shed light on the genetic mechanisms underlying gene expression variation in maize.

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P173

Estimation of influence of continuously changing environmental variables on hybrid maize performance across the United States Corn Belt

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In commercial breeding programs, hybrid maize is evaluated in unreplicated experiments in multi-location trials. Continuously changing environmental factors in these locations affect the overall performance of hybrids due to the effects of environment and genotype by environment interactions (GE). Many commonly used GE analyses treat each location as a unique environment and count environmental events as episodical. However, locations do not always represent distinct environmental events. To address this challenge, random regression models (RRMs) are being used to incorporate continuous changes in environmental variables as a means to obtaining more accurate estimates of GE. In addition, these models are being utilized to understand the relationships among locations in multi-location trials. In this study, principal component analysis (PCA) was used to decompose environmental variables and these components were incorporated into RRMs for unreplicated multi-location trials. The predictive ability of these models was examined using cross-validation techniques. Preliminary results indicate that RRMs provide a powerful tool for understanding GE. This information can be used to inform breeders, researchers, and farmers, about the magnitude and nature of GE, causes of interaction, adaptation of particular cultivars, and behavior of these cultivars in diverse environments.

Funding acknowledgement: Dow AgroSciences, Purdue University

P174

Evaluation of tolerance to salinity stress in maize bassed on indices of stress tolerance or susceptibility to stress for grain yield

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For checking indices of stress tolerance to salinity stress in different hybrids of maize, the experiment in 1385 in fram of research center of natural sources and agriculture jehad of Qom province had performed. This experiment cultivated in block model as full accidental in 3 repetition at 2 separate experiment in normal and salinity term. And 27 hybrid studied soil tissue with electric direction (EC) in 0-30 depth, 8.5 desi zimense and in 30-60 depth, was 9.9 desi zimense and soil's pH was equal with 7.6. from 5 indices that had calculated in names of yield in normal term (YN), yield in stress term (YS), tolerance index (TOL), arithmetic mean (MP), susceptibility to stress (SSI). Index of tolerance to stress (STI) and geome tric. Mean (GMP), comparatine quantities of special vectors in first component, showed that, indices of YS, MP, GMP, and STI have high correlate with first component (The component tolerance to salinity). Second component with 35 persent of total changes showed that indices of 6n, TOL and SSI had high correlate with second component (the sensitive to stress component). This component can show us hybrids with few yield stability (sensitive to salinity) and where the few amount of SSI is desirable, so based on this component hybrids with high YN and little SSI, have average mean in rwo normal and saline environment. Totally, with transmittance of hybrids on by plat that produt, classified two first component of hybrids in 4 group. Hybrids with number KSC250, KS301, G-54190, G-3337, OSSK373, BC504, NS540 had known, on viewpoint grain yield tolerance to salinity, hybrids with numbers KSC 260, G-54185, OSSK444, BC354, BC354, BC282, KS350, ZP341 with high mean of yield in every two nenvironment had been recommended. Hybrids ZP434, KSC500, KSC340, G-54193, BC572, and BC418 had good yield in normal in term but in salin term, they hadn't good yield and so, hadn't been recommended for cultivation in normal areas. Hybrids in group 4 , in every two environment were fev production and were week and hadn't been recommended for implant in area.
Key word: Maize, Salinity stress, Hybrid, Component, Correlation

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P175

Farmer's Voices On Mother-baby Trials

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Maize is the chief food crop in Malawi for more than 90% of the population. Malawian farmers still lack experience to improved varieties before buying on the market. Mother-baby concept is the answer since farmers are showcased both experimental and released varieties and farmers are asked to choose so that Breeders dance to farmers' tune. For instance, if farmers choose an experimental hybrid/OPV, the experimental variety gets released. On this research, 5 OPVs and 7 hybrids, all released, were used. Mother trial had 2 reps one green and the other yellow dependent on fertilizer applied. Green part got recommended fertilizer application while the yellow part got half of green application. Farmers preferred OPVs to hybrids despite the low yields. Malawian farmers go for flint maize because of processing. In most of the trial sites were on-farm to depict the real choice of the subsistent farmer. Consequently, both government and seed selling companies follow what the farmer is looking for in the following order: higher yielding, flint, drought resistant and free from foliar diseases.

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P176

Fine mapping and candidate gene analysis of *hrml1 (hypersensitive response modulating locus-1),* a QTL modifying the expression of *Rp1-D21* in maize (*Zea mays L.*)

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The *Rp1-D21* gene is a semi-dominant allele of the maize *Rp1* rust resistance gene causing autoimmunity. Using this autoactive phenotype of *Rp1-D21* as a reporter in a novel genetic modifier-discovery strategy called MAGIC (Mutant-Assisted Gene Identification and Characterization), a major QTL *hrml1* was discovered and mapped to chromosome 10 by crossing the *Rp1-D21* gene into the IBM mapping population (Chintamanani et al, 2010). The effect of this QTL was further confirmed with near isogenic lines (NILs) containing Mo17 fragments in the background of B73. About 50 genes are annotated within this QTL interval based on the B73 reference genome and further analysis of IBM RILs with extreme phenotypes has narrowed down the number of candidate genes to 1/3rd. Non-synonymous SNPs were found in the coding regions of some interesting candidates between B73 and Mo17. To fine map this QTL further, segregating populations were established through the selfing of B73-Mo17 NILs heterozygous in this locus with B73 as the recurrent parent. In addition, two Mu tagged mutants, 31 Mu tagged somatic sectors and 17 EMS mutants were generated for the validation of *hrml1* identity. The cloning of *hrml1* is likely to provide new information on the mechanism of HR cell death in plants.

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P177

Fine Mapping of a QTL for Prolificacy in Maize

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The reduction of the number and increase in size of inflorescences is a common aspect of plant domestication. When maize was domesticated from teosinte in southwest Mexico approximately 9,000 years ago, the number and arrangement of the female inflorescences (ears) changed dramatically. In teosinte, the long lateral branches are tipped with tassels and multiple ears are found at the lateral nodes of the branch. In maize, the greatly shorted branches are tipped with a single large ear and no ears are formed at the other nodes. To investigate this change in the number of ears on the lateral branch, we performed a QTL analysis in a BC2S3 maize-teosinte mapping population with maize as the recurrent parent. The population was scored for prolificacy, which is the number of ears on the primary lateral branch. A large QTL was detected on the short arm of chromosome one, in a location that has previously been shown to influence multiple domestication traits. Individuals that were recombinant in the QTL interval were then selfed and recombinant chromosome near isogenic lines (RC-NILs) were created from the homozygous recombinant offspring. Prolificacy was then scored for five individuals each for the 23 RC-NILs grown in four randomized blocks. The QTL was fine-mapped to an interval only 4.8 Kb in size in the B73 genome which contained no genes. A panel of diverse maize and teosintes were sequenced for this interval and potential causative polymorphisms were identified that appear to alter the expression the next gene downstream.

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P178

Fine Mapping of Two Quantitative Disease Resistance Loci in Maize

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To elucidate the mechanisms of quantitative disease resistance, we are examining quantitative trait loci (QTL) for resistance to Setosphaeria turcica, the causal agent of northern leaf blight (NLB), an important disease of maize. A map-based cloning approach, complemented by association mapping and mutant analysis, is being used to uncover genes associated with two previously identified NLB QTL at maize bins 1.02 and 1.06, designated qNLB1.02 and qNLB1.06. Each of these loci exhibits unique resistance characteristics. qNLB1.02 was found to be effective mainly against colonization (Chung et al., 2010). Aside from NLB, qNLB1.02 also conditions resistance to Stewart's wilt, caused by Pantoea stewartii, and common rust, caused by Puccinia sorghi. qNLB1.02 is nonspecific with respect to S. turcica races 0, 1, 23, and 23N. qNLB1.02 currently comprises a region of 3.41 Mb, encompassing 78 genes. Association analysis has revealed ROUGH SHEATH2 interacting KH protein (Rik) as a candidate gene for qNLB1.02. RIK interacts with the transcription factor ROUGH SHEATH2 (Phelps-Durr et al., 2005), which is known to be involved in plant defense (Nurmberg et al., 2007). Rik was shown to be upregulated in response to S. turcica. Mutant analysis and fine mapping will be used to confirm Rik. In contrast, qNLB1.06 confers resistance to NLB and Stewart's wilt and was found to be effective mainly against fungal penetration (Chung et al., 2010). qNLB1.06 spans a region of 5.92 Mb, which harbors 88 genes, including Pan1. PAN1 is a receptor-like protein that promotes polarization during asymmetric cell division (Cartwright et al., 2009). Mutant analysis shows that Pan1 plays a role in maize-S. turcica interactions. Pan1 will be tested as a candidate by fine mapping.

P179

Functional Stay-green in Maize

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The ability for annual crop species to delay senescence or "stay-green" throughout the grain filling period has been associated with increased productivity. The goal of this research was to investigate functional stay-green in maize through multiple approaches. In the first approach, germplasm characterization studies were conducted to identify potential sources of the stay-green trait. Relative greenness and chlorophyll fluorescence were used to quantify visual and functional stay-green in replicated field trials. These studies showed that Mo20W, an inbred line, expressed exceptional stay-green characteristics under abiotic stresses when compared to B73 and Mo17. Genetic analyses of the stay-green trait in Mo20W were conducted by crossing and then backcrossing Mo20W with B73 and Mo17 to produce two BC2F4 recombinant inbred line populations with B73 and Mo17 as the recurrent parents. Results from trials at three locations showed phenotypic variation within each mapping population for visual and functional stay-green at 1000 growing degree days. A selective genotyping linkage analysis indicated three major loci associated with the functional stay-green phenotype derived from Mo20W. In related studies evaluating stay-green in unselected populations, we phenotyped testcross hybrids of the Nested Association Mapping (NAM) population of maize. A set of 1320 NAM RILs with silking dates similar to B73 were crossed with PHZ51 to produce testcross hybrids for phenotypic analysis. The testcross hybrids were evaluated in trials conducted in four diverse environments using a lattice design with differences in stay-green measured at 1200 growing degree days after silking. Joint-QTL analyses were conducted and identified two major QTL. These studies of selected and unselected germplasm identified five major QTL for stay-green in maize. Near-isogenic lines for each of QTL are being developed to investigate the phenotypes and potential candidate genes associated with each locus.

Funding acknowledgement: United States Department of Agriculture (USDA), Agricultural and Food Research Initiative (AFRI)

P180

Genetic Analysis and Characterization of Quantitative Disease Resistance in Maize

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We have used the NAM and other populations to comprehensively describe the loci involved in natural variation for resistance to southern leaf blight. We have also fine-mapped two of the larger-effect loci to sub-centiMorgan intervals and have examined their effect on yield under diseased and non-diseased conditions using a set of near-isogenic hybrids. All these data will be presented. We will also describe our work on multiple disease resistance and the creation of a set of near-isogenic lines designed specifically to identify and characterize loci controlling variation in resistance to multiple diseases. Finally we will describe our plant genetics outreach collaboration with the NC Museum of Natural Sciences with 7th Grade and high school students and the general public.

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P181

Genetic analysis of haploid induction ability in maize (Zea mays L.)

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In-vivo haploid induction are routinely applied in many commercial hybrid maize (Zea mays L.) breeding programs but the genetic basis underlying it were known a little. Here we used two biparental populations (named as 1680-UH400 and CAUHOI-UH400) to do comparative QTL mapping of haploidy induction ability, and subsequently evaluate their genetic effects. Among which, inbred line1680 have no induction ability but excellent agronomic performance, UH400 has a haploid induction rate (HIR) of approximately 8%, CAUHOI is a haploid inducer with high kernel oil content and a HIR of approximately 2%. Two main QTL with large effect have been detected, one was detected in bin 1.04 in 1680- F2 (R2 =51.52%) and 1680- F3 (R2 = 48.4%), while another QTL was detected in bin 9.01 in CAUHOI-UH400 population having R2>20% in three generations of this cross. But no QTL was detected in bin 1.04 in CAUHOI-UH400 populations. These two QTLs greatly increased the HIR, especially for the QTL in 1.04 which may determine whether genotypes have haploid induction ability. The flanking markers around these two QTL also can be used for Marker- assisted selection for inducers in the maize breeding program.

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P182

Genetic Analysis of Kernel Traits in Maize-Teosinte Introgression Populations

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Kernel number and weight are two of the most important factors affecting yield in cereal crops. In maize, kernel number is mainly determined by the kernel row number and ear length, while kernel weight depends on the size and shape. Kernel traits have been selected at domestication and manipulated for thousands of years in agrarian societies. However, few studies on kernel size and shape have been reported in maize due to difficulty in phenotyping. Studies show that teosinte (Zea mays ssp. parviglumis) has more genetic diversity compared to maize inbreds and landraces. To examine the full range of genetic diversity in maize, near-isogenic, introgression lines have been developed from ten parviglumis accessions in the B73 background. A preliminary analysis of eight populations identified several large effect quantitative trait loci (QTLs) for kernel weight and kernel row number. In order to study how size and shape affect kernel weight, we have begun analyzing kernel morphometric traits using digital imaging software. We have identified QTLs for kernel area and length with moderate allelic effects that co-localize with kernel weight QTLs. Our results both confirm prior reports of kernel domestication loci, and identify novel QTLs, with a range of allelic effects enabling future research into the genetic basis of these traits.

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

P183

Genetic Analysis of Orange Endosperm Color in Maize

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Vitamin A deficiency (VAD) is a problem in many developing countries in Africa where maize (*Zea mays* L.) is a staple food. Maize grain with white endosperm is preferred for human consumption and contains little or no carotenoid compounds, including the vitamin A precursors β-carotene and β-cryptoxanthin. Maize grain with orange endosperm, which does contain pro-vitamin A compounds, could help alleviate this problem, but is uncommon in Africa. When combined with education, color has implications for acceptance because consumers can associate orange grain color with provitamin A. A QTL mapping study was undertaken to determine the genetic basis of orange endosperm color in maize. Linkage analysis conducted across and within ten of the nested association mapping families identified five common quantitative trait loci (QTL), and six rare QTL related to the relative intensity of orange grain color. Of the identified QTL, most coincide with carotenoid biosynthetic pathway genes, including phytoene synthase (y1/psy1), ζ -carotene desaturase 1 (zds1), lycopene epsilon cyclase (*lcyε*), and β-carotene hydroxylase 1 (*crtRB1*), suggesting that selection for orange color will successfully increase total carotenoid levels in the grain. We further show the relationship of visual and spectral grain color measurements with carotenoid levels in grain quantified by high performance liquid chromatography. Such grain color phenotyping methods could be cost-effectively integrated with marker-assisted selection to improve the efficiency of breeding for improved provitamin A in maize grain.

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P184

Genetic architecture of European maize: multiparental QTL mapping and insights into heterosis

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Understanding the genetic bases underlying heterosis is a major issue in maize (Zea mays L.). We extended the North Carolina design III (NCIII) by using three populations of recombinant inbred lines derived from three parental lines belonging to different heterotic pools, crossed with each parental line to obtain 9 families of hybrids. A total of 1253 hybrids were evaluated for grain moisture, silking date, plant height and grain yield. Quantitative trait loci (QTL) mapping was carried out on the six families obtained from crosses to parental lines following the "classical" NCIII method and with a multiparental connected model on the global design, adding the three families obtained from crosses to the non parental line. Results of the QTL detection highlighted that most of the QTL detected for grain yield displayed apparent overdominance effects and limited differences between heterozygous genotypes, whereas for grain moisture predominance of additive effects was observed. For plant height and silking date results were intermediate. Except for grain yield, most of the QTL identified showed significant additive-by-additive epistatic interactions. High correlation observed between heterosis and the heterozygosity of hybrids at markers confirms the complex genetic basis and the role of dominance in heterosis. An important proportion of QTL detected were located close to the centromeres. We hypothesized that the lower recombination in these regions favors the detection of linked QTL in repulsion phase leading to apparent overdominance for heterotic traits and linked QTL in coupling phase reinforcing apparent additive effects of linked QTL for the others.

P185

Genetic architecture of nitrogen use efficiency traits in the maize IBM high-resolution genetic mapping population.

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Genetic improvements in nitrogen use efficiency (NUE) advance significant economic and environmental potentials to crop production. Prior efforts to improve NUE for grain yield in maize (Zea mays, L.) have been hampered by the complexity of this trait and difficulties in identifying genetic factors that contribute to maize NUE. The objectives of this research were to elucidate the genetic architecture and identify quantitative genetic loci (QTL) controlling NUE and related traits in maize by using the high resolution intermated B73 × Mo17 recombinant inbred line (IBMRIL) population. The IBM population was test-crossed to Illinois High Protein 1 (IHP1), the known genetic extreme for grain protein and Nitrogen (N) uptake capacity, and the resulting hybrids were grown with (N+) and without (N-) 252 kg ha-1 supplemental N in two years and evaluated for NUE traits. QTL mapping results identified genomic regions controlling N utilization as well as its component traits. Some of the QTL regions were further downsized to a short list of candidate genes by saturating the region with dense SNP markers generated from Genotyping-by-Sequencing (GBS) technique on the IBMRIL population. Putative candidate genes were assigned to several robust QTL, including two genes specifically affecting grain N concentration on Chromosome 6 and 9. Results from this research provide insight into the genetic architecture of the NUE traits and facilitate future marker assisted breeding and positional cloning of these QTL.

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P186

Genetic diversity of water use efficiency and carbon isotope discrimination in maize

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Climate change and increasing incidence of drought worldwide have led to the need for improvement of water use efficiency (WUE) in many crops and is one of the key components to improving maize production in the world. We are using the nested association mapping (NAM) genotypes and testcrosses made from these genotypes to identify genes regulating WUE in maize. Instantaneous water use efficiency (WUE_i) ranged from 4.03 to 9.20 µmol $mmol^{-1}$ over two measurements and two years among genotypes. Two whole plant water use efficiency (WUE_b) experiments ranged from 3.97 to 8.38 g kg^{-1} among genotypes. Mo17 and CML333 were high WUE genotypes in both the field and greenhouse environments. NC350 was consistently the lowest WUE genotype in the field while, IL14H was the lowest in the greenhouse. Field and greenhouse WUE measurements were not correlated. Adaxial and total stomatal density collected in the field had a weak relationship with WUE_i. Nighttime water loss accounts for a significant water loss without CO_2 assimilation and may be a factor in WUE among genotypes. Tx303, HP301, and CML228 had lower nighttime water loss than all other genotypes, but this trait was not correlated with WUE among these genotypes. Carbon isotope discrimination ($\delta^{13}C$) is related to WUE in some crops such as wheat. Heritability of leaf $\delta^{13}C$ among the NAM founder lines over two years was 0.51 and ranged from -11.78 in Tx303 to -13.08 ‰ in Mo17. Carbon isotope composition was positively correlated with transpiration (E) and net assimilation (A), but was not correlated with WUE_i or stomatal density. We are identifying QTL for leaf $\delta^{13}C$ using hybrids of the NAM population. The use of these hybrids negates any effects of flowering time on isotopic composition. The mapping of QTL will then allow for gene identification, and tests of the relationship between $\delta^{13}C$ and WUE in this species.

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P187

Genetic Variation for Nitrogen Utilization in Historical and Improved Maize Germplasm

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Increased nitrogen use efficiency (NUE) is an important target for future maize improvement. Essential to the design of an effective breeding program to select maize hybrids with enhanced NUE is an understanding of past progress, variation among maize germplasm for NUE and its component traits, and identification of phenotyping approaches to optimize genetic gain. We documented genetic variation for NUE and its component agronomic traits among a diverse collection of historical and recent elite maize inbreds and hybrids grown in field trials with different levels of soil N supply. Many of the genotypes evaluated also represent important resources for maize functional genomics. The results confirm previously reported trends for modern elite compared to historical hybrids, where grain yields have increased as a result of superior tolerance to higher plant densities, greater harvest index, and reductions in grain protein concentration. In addition, we demonstrate that past breeding has likely optimized N uptake for high grain yields, but that significant opportunities exist to further improve how maize plants utilize acquired N. We developed a phenotyping approach that estimates N utilization as the ratio of total biomass relative to total plant N, which effectively controls for the significant impacts of N-level, relative maturity, and heterosis on this trait. Using this measure of total N utilization, we identify genotypes with promise as sources of enhanced N utilization with lower N inputs.

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P188

Genetic variation for tolerance to Goss's wilt among elite inbred lines of maize

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Goss's bacterial wilt and leaf blight is a damaging disease of maize threatening food, feed and biofuel production. After 2006, the disease incidence of Goss's wilt dramatically increased for unknown reasons. Previous studies have shown that tolerance to Goss's wilt is quantitative in nature, but is poorly understood. To better understand the genetic basis of tolerance to Goss's wilt, a panel consisting of 200 public and expired Plant Variety Protection Act (ex-PVP) lines was evaluated for Goss's wilt tolerance. The lines in the panel represent the genetic diversity currently used in the hybrid seed industry. Phenotypic measurements were collected at two locations using one of two inoculation methods: a "leaf cut and dip" method and a machine method. Broad-sense heritability was 0.36 for "leaf cut and dip" method and was 0.95 for the machine inoculation. While the machine inoculation allowed a better separation of the lines according to Goss's wilt tolerance compared to the "cut and dip" method, the majority of lines were rated as either tolerant or semi-tolerant. Based on the performance of susceptible checks, we concluded that the infrequency of susceptibility was due to poor environmental conditions for bacterial growth. When the lines were grouped according to genetic background, significant variation between groups was observed for tolerance to Goss's wilt. This preliminary data provides information about the variability of tolerance to Goss's wilt within maize germplasm and identifies sources of both tolerance and susceptibility. The results from this experiment can help future experiments aimed at dissecting the genetic basis of tolerance to Goss's wilt.

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P189

Genetics of Combined Abiotic Stress—Mapping Genes for Synergy Using Dose-Response Surfaces

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Suboptimal environments are the most important limit for maize improvement, and environmental variation will increase with future climate change. We previously determined that the set of genes important for abiotic stress resistance is different in combined, two-stress environments as compared to single managed stress environments. It is not known whether this is due to synergy through shared signaling steps, or via threshold effects. Response surface methodology is widely used in drug and toxin analyses and in product optimization, but has not been applied for detection of genetic control. We mapped the maize genes important for differences in shape parameters of the 3D dose-response surface for drought and fertilizer stress. We used the IBM94 mapping population and five different levels of drought and nitrogen arranged in a cubic-centered face design. Global quadratic fit was compared to the response surface fit for each marker state to identify significant loci. Loci that confer increased growth under intermediate and high levels of combined stress are candidates for further field evaluation and for analysis of control points in abiotic stress signaling.

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P190

Genic and non-genic contributions to natural variation of quantitative traits in maize

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The complex genomes of many economically important crops present tremendous challenges to understand the genetic control of many quantitative traits with greatest importance in agriculture, adaptation, and evolution. Advances in genomic technology need to be integrated with strategic genetic design and novel perspectives to break new grounds. Complementary to individual-gene targeted research, which remains challenging, a global view of the genomic distribution of trait-associated SNPs (TASs) discovered from genome scans of quantitative traits can provide insights into the genetic architecture and contribute to the design of future studies. Here we report the first systematic tabulation of the relative importance of different genomic regions on natural variations of quantitative traits in maize. We found that TASs were enriched in the non-genic regions, particularly within a 5 kb window upstream of genes, suggesting that changes in gene regulation are quantitatively important than other sequence polymorphisms in shaping the natural variation in quantitative traits. Consistent with these findings, TASs collectively explained 44~59% of the total phenotypic variation across maize quantitative traits, and on average, 79% of the explained variation could be attributed to TASs located in genes or within 5 kb upstream of genes, which together comprise only 13% of the genome.

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P191

Genome-wide association study identifies 74 loci in lipid biosyntheses in maize kernel

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Increasing oil content and the proportion of unsaturated fatty acids in maize kernel are considered as important breeding objectives especially for feeding humans and livestock. As a polygenic trait, there has been limited success in identifying the QTLs/genes underlying the natural variations of maize kernel oil. We used genome wide association(GWA) study from 368 inbreds and high density SNPs markers including 1 million expressed sequence based SNPs (eSNPs) by whole genome RNA-sequencing and 50K SNPs from Maize SNP50 chip (gSNPs) to identify genetic variants influencing maize kernel oil content and then components. In total, 74 loci were reported for total oil content and components that included 26 significant loci associated with total oil content and 22 loci located in the known fatty acid biosynthesis pathway. The peak signals at three loci were in or tied closely to previously identified genes (DGAT1-2, FATB and FAD2). Our results also identified several novel loci associated with oil or oil components. Finally we validated three of the novel genes—ZmLACS, ZmACP and ZmCOPII and two known genes—ZmFAD2 and ZmWRI1a— by re-sequencing, linkage and expression analysis. Combined all the variants, 84% phenotypic variation was explained of total oil content. Simple additive model can predict the oil content well in maize kernel reaching 81%. Our findings provide the foundations to understand the genetic architecture of fatty acid biosynthesis and to identify beneficial alleles for molecular breeding.

P192

Genome-wide association study of root and shoot traits under normal and waterlogged conditions in maize seedling stage

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The decline of accumulation rate of biomass is a critical response of maize seedlings to waterlogging stress. To investigate the genetic basis of maize seedlings in response to waterlogging, we report the first findings from a genome wide association study (GWAS) of biomass of maize seedlings under normal and waterlogged conditions in a panel of 144 inbred lines. In addition, an advanced backcross population developed from a combination of two inbred lines, which present significant difference in waterlogging tolerance, was analyzed to confirm quantitative trait locus (QTL) for root and shoot-associated traits under normal and waterlogged conditions, respectively. Whole genome scan detected three strong peak signals at single nucleotide polymorphisms（SNPs）that were significantly associated with stress response on chromosomes 5, 6 and 9. SNP 4784 with strongest association with the fresh and dry weight of root and shoot were identified in bin 5.04 and co-localized with QTL for the same traits in an advanced backcross population. The results will help to understand the genetic basis of differential response and tolerance to waterlogging stress among maize inbred lines, as well as provide some new loci for improving waterlogging tolerance of maize inbred using marker-aided selection.

P193

Genotyping by Sequencing of Eight Thousand Maize Lines Revealed Vast Amount of Loci Associated with Agronomic Traits

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Identification of specific loci associated with agronomic traits in maize has been a long-term goal of maize researchers and breeders. While many efforts have been made through a variety of platforms, such as Recombinant Inbred Lines (RILs), Near Isogenic Lines (NIL) and released inbred lines, only a handful of causal alleles have been robustly identified for complex agronomic traits. Common limitations in past studies include low minor allele frequencies, population stratification, limited sample size, and low genetic marker density. To overcome these problems, we united a panel of approximately 5,000 NAM (Nested Association Mapping) RILs and approximately 3,000 maize inbred lines in a Genome-Wide Association Study (GWAS) that queried over half a million SNPs obtained through Genotyping-By-Sequencing (GBS). The analyses, performed using a new R package (GAPIT), revealed a vast number of loci significantly associated with agronomic traits. For some of the known genes (e.g., Ghd7 and Vgt1 which control flowering time), the associated SNPs were only 50 to 200 Kb away. However, current marker density is still not high enough to guarantee complete linkage disequilibrium with causative SNPs. As such, increasing marker density and imputation with the maize HapMap SNP sets are necessary.

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P194

Harnessing the power and precision of isoline populations for investigating rootworm tolerance in corn

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The western corn rootworm (WCR) is a major economic pest of maize accounting for over $1 billion in annual management costs and yield losses. Despite widespread use of crop rotation, insecticides, and transgenic corn, substantial damages occur year after year. Evidence suggests the loss of efficacy of these strategies is a consequence of adaptations in WCR populations. A major focus of our group has been to characterize the underlying genetic variation that accounts for differences in host-plant tolerance, however, a central challenge has been to validate these genetic mechanisms across biological replications and environments. The use of isogenic populations fixed for recombination events between parental alleles provides a powerful resource for disentangling genetic and environmental effects. Here we describe the deployment of the IBMRILs and their reciprocal hybrids with B101 to assess variation in shoot architecture, reproductive biomass, and WCR tolerance. The IBMRILs and their hybrids were grown in an alpha lattice design with 4 reps per genotype over the course of two consecutive years. Genotypes at 2,019 markers were used to map QTL for plant height, ear height, germination, lodging, stalk strength, and grain fill traits. Here we report the identification of genomic loci accounting for variation between lines and the effects of rep, year, and environment. We also describe the ongoing development of a set of doubled haploid lines generated from exotic germplasm and fixed for different combinations of native resistance alleles to the WCR and their functional use for interrogating host-plant resistance to this important pest.

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P195

High-throughput Phenotyping – A Boost for Genomics in the 21st Century

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Due to the development of highly automated genetic analysis, plant genomics has immensely enlarged our understanding of the genetic structure of plants over the last two decades.
The fast evolving need to identify interactions between genes and environmental factors (*biotic and abiotic*) that brings about a certain plant phenome made it necessary to develop quantitative, reproducible and highly automated plant phenotyping systems for large plant numbers.
Phenotyping systems such as these have to integrate reproducible plant management (randomization, watering) and comprehensive imaging of root and shoot far beyond human vision (*visible light, fluorescence, near infrared, infrared, hyper spectral, NMR, X-rays, THz*) as well additional chemical analysis methods. Immediate and automated image analysis of the stored images and further data transformation using plant shape and plant growth models are the important intermediate steps before undertaking statistical data analysis of the phenotyping results to characterize plant phenotypes quantitatively. Such quantitative data contributes in a decisive way to the further analysis of gene functions (*tilling, QTL etc.*), especially under fluctuating or stress-induced environmental conditions with a special focus on complex traits like yield or drought tolerance.
This presentation will provide a survey on phenotyping technology and the close interaction between phenotyping technologies, modelling approaches and the new opportunities of fast and automated high-throughput genomics.

P196

Hybrid Vigor in the Maize Nested Association Mapping Population

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Prior studies of the maize NAM (Nested Association Mapping) population and analysis of the genetic diversity characterized in the first generation maize haplotype map suggest that there is increased residual heterozygosity in pericentromeric regions of the maize genome. From these observations we infer a direct relationship between recombination rate and residual heterozygosity. The primary goals of this study are to enhance our understanding of the physical and genetic architecture underpinning quantitative trait loci explaining variation in hybrid maize yields, and to provide an empirical foundation of data on which genomic selection models may be trained. After sufficient development and cross-validations, these modeling algorithms may be used to successfully predict hybrid maize yields worldwide.
We utilized hybrids created by crossing a subset of the NAM population and PHZ51, a non-stiff stalk expired PVP line. The hybrids have been evaluated for a range of developmental traits, as well as yield, in nine environments across the U.S. over the past two years. The inbred lines were genotyped using GBS (Genotyping by Sequencing). This data will be used together with data from the second version of the maize haplotype map to examine genetic characteristics underlying hybrid vigor. We will present preliminary findings from this study.

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P197

Identification and fine mapping of ear branching modifiers using the IBM population

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The architecture of the maize inflorescence reflects the fate of lateral meristems borne along its central rachis. In the male inflorescence, a few of these meristems are indeterminate and will give rise to the long branches located at the base of the tassel. The lateral meristems of the female inflorescence, however, are determinate, resulting in unbranched ears with tightly packed kernels. The *ramosa1 (ra1)* gene affects inflorescence architecture by imposing this determinate or short branch identity on lateral meristems as they are initiated. Thus, tassels of *ramosa1* mutants display increased branching, while ears exhibit unorganized rows and/or lateral branching. The severity of the *ra1* mutant phenotype varies widely according to genetic background. In a B73 introgression, for example, the mutant allele *ra1-63* conditions highly branched tassels and ears. In Mo17 introgressions, however, *ra1-63* displays relatively mild effects including a few additional branches in the tassel and ears with crooked rows and an occasional branch. We exploited these phenotypic differences by using the IBM population to identify loci that affect the number of ear branches in *ra1-63* mutants. Each RIL in the IBM-94 population was backcrossed to Mo17- and B73-introgressed *ra1-63*, resulting in two sets of F1BC1 populations. We then mapped modifiers of branching from the B73 and Mo17 backgrounds, and identified several putative modifier QTL. In the B73 backcross population, a particularly significant peak was identified on chromosome 1 with an effect of approximately 3 branches. Near isogenic lines (NILs) were used to generate additional recombinants within the QTL region, which were then backcrossed to B73 introgressions to create lines fixed for *ra1-63* and segregating for the new recombinant chromosome. These lines will be used to further delineate the boundaries of the QTL region. Finally, by integrating data from several existing genome-wide datasets such as from transcript profiling and ChIP-seq experiments, we hope to identify candidate genes within the interval that may contribute to the QTL effect.

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P198

Identification and Validation of Maize Loci Controlling a Yield Component Trait via 2nd Generation Bayesian-based GWAS

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Advances in next generation sequencing (NGS) technologies and the development of appropriate statistical methods have enabled the genome-wide dissection of the genetic determinants of phenotypic traits. Several yield component traits were collected from 4,892 recombinant inbred lines (RILs) in the Nested Association Mapping (NAM) population. A set of 4.2 million genic SNPs was identified via the analysis of RNA-seq data from four tissues from each of the NAM parents. These genic SNPs were combined with HapMap1 SNPs and projected onto the NAM RILs based on RIL genotypes as deduced from ~1,000 tagging SNPs. A joint composite QTL analysis was conducted using the ~1,000 tagging SNPs. Next, a Bayesian-based GWAS method was used to simultaneously fit all quality-checked SNPs and thereby estimate their effects on the yield component traits. Approximately 100 Trait Associated SNP (TAS) were identified, most of which are within or near the identified QTL intervals. As compared to control SNPs, many TASs are associated with variation for yield component traits in one or more of three unrelated populations, thereby validating these TASs. Hence, this methodology outlines a Bayesian-based paradigm for 2nd generation GWAS.

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P199

Identification of a major *Zea mays* QTL that affects primary root elongation rate

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The maize primary root is an ideal system for studying plant growth. Cell division and cell elongation occur within a small region of less than 10 mm in the apical tip. We mapped QTL that explain variation in primary root elongation among 206 inbred lines from the intermated B73xMo17 (IBM) population and identified 12 quantitative trait loci (QTL). Each explained 4-22% of the variance with the fast-growing parent B73 contributing the favorable allele in all but one case. The largest and most consistent effect QTL, *enhancer of primary root growth 1 (eprg1)*, was located on the short arm of chromosome 1. We identified a BC_3S_2 introgression line, Mo17.B73-*eprg1*, homozygous for *eprg1* from B73 in the Mo17 background but homozygous for Mo17 at the remaining QTL. The introgression line Mo17.B73-*eprg1* had enhanced root growth rates and explained 66% of the growth rate variation when compared to a non-introgressed line Mo17.Mo17-*eprg1*. We identified 15 genes whose transcript levels differed between Mo17.B73-*eprg1* and Mo17.Mo17-*eprg1*. Two genes associated with the reactive oxygen species scavenging system were differentially expressed suggesting a role for free radical scavenging in explaining natural variation for root growth rates.

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P200

Identification of Flowering Time and Plant Height QTL in Sorghum using Introgression Mapping

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Sorghum is a photoperiod-sensitive, short-day tropical species that shows long delays in flowering at temperate latitudes. Most temperate-adapted sorghum cultivars are photoperiod-insensitive and dwarfed for grain production. Classical segregation studies predict that temperate adaptedness involves four major loci each for flowering time and dwarfing. Two major flowering loci, Ma1 (PRR37) and Ma3 (phytochrome B), and a single major dwarfing locus, Dw3 (PGP1/br2), have been cloned. We used introgression mapping to map sorghum flowering time and plant height QTL in a set of ~800 sorghum conversion (SC) lines. SC lines are exotic varieties that have been introgressed with early flowering and dwarfing QTL from a common, temperate-adapted donor through backcrossing. Illumina genotyping-by-sequencing was used to identify polymorphisms in the SC lines, their exotic pre-converted (PRE) progenitors, and the temperate-adapted donor (BTx406). The frequency of BTx406 introgressions, mapped in 10kb intervals across the genome, identifies both the major cloned genes and several uncloned loci. To assess the phenotypic effects of individual introgressions, near-isogenic lines (NILs) can be generated by crossing an SC line to its corresponding PRE line. Because ~800 SC/PRE pairs are available, NILs for common introgressions can be studied across a multitude of genetic backgrounds. Six large SC/PRE F2 populations were selfed in the 2011-2012 winter nursery. In summer 2012, F3 populations will be grown to assess the phenotypic effects of the segregating introgressions, both alone and in combination, and to fine-map the underlying uncloned loci.

P201

Identification of Genetic Loci in ex-PVP Maize Inbreds Contributing to Agronomic Performance

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Elite inbred lines developed by Pioneer Hi-Bred and Holden's Foundation Seeds, which were formerly used extensively for elite commercial hybrid seed production, were recently released from Plant Variety Protection (PVP) or patent protection (ex-PVP inbreds). Prior to the expiration of their patent or PVP, ex-PVP inbreds were not publicly available to other organizations for crossing. The recent public release of these inbreds represents a unique opportunity to evaluate genotypic and phenotypic variation in inbred progeny from two historically and commercially important germplasm lineages. An F2:3 mapping population was derived from PHG35 (Oh7-midland/Iodent) and LH51 (a backcross recovery derived from Mo17), and testcrossed to LH119, PHG39, and a Dow AgroSciences (DAS) proprietary tester. The testcross progeny were evaluated for agronomic phenotypic performance and compared to commercial checks in 2010 and 2011. QTL analysis on these datasets identified significant QTL for agronomic traits, including grain yield, plant height, ear height, grain moisture, and root and stalk lodging. Favorable alleles were identified in both parents, indicating that transgressive segregation could produce inbred lines that out-perform both parents. Yield trial results also indicate that several of the F2:3 progeny compete with commercial checks and could be used for research and breeding in the public-sector.

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P202

Improving haploid induction and doubled haploid generation in maize

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Production of double haploid maize lines uses pollen-mediated induction of gynogenetic haploids followed by chromosome doubling in the seedling meristem to restore fertility. The molecular mechanism of gynogenetic haploid induction is undetermined and the limitations to and optimized methods for doubling are not publicly available. We have set out to optimize two aspects of this process. Populations generated by crossing inductively competent maize lines into backgrounds with heat, drought, and Maize Streak Virus resistance have been evaluated for induction rate. We have also made crosses into lines with compromised epigenetic regulation to test for the role of DNA methylation, chromatin marks and small RNA in the mechanism of haploid induction. To optimize doubling, we have evaluated several microtubule-binding herbicides as less toxic alternatives to colchicine, as well as different methods of herbicide delivery. Flow cytometric analysis of nuclear DNA content was used to rapidly compare doubling agents and treatment methods for efficacy. While many drug delivery methods did not work, surprisingly, we have found that direct delivery of drugs to the meristem of maize is possible by low speed centrifugation of intact seedlings. By altering the chromosome doubling agent and the method of delivery to the meristem we are confident that DH breeding can be accomplished with minimal equipment and without electricity or refrigeration. This would enable doubled haploid methods to be put to use in the preservation of genetic diversity and in the improvement of locally adapted maize varieties.

P203

Introgression of Teosinte Genes for Improving Yield and Disease Resistance in Maize

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Exotic germplasm, including wild species, is important to increase genetic diversity in domesticated crops. A set of inbred lines (teo-lines) were developed by first crossing *Zea Mays ssp. mexicana* Race Chalco with BSSS, and then followed by three rounds of backcrossing to elite maize lines. Data suggest that the testcrosses of the teo-lines have on-par yield performance and significantly lower aflatoxin accumulation as compared to the commercial hybrids.

P204

Investigating the Genetic Architecture of Grain Protein Concentration in the Illinois Protein Strains

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Identification of the genetic mechanisms underlying phenotypic variation between and within populations is essential to understanding natural selection and evolution. Changes in gene expression are an important source of phenotypic variation and may arise due to a combination of *cis*- and *trans*-regulatory variation within complex genetic networks. This complexity has hindered identification of genomic targets of both natural and artificial selection. Long-term phenotypic selection experiments in plants and animals provide an invaluable resource for studying evolutionary change in natural populations, with implications for enhancing the efficiency of genetic gain in plant and animal breeding. The Illinois Long-term Selection Experiment for grain composition is a classic in plant genetics, and although conducted for more than a century, the tools for studying genome-wide responses to selection are just becoming available. Previous QTL mapping studies suggest that changes in protein concentration in the Illinois experiment are governed by many genes of small effect, but these studies are limited by insufficient marker density to capture local changes in linkage disequilibrium. Here we investigate the contribution of *cis*- and *trans*-regulatory variation in the Illinois Protein Strains using functional genomics approaches. We provide evidence for at least one major-effect factor contributing to high seed protein concentration in Illinois High Protein (IHP), and another major-effect factor associated with the response to reverse selection from IHP, which acts by a distinct regulatory mechanism. We also outline ongoing genomics approaches for further study of the genetic mechanisms underlying phenotypic variation in the Illinois Selection Experiment, including the use of an association mapping population and sequence capture array for high-density genotyping.

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P205

Joint linkage analysis and GWAS in the NAM population identifies genes associated with carotenoids and tocochromanols in maize grain

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Maize is a primary food source for over one billion people in sub-Saharan Africa and Latin America. However, maize typically grown in these regions does not provide enough provitamin A and vitamin E to meet daily recommended intake levels. Subsequently, vitamin A deficiency (VAD) and, although not as prevalent, vitamin E deficiency (VED) are major health issues that affect many people. Biofortification of maize grain, in which carotenoids with provitamin A activity (e.g., β-carotene) and tocochromanols with vitamin E activity (e.g., α-tocopherol) are boosted through marker assisted selection (MAS) on target genes, is one solution for reducing VAD and VED. To identify these genes, we conducted over 8,500 high-pressure liquid chromatography runs to quantify 14 carotenoid and 7 tocochromanol compounds in grain from the nested association mapping (NAM) population. The genetic architecture of these compounds, as well as their relevant ratios, was characterized through joint linkage analysis and genome-wide association studies. We identified an average of six QTL associated with each carotenoid compound and an average of thirteen QTL associated with each tocochromanol compound. Typically, each detected QTL is common to as few as 2 and as many as 22 NAM families. The largest effect sizes of QTL for β-carotene and α-tocopherol suggest fold increases of 1.3 and 1.7 relative to B73, respectively. There are common QTL with effects estimates that are higher, lower, or equal to B73, suggesting the presence of an allelic series. Moreover, the same QTL are often found for multiple traits (i.e., pleiotropy), some of which coincide with carotenoid or tocochromanol biosynthetic pathway genes. The architecture of tocochromanol and carotenoid traits appear to be less complex than developmental and morphological traits, thus suggesting that these biochemical traits are highly amenable for MAS in biofortification maize breeding programs.

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P206

KeyPoint Mutation Breeding: Novel Traits in Several Months

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Elite plant varieties are complex mosaics of carefully combined loci (haplotypes). It is often desirable to introduce new traits as simple add-ons, without haplotype disintegration by cross breeding, and with short time-to-market. We present Keypoint® MB, a new targeted mutation-selection technology that delivers plants with substantial novel sequence variation in specific genes in ~4 months, starting from any desired (elite) seed material. This new variation is then used to select improved trait performance. We illustrate our technology with examples of improved traits in tomato and oilseed rape and present preliminary technical results in maize. Using large allelic series in a hormone response gene, we demonstrate how subtle new polypeptide modifications can improve the growth properties of these crop plants. Our technology has been specifically designed to support practical breeding. Keypoint® MB has wide potential to improve specific traits in crop plants including maize in a very flexible, quick and economical way.
The KeyPoint MB technology is covered by patent applications owned by Keygene N.V.

P207

Large Effect QTL Explain Natural Phenotypic Variation for the Developmental Timing of Vegetative Phase Change in Maize

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Vegetative phase change is an intrinsic component of developmental programs in plants. Juvenile and adult vegetative tissues in grasses differ dramatically in their anatomical and biochemical composition. These traits affect the utility of specific genotypes for use as animal feed and as a biofuel feedstock. The molecular network controlling the process of developmental transition is incompletely characterized. In this study, we used scoring for epicuticular wax as an entry point to discover quantitative trait loci controlling phenotypic variation for the developmental timing of juvenile to adult transition in maize. We scored the last leaf with epicuticular wax on 3875 recombinant inbred lines from the Nested Association Mapping (NAM) population, 302 lines of the intermated B73xMo17 (IBM) population, 277 recombinant inbred lines from Oh43xW64A, 243 recombinant inbred lines from Ny821xH99 populations, and 573 inbreds found in the Wisconsin Diversity Panel across multiple seasons. The last leaf with epicuticular wax ranged from leaf 4.5 to 14.2 across all 5270 unique genotypes with a heritability of 0.6 in the IBMs, 0.53 in the diversity panel and a repeatability of 0.72 in the NAMs. Three major QTL located on chromosome 2, 3 and 9 were detected in all three biparental RIL populations and 22 NAM families. A genome wide analysis of the NAMs identified the most significant marker on chromosome 9 positioned within *Glossy15* , a gene controlling expression of adult leaf traits, as well as a candidate gene on the end of chromosome 2 adjacent to the most significant marker (LOD = 212.4). The most significant gene on chromosome 2 is a homologue of the Arabidopsis transcription factor, *enhanced downy mildew-2* , which has been shown to regulate disease response, flowering time and to promote the transition from juvenile to adult vegetative phase. Overall, these results show that several major QTL and potential candidate genes underlie the extensive natural variation for this developmental trait.

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P208

Leaf epiphyte function: how abiotic stress and fungal disease organisms interact with community structure

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Plant leaves are inhabited by a diverse population of microorganisms that are important contributors to the overall health of the plant species. We previously examined the relationship between bacterial diversity and pathogen resistance in Zea mays L. and it has been proposed that the presence of certain species of bacteria on corn leaves could increase resistance to fungal infection. It is important to look at how microbial diversity on corn plant leaves changes in relation to changes in the environment and how these changes contribute to the overall health of the plant. The purpose of this experiment is to examine how microbial communities change when exposed to multiple biotic and abiotic stresses. In order to determine appropriate sample sizes for each variable or combination of variables a power analysis was conducted. The permutated data was analyzed using ANOVA methods to determine the power for various sample sizes.
Two inbred genotypes of Zea mays L. will be planted at the Central Crops Research Station in Clayton, NC and exposed to environments with multiple stress factors. These factors include normal conditions (standard water availability and use of fertilizer in the soil), drought, low nitrogen, and southern leaf blight inoculation. Leaf wash samples will be taken from plants early in the season (before pollination) and analyzed for microbial community composition. The differences in these populations will be correlated with pathogen resistance that will be observed later in the season.

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P209

Maize *ZAR1* transgene by environment effect on yield and relation to crop breeding

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Genotype by environment interaction in complex agronomic traits is a well-known important factor in crop breeding. However, in biotech crop improvement, transgene performance tested under different environments has rarely been evaluated in relation to environmental classifications. Here we report that overexpressing *ZAR1 (**Z**ea mays **AR**GOS1)*, a maize ortholog of Arabidopsis ARGOS gene (***A***uxin ***R***egulated ***G***ene involved in ***O***rgan ***S***ize), enhanced plant and organ growth, grain yield, and also drought stress tolerance. Yield testing under different environment classes showed that the *ZAR1* transgene effects on yield are subject to environment interaction, with significant yield increase or reduction depending upon specific environment classes. Natural allelic variations in the *ZAR1* gene also associate with drought stress tolerance. Moreover, two alleles are predominant in the elite germplasm through breeding selection. These two major alleles are differentially expressed in the hybrid and encode protein variants. Each allele is represented and conserved in the Stiff Stalk and Non Stiff Stalk heterotic pools, respectively, suggesting a contribution of *ZAR1* to hybrid maize development. This work demonstrates a link between transgenic and natural crop breeding selection.

P210

Mapping domestication QTL in a Maize-Teosinte BC_2S_3 population using GBS data

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We developed a set of 866 maize-teosinte BC_2S_3 RILs for which W22 was the maize and the recurrent parent. The lines were scored for ~50,000 marker loci using genotyping-by-sequencing (GBS) technology and phenotyped for 18 traits during three field seasons (years). Since BC_2S_3 is a non-standard breeding scheme, we modified the multiple QTL mapping tools in R/qtl to accommodate this scheme or other schemes with any number of generations of backcrossing, self pollination, or backcrossing followed by self pollination. The new set of RILs and mapping techniques have allowed us to find larger numbers of QTL than identified in previous QTL mapping studies with teosinte. Many large effect QTL were identified and exhibit narrow 1.5 LOD intervals, making them attractive targets for fine mapping. For instance, while previous mapping efforts have identified up to 11 QTL for days to flowering, our analysis identified 23 QTL controlling this trait. The largest of these QTL falls over *ZmCCT* on chromosome 10. The 1.5 LOD support interval for this QTL is 0.65 cM and 7.6 Mb and encompasses only 179 known genes. We identified 4 QTL for ear shattering including one on chromosome 5 with a 1.5 LOD support interval that includes only 30 known genes. Similarly, we identified 11 QTL for prolificacy, including one which falls over *gt1*, a strong candidate gene for this trait. The *gt1* 1.5 LOD support interval spans 372kb and 0.62cM and encompasses only 9 known genes. This set of lines should be a useful resource for maize the community both for QTL mapping and as a starting point for fine mapping of QTL to single genes. The seed of these lines is available from the Maize Genetics Cooperative and the marker data is available at *panzea.org*.

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P211

Mapping Maize Susceptibility to Pre-Emergent Treatments of Saflufenacil

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In modern commercial production of Maize, herbicide tolerance provides a great advantage in integrated management practices to produce the highest quality and yield. BASF product SharpenTM powered by KixorTM, has the active ingredient Saflufenacil. This is a labeled pre-emergent herbicide for use in maize. In multiple trials, genotypes were selected for susceptibility to this product. Having found susceptible genotypes, it is possible to map the genes that underlie the biological process by which this herbicide affects maize, and how tolerance is achieved.

Nested Association Mapping (NAM) founder lines were screened with the Herbicide as a pre-emergent, and lines showing symptoms were advanced for continued testing. NAM founder line "Ki3" showed very identifiable chlorosis at early leaf stages. A selected 200 RIL's from NAM family 13, as well as the Association panel consisting of 282 lines were grown and treated with the herbicide pre-emergent, and used to map loci involved in the susceptibility of this herbicide. The goal of this study is to find loci associated with the activity of this chemical herbicide in maize and begin to understand the specific mechanism of susceptibility vs. tolerance. With this knowledge it is possible to design and understand new chemistry in future herbicides, as well as to select for genetics that will tolerate new or old chemistries. Preliminary results will be presented.

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

P212

Mapping of a major QTL, qPH3.1, for plant height using near-isogenic introgression lines and cloning of the candidate gene in maize

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In maize, plant height (PHT) is closely associated with biomass, lodging resistance, and grain yield. The characterization and cloning of the genes for PHT contribute to the understanding of its genetic basis, which can guide the genetic improvement of crops. In this study, a quantitative trait locus (QTL), qPH3.1 for PHT, was identified on chromosome 3 using populations derived from
the crossing of SL15 and its near isogenic line (NIL), Zong3. Remapping using a small NIL-F2 population placed qPH3.1 within a 4.0 cM interval, explaining 22.1% of the phenotypic variance.Two larger NIL-F2 populations containing 617 and 2153 individuals were also used to develop a sub-contiguous near-isogenic line (sub-NIL). Substitution mapping with eight sub-NILs further
narrowed down the qPH3.1 within a 12.6 kb interval. One ortholog of OsGA3ox2, designated as ZmGA3ox2, encoding a GA3 β –hydroxylase was successfully cloned. Association mapping identified seven polymorphisms in the ZmGA3ox2 that were significantly associated with PHT across two environments. Quantitative RT-PCR (qRT-PCR) showed that SL15 had significantly higher levels of ZmGA3ox2 transcript in stem apex than in Zong3. DNA sequencing and linkage assay confirmed that a large deletion in encoding region of ZmGA3ox2 is responsible for dwarf mutant d1-6016 . We have successfully cloned qPH3.1 which provides novel information on
genetic basis of plant height in maize, as well as an opportunity for the improvement of plant architecture in maize breeding.

P213

Mapping of genes involved in carbon partitioning

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Carbon partitioning (CP) is the process where carbohydrates assimilate in photosynthetic tissues and are translocated through the phloem into other parts of the plant such as roots, flowers and fruits to facilitate growth and development. Plants with a carbon partitioning defective (cpd)-like phenotype were identified among 9224 families of the Maize TILLING population and outcrossed to the inbred Mo17 to generate mapping populations. Mutations underlying these cpd-phenotypes were genetically mapped using bulked segregant analysis (BSA). When grown in common environments, some sucrose transport mutants show phenotypic variation that is dependent on genetic background. The natural diversity of maize and the extensive characterization of the maize Nested Association Mapping (NAM) population will be utilized to identify interacting genes and their roles in carbon partitioning.
Most previous studies have investigated the role of CP genes in source tissue; however, sucrose transporters can also be involved directly and indirectly in sucrose off-loading from the phloem and their roles in sink tissues are less clear. Mutants of *sucrose transporter 1* (*SUT1*) were used to characterize the effects on root growth and root architecture in soil-filled columns. The root systems of these plants were extracted and length and dry weight measured at different growth stages compared to non-mutant controls. No significant difference was observed between the *sut1* mutants and the controls at two different growth stages. Root : shoot ratios were also unaffected by the *sut1* mutation. These data suggest either that *Sut1* may not play a role in sink strength or that its role is redundant with other proteins.

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P214

Mapping QTLs for Heat Tolerance Traits in Maize Using NAM populations

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As global climate change becomes inevitable, the sustainability of agricultural production in US and worldwide faces serious threat from extreme weather conditions, such as drought and high temperature (heat stress). Heat and drought stresses occurred during maize growing season can cause severe damage to maize developing leaves (leaf firing) and reproductive tissues (tassel blasting, delayed silk emerging etc) and resulting in reduction in pollen production, pollination, kernel numbers, kernel size, and total grain yield. Understanding high temperature adaptation mechanisms in maize is crucial to the success of developing high temperature tolerant hybrids. However, little is known about the mechanisms of high temperature tolerance in maize. To dissect the genetic control of heat tolerance in maize, we screened a large number of maize accessions for variation in heat tolerance under field condition. In this study, 2 RIL populations were selected from publically available NAMs population and used for heat tolerance evaluation in 2011. Various heat tolerant phenotypes were evaluated at different developmental stages during growing season. Genetic analysis suggests several genetic traits contributing to the heat tolerance in maize, suggesting complex genetic nature of heat tolerance in maize.

Funding acknowledgement: United States Department of Agriculture (USDA)

P215

Marker imputation prior to genomewide prediction in a mixed maize population

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Genomewide predictions for mixed populations require a relatively high marker density to achieve acceptable prediction accuracy, but high density genotyping of an entire population can be prohibitively expensive. Marker imputation may deliver the benefits of dense marker coverage at a fraction of the cost. We used a population of 288 historical Minnesota and non-Minnesota inbreds that were genotyped with the Illumina MaizeSNP50 BeadChip and phenotyped for several traits. The inbreds were randomly partitioned into 96 lines where the high density marker genotypes were retained, and 192 lines where 96, 192, 288, 384, 768, 1536, 3000, or 6000 markers were retained and all other marker genotypes assumed unknown. The program fastPHASE was used to impute the missing markers and run 100 times; the mean genotype call was used in subsequent genomewide predictions. Accuracy of imputation ranged from about 70% (96 markers) to nearly 90% (6000 markers). We will present results on genomewide prediction accuracies using the original marker dataset, the imputed marker dataset, and the marker subset and show how the imputed markers affect prediction accuracy.

Funding acknowledgement: Minnesota Agricultural Experiment Station

P216

Numerical optimization of a marker-assisted backcrossing scheme for introgression library construction

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We are developing QTL-targeted tiling paths of near-isogenic line allelic series' to study the genetics of maize photoperiod sensitivity and environmental adaptation. While methods have been developed to backcross a single genomic interval from a donor parent into a recurrent parent genetic background, methods to produce an introgression tiling path are not available. We propose a method for marker-assisted backcrossing that uses marker and pedigree information to form such tiling paths across chromosomal regions of interest. Our method optimizes the construction of tiling paths, throughout a series of successive backcrosses, by finding a *minimal spanning set* of individuals with crossovers in the region of interest. Using simulations, we compute the number of families and individuals per family that need to be genotyped over successive generations to produce an optimal set of iso-lines that will constitute an introgression library. In this study, tiling path construction across four distinct regions of the maize (*Zea mays*) genome was investigated, using the nested association mapping linkage map as a basis for the simulation parameters. Assuming a count-location model for recombination, we observed that the number of best individuals obtained depends on length of the chromosome, length of target region, and a chromosome-specific Poisson parameter that determines the number of crossovers in each cross. We found that it is more efficient to "pool progeny" in each generation and to genotype more individuals towards the end of the backcross scheme.

Funding acknowledgement: United States Department of Agriculture (USDA)

P217

Phenotypes and genes associated with root traits to search for phosphorus acquisition efficiency in maize

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Phosphorus (P) is an essential nutrient to plants and is acquired as inorganic phosphate from the rhizosphere solution. P is one of the least available nutrients particularly in highly weathered, tropical soils, limiting substantially plant growth. An interesting approach to circumvent P deficiency in tropical areas is to explore the genetic diversity available in plants to breed cultivars more efficient in P acquisition. It has been shown that root traits, such as root length and volume, are important to determine if a genotype is P efficient. This study aimed to study root traits that could be involved with P acquisition efficiency and to identify candidate genes with an expression profile consistent with a possible role in root morphology. Field phenotyping results under low and high P conditions enabled us to define two contrasting genotypes for P acquisition efficiency that were used for root traits studies. We standardized the nutrient solution conditions in order to find the best phenotyping parameters for root early screening. Root traits presented overall a high heritability and a low coefficient variation. Also, out of 24 root traits analyzed, 10 presented a correlation above 0.9.These results together with PCA allowed us identify four root traits which adequately represent the variation observed among genotypes. The information described in this study is important for designing early selection strategies for P efficiency in maize, which are needed to support advanced molecular and physiological studies.

Funding acknowledgement: Embrapa, Fapemig, McKnight Foundation and Generation Challenge Program (GCP)

P218

Precision phenotyping gray leaf spot disease using real-time PCR and digital image analysis

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Gray Leaf Spot (GLS) disease of maize is caused by either of two fungal species *Cercospora zeae-maydis* or *Cercospora zeina*. Whole plant phenotyping is currently used to score for resistance to GLS in the field, however methods were lacking for precision phenotyping in glasshouse trials or field screens for quantitative resistance. We developed a specific and sensitive real-time PCR assay for determining the amount of Cercospora DNA relative to maize DNA in a diseased leaf, which is effective for both species of Cercospora. In addition we quantified GLS lesion area by digital image processing of leaf photographs using ASSESS 2.7 software. The methods were validated in glasshouse samples as well as field samples of two maize lines differing in resistance to GLS based on whole plant phenotyping. The amounts of Cercospora DNA within leaves of the susceptible and resistant inbred lines were significantly different (*T*-test, $p<0.001$). The amount of Cercospora DNA detected by the real-time PCR assay correlated well with calculated lesion area (Pearson correlation coefficient = 0.8). These assays will be useful for both basic research into understanding the GLS-maize pathosystem, as well as breeding programmes.

Funding acknowledgement: Technology Innovation Agency (TIA) South Africa, National Research Foundation (NRF) South Africa

P219

Principal component analysis in hybrid corn in terms of salinity

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In this study 21 hybrid corn in block shape as chance in 3 repetition at 2 experiment in separate state at normal terms and salinity in 1385 in from conter seruting of natural sources and agriculture jehad in Qom province had been evaluated. Soil tissue with electric conduction (EC) in depth 0-30, was 8.5 desi zimence and in 30-60 depth, 9.9 desi zimence and soil pH was equal with 7.6.

In tension environment:
Special amounts outcome of component 1 to 8 were more than one, and 23, 15, 13, 10, 7, 5, 7, 4. percent, respectively and totally, they had explanted 84 percent of total variance of variables. Comparative amount of special vectors coefficient in first component showed us that, corn weight lineament, bush height, the width of corn leaf and the surface of leaf flag, were the most important quality for grouping hybrid in claster analysis. In second component, the surface of corn leaf, the length of flag's leaf and exterior pedankel and in third component the numbers of seed in row, the number of leaf, altitude to corn leaf and the longitude of corn leaf had more coefficients of special vector. In this order, in forth component, the number of dry leaf, total operation and seed operation and removal index and in fifth component, corn diagonal, corn length, abortion seed and the depth of seed and in sixth component, diagonal of haulm and inside pedankle and in seventh component of TAji flower, the number of corn in bush and the weight of thousand seed, slink seed and in eighth component of latitude of flag's leaf and the diagonal of corn's wood. Have more special coefficients of vectors.

Key words: corn, salinity, hybrid, principal component.

Funding acknowledgement: United States Department of Agriculture (USDA)

P220

Quantitative genetic analysis of grain yield under drought and low soil N stress conditions in the IBM population

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The development of corn (Zea mays L.) hybrids that combine high and stable grain yields under abiotic stress with high yields under normal conditions can lead to sustainable corn production in a changing and highly unpredictable climate. This study aimed at identifying chromosomal regions and secondary traits that are associated with high grain yield under drought and low N stress in the intermated B73 x Mo17 (IBM) mapping population. Testcrosses of 238 recombinant inbred lines were evaluated in multi-location trials under managed drought stress and low N stress conditions in Minnesota for two years. The phenotypic data showed that delayed leaf senescence and high leaf chlorophyll content are favorable for high grain yield under drought stress and low N stress and therefore can be used for indirect selection for grain yield. Heritability estimates for grain yield decreased from 0.60 under normal conditions, to 0.47 under low soil N stress to 0.37 under drought stress conditions. Quantitative trait loci (QTL) mapping analysis revealed a total of 21 QTLs for grain yield and two mapped on the same region on chromosomes one and four (bin 1.09 and bin 4.08) across the three different management levels. Co-locations of QTLs for grain yield, leaf senescence, ASI, plant height were common under both drought and low N stress conditions, confirming the strong genetic correlations that had been observed. Our results indicate that there is enough phenotypic and genotypic variability in the IBM population for drought and low N stress tolerance which when coupled with information from secondary traits, can help understand the genetic basis of stress tolerance.

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P221

Raiders of the Lost "A" Lines: Exploring Linkage Disequilibrium and Population Structure of the Historic Minnesota Breeding Program

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From the 1920s to the 1980s, the University of Minnesota developed inbreds adapted to the northern U.S. Corn Belt, including some that were widely used as single cross parents (e.g., A632, A619, and A654). The Minnesota "A" lines represent an early-maturing genetic background that is different from the later germplasm (e.g., B73, Mo17, and Oh43) that has been used widely in other parts of the U.S. Corn Belt. Since the 1980s, the Minnesota collection of A lines has been underutilized and has become more of a seed morgue than a seed bank. The availability of dense SNP assays and advancements in statistical procedures provide new ways to explore the variation present in the A lines. The objectives of this project are to characterize the extent of linkage disequilibrium (LD) and population structure present in the A lines and a collection of public and private inbred lines. A total of 143 A lines and 141 ex-PVP and public lines were assayed using Illumina's MaizeSNP50 Beadchip, providing more than 56,000 SNP markers. The decay of linkage disequilibrium ($r^2 = 0.1$) ranged from about 2kb to almost 500kb among chromosomes. LD decay also varied slightly when comparing the A lines versus the entire inbred panel. Subpopulations within the panel were identified using Structure software. This research is providing insights into the organization of early maturing corn inbreds and background information for implementing genomewide association mapping to identify useful loci present in the A lines but absent in other genetic backgrounds.

Funding acknowledgement: Pioneer Hi-Bred University Plant Breeding Fellowship, Minnesota Agricultural Experiment Station

P222

Real-Time Genome Wide Association Study (GWAS) of Plant Growth in Maize

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Recent advances in maize (Zea mays) population development, high throughput marker technologies, and sophisticated QTL and association analysis methods have provided an opportunity to gain a greater understanding of the complex genetic architecture underlying numerous traits. Resources currently available in maize have provided an excellent opportunity to examine the relatively unknown genetic architecture of maize plant height and growth rate over time (i.e. emergence to flowering). In the summer of 2011, we planted a subset of the 282 inbred association panel comprised of 103 inbred lines at two field locations in Missouri, with four replicates each. Plant height was measured weekly from emergence to flowering which enabled us to calculate the weekly growth rate of each line. Additionally, plant height and growth rate were calculated for the vegetative phases of V5, V10, and final plant height (FPH). Genome-Wide Association analysis was performed on the weekly and vegetative phase plant height and growth rates using the multiple linear model (MLM) method in TASSEL 3.0 and a 132,842 SNP marker set. After multiple test correction, numerous significant associations were detected for both plant height and growth rate throughout the maize growth cycle. Interestingly, many of the significant associations detected during the maize growth cycle for plant height were not significant for FPH. Significant associations for plant height were gained and lost during the maize growth cycle, suggesting that important plant growth QTL are not detected when examining only FPH. Based on these results from this study, it appears that traits related to growth need to be examined over time to gain a more thorough understanding of the genetic complexity underlying many of these traits.

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

P223

Root traits analysis of recombinant inbred lines underlying phosphorus acquisition efficiency in maize

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Phosphorus (P) is essential macronutrient for plants, which is acquired from the rhizosphere solution as phosphate (Pi), primarily in the form of H2PO-4. The concentration of Pi in the soil solution is often low, therefore the supply of Pi to the root surface by diffusion is slow. Hence, P is one of the least available mineral elements in the soil and frequently limits plant growth. Crop production always requires P fertilizers to maintain yield and quality. An alternative to ameliorate this problem is to explore the genetic diversity available in plants and breed more Pi-absorption efficient cultivars in combination with soil management practices. This study aimed to analyze maize root traits that could be involved in P acquisition efficiency. We used a paper pouch system with Magnavaca´s nutrient solution (low P - 2.5 uM) under a controlled environment to evaluate four root traits (length, volume, diameter and volume of fine roots) in a population of 145 recombinant inbred lines (RILs) derived from a cross between maize genotypes L3 and L22, which have contrasting adaptation to low P availability in the field. High heritability (79.1 to 86.1%) and low coefficient of variation (7.1 to 22%) were detected for the root traits. Additionally, Principal Component Analysis (PCA) enabled us to differentiate contrasting maize RILs based on the selected root morphology traits. The first principal component (PC1) explained 66.2% of the variation while the second principal component (PC2) explained 31.2%. PC1 had positive eigenvector coefficients for all variables, except for root diameter. PC1 was explained most by root volume and PC2 was explained mostly by root diameter, allowing identification of the lines with contrasting root system traits. These phenotypic results will be used in the discovery of root morphology QTLs that are also involved on P acquisition efficiency in maize.

Funding acknowledgement: Embrapa, CNPq, Fapemig and Generation Challenge Program (GCP)

P224

Seeds of Discovery; Opening and utilising the black box of maize genetic diversity

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Mexico and neighbours in Central America comprise the centre of origin and diversity for the world's most widely grown crop. In the 1960s and 1970s, CIMMYT's maize breeding program extensively sampled landraces to form the genetic base from which many modern inbred lines and populations have been derived. Nonetheless, only a small fraction of the total diversity in the region has been exploited, and many alleles of potential value may not have entered breeding programs. Through Mexican Government funding, the collaborative Seeds of Discovery (SeeD) initiative aims to systematically characterize and mobilize the underutilized genetic diversity within the maize germplasm collections of CIMMYT and Mexican partners to benefit Mexico and the world. SeeD will assay the genetic structure of Mesoamerican maize landrace accessions, and will identify novel alleles for important traits through genome-wide association mapping in broad samples of the collection, and by the study of allele frequency gradients in accessions sampled from stress-prone regions. Breeding populations containing high value underutilized alleles will be formed and improved rapidly through genomic selection.

In 2011, SeeD started an extensive association mapping project conducted in indigenous maize germplasm by testcrossing single plants from the CIMMYT Maize Breeder's Core Collection of landraces to adapted hybrid testers. The male landrace parent of each testcross is being genotyped via GBS at Cornell. The testcrosses are being evaluated for yield, agronomic characteristics, phenological traits, and grain quality at many locations in Mexico in a collaborative testing effort by INIFAP, Mexican universities, and CIMMYT. The experiment will provide a snapshot of diversity in the collection relative to improved germplasm. It will also identify haplotypes consistently and reliably associated with biotic and abiotic stress resistance, phenology, plant type, and grain quality, and serve as a robust training population for initiating genomic selection in populations derived from Mexican landraces.

Funding acknowledgement: SAGARPA

P225

Steps towards cloning a gene involved in the release of phytosiderophores in maize

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Iron (Fe) deficiency in plants leads to chlorosis, reduced yield, and decreased nutritional quality. Graminaceous plants follow strategy II, in which chelating substances called 'phytosiderophores' are produced and secreted by an unknown transporter, potentially encoded by the *yellow-stripe 3 (YS3)* gene, into the rhizosphere. The Fe(III)-phytosiderophore complex is then taken up by the specific transporter, the *yellow-stripe 1*. The objective of this research is to identify the *YS3* gene using a combination of map-based cloning and transposon tagging approaches. The map-based cloning strategy consisted of phenotypic and genotypic evaluation of about 9,000 F_2 individuals. Transposon based gene isolation was also performed using the Ac and Mu transposable elements. A transcriptome profiling experiment was carried out using F_2 individuals and two different Fe-concentrations in order to identify polymorphisms, novel genes, and differentially expressed genes. The region of interest is located on chromosome 3 near the centromere spanning 1.74 Mbps and consisting of 12 BACs and 17 genes. One *ys3* mutation *(YS3:Mu)* was identified in the Mu tagging population and used to amplify genomic sequences flanking Mu transposons via a Digestion-Ligation-Amplification strategy.

Funding acknowledgement: Max Planck Society, DFG

P226

Study correlation analysis between characters in maize hybrids in salinity stress condition

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In this study 27 maize hybrids in full block shape in random state at 3 repeat in 2 separate test under normal and salinity condition in 1385 farm of natural source of studying center and agriculture Jahad of Qom province had been evaluated. Soil tissue with electrical conductance (EC) in 0-30 depth was 8.5 ds and in 30-60 depth, it was 9.9 ds and soil pH was equal with 7.6. Results of correlation analysis between elaracters in salinity stress condition determined that the character of leaf number with maize length characters, seed number in row and maize weight respectively in probability levels 0.5 , 0.1 , 0.5 have meaningful positive correlation and also the weight of thousand seed with maize length character in probability level has 0.1 meaningful and positive correlation. The character of seed number in every row with removal index in posibile levels has 0.1 meaning. The characters of flag leaf level and external peduncle with total operation respectively in possibility level have 0.1 and 0.5 positive and meaning full correlation. The weight of thousand seed and maize leaf level with seed operation respectively in possibility levels has 0.5 and 0.1 meaningful and positive correlation. Total operation with removal index in possibility levels has 0.5 meaningful and negative correlations. The existence of negative correlation between two character show that if total operation was further, the removal index is fewer (-0.44*) and also seed operation with removal index in possibility level have 0.1 meaningful and positive correlation.

Key words: maize, salinity stress, hybrid, correlation, analysis

Funding acknowledgement: United States Department of Agriculture (USDA)

P227

The Effects of High Plant Density on Morphological and Female Inflorescence Development in Two Reciprocal Recurrent Cycles of Iowa Stiff Stalk Synthetic and Iowa Corn Borer Synthetic Populations

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The Iowa Stiff Stalk Synthetic population, BSSS(R), and the Iowa Corn Borer Synthetic #1 population, BSCB1(R), have undergone 17 cycles of reciprocal recurrent selection. Primary emphasis of selection has been for grain yield and agronomic performance. Recent studies have shown that selection for grain yield has greatly increased the adaptation of the population to high plant density. Advanced cycles achieve maximum grain yield at much higher densities than early cycles. However, less is known about the impact of high plant density of early plant development and morphology. The objective of this experiment was to examine the effect of plant density on morphological and female inflorescence development in BSSS(R) and BSCB1(R) populations. Unselected base populations and the cycle 17 populations and population crosses were planted at four different densities in two replicates ranging from 3.8 to 12.4 plants m-1at the Iowa State University Agronomy Farm outside Ames, Iowa. High plant density reduced rate of morphological development and the rate of female ear development beginning early in development suggesting that understanding the effects of plant density on agronomic performance will require characterization of the effects of high plant density on early plant development.

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P228

The Maize ATLAS project: implementation of an experimental framework for studying adaptation

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Plant responses to environmental variables such as day length and temperature have limited the adaptation and use of 'exotic' sources of maize for N. American production. The USDA-NIFA funded Maize ATLAS (Adaptation Through Latitudinal Artificial Selection; http://www.maizeatlas.org/) project aims to: 1) phenotypically, genetically, and ecologically characterize genomic loci limiting the adaptation of tropical maize to temperate environments to enhance breeding speed and progress; 2) increase knowledge about the genetic basis of response to artificial selection that is fundamental to plant breeding. We are developing new germplasm and genomic resources, methods for crop improvement, and statistical approaches for dissecting the genetic architecture underlying environmental adaptation and response to selection. Here, we provide an overview of the project.

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P229

The role of *Tasselseed1* and *Tasselseed2* in defense response

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Northern Leaf Blight (NLB), caused by *Setosphaeria turcica*, is an important foliar disease in maize, and is controlled by both qualitative and quantitative genetic resistance mechanisms. Many effects are postulated to control the underlying mechanisms of quantitative disease resistance. A SNP in the *Tasselseed2* (*Ts2*) gene was identified through association mapping in a ~300 maize inbred line diversity panel (Flint-Garcia *et al*., 2005) to confer resistance to NLB. Analysis of the *Ts2* gene in the diversity panel identified significant non-synonymous SNP in linkage disequilibrium with the originally identified SNP. Two *ts2* mutants, *ts2-ref* and *ts2-N2409*, were shown to have significantly greater susceptibility to NLB than their wild-type counterparts, with a difference of 8-9% difference in average diseased leaf area. The tasselseed1 (*ts1*) mutant, *ts1:Ds*, was also examined for association with resistance to NLB. The *ts1:Ds* mutants were found to be more susceptible to NLB, with a difference of approximately 20% in diseased leaf area compared to wild type plants. A QTL identified through the Nested Association Mapping population was previously identified on chromosome 2, in a region that encompasses the *Ts1* gene. *Ts1* is a viable candidate gene for resistance to NLB underlying the NAM population QTL. TASSELSEED1 is a lipoxygenase gene, and TASSELSEED2 is an alcohol dehydrogenase gene, both with suspected roles in the jasmonic acid pathway determining sex differentiation in the floral architecture of maize. Our study highlights the importance of the hormonal regulatory process in quantitative resistance mechanisms.

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P230

Use of Public Germplasm Resources for the Research of the Genetic Inflorescence Architecture in Maize

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The availability of public research germplasm has been a useful tool in the understanding of the genetic relationships in maize inflorescence architecture. Public resources such as the Nested Association Mapping population (NAM), the NAM test crosses (TC) and the Ames Association Panel TC sets are currently used to phenotype tassel and ear traits. Tassels and ears are first collected from experimental fields. Phenotypic information is then collected via electronic data capture using Ipads and bluetooth scanners for nine ear traits and 12 tassel traits. Genotypic information is publically available for some of these sets. Previous QTL findings in the NAM population have led to the targeting of quantitative trait loci (QTL) regions for fine mapping of tassel branch number. Preliminary results will be presented. Phenotyping of tassels and ears are underway for the AMES TC set with tassels collected in IN, MO NE, NY and IA with a total of over 6000 tassels collected in 2011. The NAM TC set contains over 5000 tassels collected in three locations: IN, NY and IA. We are also examining the AMES set per se collected in IA with 3150 tassels. This TC material will enable us to look at expression of QTL in a hybrid context and with much more power to test candidate genes than the 281 line Goodman Association Panel. Preliminary QTL and statistical analyses will be presented.

Funding acknowledgement: National Science Foundation (NSF)

P231

Using GBS data to improve resolution for the maize Nested Association Mapping (NAM) population

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Previous studies have described the use of 1106 markers from an Illumina Golden Gate array to conduct joint linkage analysis and to project SNPs from densely genotyped parents for genome-wide association (GWA) analysis. Low to moderate coverage genotyping by sequencing (GBS) data now provides a lower cost alternative for generating data for very large number of SNPs and the opportunity to improve mapping resolution compared to the earlier array-based markers. However, use of GBS data for bi-parental populations has challenges. The main one is that coverage is low enough for individual SNPs, that at heterozygous loci, often only a single allele is sampled and the locus appears to homozygous. We present a method using a hidden-markov model (HMM) that can use GBS data to call heterozygotes with a high degree of accuracy and is flexible enough to correct other types of errors as well. The use of GBS data to improve resolution in analyses of the NAM population is demonstrated.

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

P232

How well do you know YOUR maize research community?

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MaizeGDB is a database of maize genetics and genomics information accessible to anyone in the world. Researchers use MaizeGDB regularly for biological investigations, but we've leveraging it in a different way; to better understand the evolution of the research community. Data mining reveals details such as distribution of maize cooperators by country and, within the US, by state. By analyzing the collection of articles curated into MaizeGDB, we've determined which words are the most prevalent, which authors are listed on the most publications, and much more. Stop by to learn some lesser known facts about the maize research community!

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P233

Alpha.MaizeGDB.org: MaizeGDB's interface redesign

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To prepare for both current and future needs of the community, MaizeGDB, the maize research community's Model Organism Database, has complete the first year of a two-year effort to redesign its interface. The redesign is both cosmetic and functional. The overall goal of the redesign is to create a clean modern interface with improved user interaction while expanding the overall functionality of MaizeGDB. Cosmetically, we modernized the appearance and simplified page organization and navigation. A key component to the redesign is community involvement. To ensure that the new interface is useful, guidance and beta testing groups will be created and consulted. To get your immediate feedback, we have released an alpha version, which can be found at http://alpha.maizegdb.org. Since this is an alpha release, be aware that not all pages are functional and there are some known bugs. A beta release is expected sometime in late 2012. The second year of the redesign will focus on functionality. We will put particular emphasis on ways to: view and compare multiple maize genomes, enable access to billions of SNPs, and deploying novel search and browse methods for phenotypes, gel images, QTLs, and other data types. Here we report our updated timelines and focus on concepts and designs, future features to be deployed (data, tools, and resources), and outline how you can be involved in changing MaizeGDB to better meet your needs.

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Learn How to Use MaizeGDB

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MaizeGDB is the model organism database for maize. In addition to serving a B73_RefGen-based genome browser with genome features, SNPs, expression data, etc., MaizeGDB also serves loci, phenotypes, alleles, genetic maps, QTLs, stocks, probes, images, and more. As datasets become larger and more complex, MaizeGDB evolves to meet emerging needs. Here we describe many new features that endeavor to make your interactions with MaizeGDB simpler and more robust. For example: in the last year a new BLAST user interface designed to make it easier to access diverse datasets has been deployed, GBrowse2 with new features that speed and simplify genome interaction has been released, a total redesign of the web interface is underway to make it easier for you to interact with the increasingly large and complex datasets, and a BioCyc-based metabolic pathway tool has been has been made available. We encourage you to stop by this poster to see how to use some of the new features, guide our development, and ask questions.

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P235

MaizeGDB – use cases that leverage new data and tools.

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Various tools have been implemented at MaizeGDB over the past year to add functional annotation to the B73 reference genome sequence. These include high quality gene expression data and computational inferences about metabolic pathways and related metabolites. The newest data and tools are presented in accompanying posters at the meeting and also in a MaizeGDB tutorial offered Thursday afternoon. This poster will present detailed workflows for use cases and is directed at persons wishing to discover candidate genes for functions of interest. Please bring your specific requests, and of course, all ideas about how we might better facilitate access to data for your research.

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Gene Expression Resources Available from MaizeGDB

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The completion of the maize genome sequence in 2009 has created both significant challenges and opportunities for maize researchers. The opportunities for understanding cellular processes underlying maize's phenomenal productivity have never been greater but this opportunity can only be seized if functional genomics software tools (FGSTs) are available to reduce the complexity of multimillion point data sets into manageable images and/or concepts. Currently, MaizeGDB is hosting numerous large gene expression data sets, and furthermore, indications from currently funded NSF Plant Genome Research Projects are that much more data will be deposited at MaizeGDB in the near future. Fortunately for maize researchers, free public domain FGSTs have been developed for other biological systems and their implementation at MaizeGDB can be accomplished with a moderate amount of effort. In this poster, we describe current efforts at MaizeGDB that focus on leveraging two of these FGSTs, the eFP browser (1) and MapMan (2) by creating strategic linkages from MaizeGDB to the sites where these FGSTs are deployed. The eFP browser projects gene expression data onto a series of pictures (pictographs) representing the original plant tissues from which the expression data was derived. Each pictograph is colored according to the level of expression for the gene on of interest. The MapMan software suite allows the visualization of a variety of functional genomics datasets in the context of well characterized biochemical processes and metabolic pathways. Our initial efforts focus on the 60 tissues within the B73 Maize Gene Atlas (3) developed by the Kaeppler laboratory at the University of Wisconsin with the expectation that additional expression data sets characterizing meristem and kernel development will be added in the near future.

1. Winter et al (2007). PLoS ONE 2(8): e718.
2. Thimm et al (2004) Plant J. 37:914-39
3. Sekhon et al (2011) Plant J 66:553-63

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P237

MaizeCyc: Metabolic Networks in Maize

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MaizeCyc is a Pathway Genome Database, and represents a catalog of known and predicted metabolic and transport pathways for corn (Zea mays), which enables plant researchers to study and graphically represent the metabolome of this cereal, thereby supporting integrated systems-biology analysis. MaizeCyc is accessible from the Gramene and MaizeGDB websites, and was created using Pathway Tools software version 15. Analyses and cross-species comparisons are supported for a variety of data, including genetic and phenotypic profiles, transcriptomics, proteomics, and metabolomics data sets. Pathways, reactions, and genes in the catalog are based on the electronic and manual annotations of 39,656 maize gene models in the RefGen_v2 filtered set (Maize Genome Sequencing Project, release 5b.60), and phylogenetically-derived projections from related plant models, including Arabidopsis and rice. This community resource includes sequence-based associations provided by Gramene, MaizeSequence, and MaizeGDB to external database entries from EntrezGene, UniProt-SwissProt, and Gene Ontology. Manual annotations of genes include mapping of classical phenotype genes to sequenced genomic loci (Schnable and Freeling, 2011), proteomics-supported functional annotations (Friso et al, 2010), and enzyme commission code mappings from literature mining. Using expression profiling data from the B73 Maize Gene Atlas transcriptomics set (Sekhon et al, 2010), we created exemplar visualizations of spacio-temporally regulated global gene expression in maize, and will provide these graphic representations freely to the community. This work is supported by the NSF (Gramene: A Platform for Comparative Plant Genomics), and the USDA-ARS (The Maize Genetics and Genomics Database).

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Diversity Data at MaizeGDB

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With the increasing quantity of diversity data, MaizeGDB is laying the groundwork for storage, retrieval, visualization, and analysis of these datasets, some of them very large. Along with personnel from the Schnable lab, iPlant Collaborative, Panzea, and the Genome Reference Consortium, MaizeGDB personnel are exploring alternative storage and database designs for maintaining large public datasets in a way that will enable researchers to access all datasets. As well, we are looking into ways to represent and store the variety of variations that appear in maize, ranging from Single Nucleotide Variations (SNPs) to the more complex Present Absence Variations (PAVs) and Copy Number Variations (CNVs) to complex rearrangements (complex loci), in addition to representing sequences in non-B73 lines that don't exist in the B73 reference genome.

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P239

A Bioinformatic Pipeline to Identify Transposon Flanking Sequences via High-Throughput Sequencing

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The Maize Gametophyte Project (maizegametophyte.org) has generated a large set of new *Ac* insertion lines, using launchpad *Ac* elements located in multiple regions of the genome, and is screening these insertions for gametophytic transmission defects. However, although high-throughput protocols to isolate transposon insertion flanking sequences exist for *Mutator* elements (Williams-Carrier 2010; Settles 2007), none have yet been published for *Ac*/*Ds*. The Transposon Flanking Sequence Finder is a data pipeline designed to locate the positions of new *Ac* insertions by taking advantage of TAIL-PCR and high-throughput sequencing. Using *Ac*-specific primers, PCR products were enriched for the sequences directly flanking *Ac* elements using a modified *Mu*-TAIL procedure (Settles 2004). Products from different lines were bar-coded, pooled, and sequenced via an Illumina HiSeq. After sequencing, the pipeline filters the raw paired-end reads into separate files based on their barcode and the presence of the *Ac*-specific primers. The barcode and primer sequences are removed from the reads and the remaining sequence and its paired read are then aligned to the maize B73 genome using BLAST. The BLAST alignment results are returned in both GFF and CSV formats, allowing the alignments to be viewable in both a genome browser and a spreadsheet viewer. Areas of the genome shown to correspond to a high read frequency represent likely locations of *Ac* elements. Due to the modular nature of the tool it can easily be modified to search for other elements or alignments to other genomes. Controls within the tool allow the user to adjust several options such as the number of acceptable mismatches, the E value for the Blast search and whether the PCR samples were sheared before sequencing. Currently the tool is in the testing phase; small-scale tests have so far been promising.

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P240

A high resolution structural and proteomics atlas of the developing rice leaf

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The C3 grass rice (*Oryza sativa*) is a major food crop and full genome sequences of two rice ecotypes greatly facilitates rice research. Leaf photosynthesis is a key factor in determination of biomass production and grain yield. Here we provide an integrated view of development transitions of a young rice leaf with clear sink- and source zones as determined by 14C-labeling. The leaf structural transitions, including vascular development, and development of the organelles from non-photosynthetic sink zone to photosynthetic source zone, were quantified by microscopy and complemented by photosynthetic pigment analysis. Large scale quantitative proteomics (140 nanoLC-ESI-LTQ-Orbitrap runs; 2 million MSMS spectra) of seven narrow leaf sections, followed by hierarchical cluster analysis and functional annotation, identified >4000 proteins (>6000 protein models) and resolved the major leaf developmental transitions. Rice proteins were annotated for subcellular location and function based on homology to maize and Arabidopsis, taking advantage of our extensive analysis of the maize and Arabidopsis proteome and the Plant Proteome Database (PPDB). This study provides a structural and proteome atlas of the developing rice leaf; we will highlight several transitions along this rice developmental leaf gradient and comment on differences with the leaf gradient of the C4 maize leaf.

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P241

A high-throughput stalk-core sampling device for the evaluation of maize biomass composition

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A major challenge in the identification and development of superior feedstocks for the production of biofuels is the rapid assessment of biomass composition of a large number of plant samples. Our goal was to develop a simple mechanical stalk-core sampling device (CSD) capable of collecting large numbers of uniform maize biomass samples of a dimension compatible with robotic processing and analysis. Empirically, we determined an output of up to 1,000 samples in an 8-hour period using the CSD. A previously described high-throughput digestibility platform was used to assess cell wall glucose and pentose yield in stalk-cores of 513 inbred lines of the Wisconsin Diverse (WIDIV) maize population. High correlations were observed between repeated measurements obtained in individual stalk-cores at two different time-periods for glucose and pentose yield. Large phenotypic variability was observed among inbred lines for the two traits evaluated. These results demonstrate that the CSD generates homogenous stalk-core samples in a repeatable and high-throughput manner and it is therefore well suited for the evaluation of large numbers of plant samples for the development of superior feedstocks.

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P242

An RNA sequencing and microarray-based gene atlas for the maize community

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Transcriptome analysis is a useful way to characterize genes and pathways underlying plant growth and development. We have previously published a maize gene atlas based on 60 unique spatially and temporally separated tissues from 11 maize organs. For this atlas, we custom designed a NimbleGen array based on the B73 draft genome sequence. This resource has been made available to the maize community via MaizeGDB and PLEXdb. To provide enhanced resolution to the maize gene atlas, we have analyzed 18 selected tissues representing five organs using RNA sequencing (RNAseq). For a direct comparison of the two methodologies, the same RNA samples originally used for our microarray-based atlas were evaluated using RNAseq. A similar percentage of genes could be detected in at least one tissue using microarray (79%) and RNAseq (74%) approaches. Differences among the technologies were evident from low correlations between expression estimates from the two technologies. RNAseq provided better sampling of classical maize genes (Schnable and Freeling, 2011, PLoS One); of 465 classical genes, 427 were detected by RNAseq compared to 390 on the microarray. RNAseq also provided the ability to discern the expression of closely related paralogs that were inseparable by microarrays. Furthermore, the RNAseq data facilitates genome annotation by providing evidence for transcripts from unannotated regions and by capturing splice junctions. We also compared co-expression derived from microarray or RNAseq data to assess similarities and differences of the network properties and results from this analysis will be discussed. The RNAseq information provides a useful complement to the maize gene atlas and can help to further understand the dynamics of transcription during maize development.

Funding acknowledgement: Department of Energy (DOE)

P243

Analysis of the Zea mays gene-space

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In 2007 NCBI created the first group of RefSeq mRNA-protein pairs for Zea mays. These were produced from mRNA sequence records present in UniGene clusters. In addition to the continual effort to recover mRNA for specific enzymes, the corn community has supported at least four projects to generate full-length cDNA leading to an increase in the number of corn RefSeq mRNA-protein pairs. With the determination of the genome gene-space sequence of B73 the need for mRNA-protein pairs from B73 was recognized. As the first step to fulfill this goal the current details of UniGene clusters and associated RefSeq mRNA-protein pairs were examinied. The details examined include the accession types comprising the UniGene cluster, the cultivar source of the GenBank record used to instantiate the RefSeq records and the distribution of full-length cDNA from the various projects among the clusters. 46,000 of the 98,000 clusters have only EST evidence. Thirteen cultivars have 14 or more accessions instantiating a RefSeq record. There are 198 cultivars instantiating a RefSeq record 10 or fewer times. Almost 18 thousand UniGene clusters contain a representative from the AGI flcDNA project. Roughly one third of the clusters contain two or more accessions from the AGI flcDNA. The basis for this will be described. The use of these resources to evaluate the completeness of the gene-space sequence of B73 will be described.

P244

Application of our plant CNS Discovery Pipeline to maize

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Functional DNA sequences change at a lower rate over evolutionary time compared with non-functional sequences. Thus functional sequences of noncoding DNA can be found through conservation of syntenic regions between related species. Conserved noncoding sequences (CNSs) are expected to function, usually as cis regulatory regions involved in transcription or chromatin structure. The specific function of almost all CNSs remains unknown. We developed a Python application to automate large-scale identification of thousands of CNSs between usefully diverged species (Ks between 0.5 and 0.9). This application works by first attempting to correct annotation errors between the two species. Afterwards, it condenses local duplicates and finds syntenic regions based on ploidy relationships. BLAST is applied to the syntenic regions between the two species to find CNSs. CNSs are found through blastn at an e-value less than or equal to a 15/15 exact base pair match. Nonsyntenic CNSs are removed along with CNS with hits to known RNA or exons. Maize and rice diverged to an extent that CNSs are expected to be shared above noise level. This pipeline has proved to be accurate for both monocot and dicot genomes without altering settings, but did not initially work well when comparing rice and maize. By adapting our pipeline, we identified over 50,000 maize-rice CNSs. We also found many previously unidentified genes, RNAs and exons. The identification of new CNSs in an automated fashion leads to a better understanding of maize gene regulation and CNS function, especially regulation following its lineage-specific paleotetraploidy.

Funding acknowledgement: National Science Foundation (NSF)

P245

COB: The Co-expression Browser— a web application for integrating and browsing genome scale transcriptional networks

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The widespread use of microarray and high-throughput sequencing platforms has produced an abundance of genome-wide expression data for maize. The ability to gain insight from this growing collection of expression profiles is often severely limited by the inability to conveniently browse or ask even simple queries. To address this need, we have developed CoB (Co-expression Browser), a database and web-based interface that supports convenient browsing of a large collection of maize expression data and co-expression networks derived from them. The database includes a large number of profiles generated to discover transcriptomic differences between a variety of *Zea mays* accessions as well as an atlas of different tissues and developmental stages. Co-expression networks were constructed by measuring covariance of pairs of genes across different genotypes, tissues or developmental stages. These networks provide a global view of putatively co-regulated genes and can serve as a powerful resource for learning about otherwise uncharacterized genes. The web-interface supports a number of different use cases including gene-centric queries for further functional characterization of genes, discovery of genes underlying QTL, and comparative analysis of co-expression relationships across different contexts. With continued development of features and the addition of more gene expression and other datasets, we anticipate that CoB will serve as a maize community portal for integrative analysis of maize gene expression profiles.

Funding acknowledgement: University of Minnesota Interdisciplinary Informatics Initiative

P246

Comparative analysis of rice and maize transcriptome through RNA-seq – building the foundations for engineering C4 photosynthesis

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C4 photosynthesis has evolved multiple times during angiosperm evolution from C3 ancestors. This highly specialized form of photosynthesis essentially eliminates the detrimental photorespiratory process and offers higher water and nitrogen use efficiencies. In hot arid environments, crops that use C4 photosynthesis are more productive than C3 crops. This C4 advantage, together with current rises in world population and food prices, has led to an ongoing engineering effort to introduce C4 traits into C3 crops (e.g. rice). This ambitious goal demands a systems-level understanding of the regulatory networks underlying C4 photosynthetic differentiation. To define regulatory nodes that mediate C3 and C4 photosynthetic differentiation, we are using a comparative transcriptomics approach. We have developed a robust strand-specific RNA-seq library construction protocol and novel data analysis pipeline and are currently using this pipeline to obtain a highly resolved developmental time series of photosynthetic differentiation along maize (C4) and rice (C3) leaf gradients. We and collaborators are also layering additional datasets along this gradient including detailed histological, metabolic and proteomic surveys to develop one of the most highly resolved –omics datasets in plants.
Here, I will discuss the use of strand-specific RNA-seq data to improve the maize and rice gene annotation for accurate gene quantification. I will discuss methods we are developing to calibrate these leaf gradients and our results of comparative transcriptomics profiling. This includes identifying gene orthologs from rice and maize and the use of several clustering methods to identify novel cis and trans regulatory elements that may be driving C4 differentiation.

Funding acknowledgement: National Science Foundation (NSF), The Bill & Melinda Gates Foundation, iPlant Collaborative

P247

Development of a Robust Method for Microscopic and Molecular Assays of Nuclear Architecture and Chromatin Structure in Maize.

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We set out to develop a robust chromatin structure assay for maize. For this purpose, we have undertaken a detailed and systematic analysis of the effect of three commonly used nuclear isolation buffers. These are (1) “Buffer A”, first developed to preserve chromatin structure for 3-D microscopy; (2) “Apel Buffer”, used for plant nuclear assays including run-on transcription; and (3) ”MNEP Buffer”, used for human cell nuclear isolation and chromatin structure assays. These buffers were used on two different maize tissues (ear shoot and seedling), replicated in two of the three possible labs. Here we describe our findings using various qualitative and quantitative assays. We found that Buffer A provides the best preservation of chromatin using 3-D microscopy of DAPI-stained nuclei. All buffers gave high quality MNase digestion patterns with typical nucleosomal ladders using EtBr-stained agarose gels. Additional findings and considerations will be presented. Careful development of a robust nuclear isolation and chromatin preparation protocol will enable us to continue our characterization of chromatin structure and genome response in maize (project website at maizenucleosome.org). Furthermore we expect this work to make a significant contribution to the rapidly advancing area of plant chromatin research.

Funding acknowledgement: National Science Foundation (NSF)

P248

Discovery, annotation and expression analysis of arginine/serine (SR) proteins in maize and sorghum using the Plant Genome Database PlantGDB

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The arginine/serine (SR)-rich family of conserved proteins across plant species plays an important role in plant growth and development and in response to environmental stress. We have searched and discovered candidate SR genes in different plant species available at the Plant Genome Database PlantGDB. These were manually annotated and the gene structures were determined by splice alignment of the available ESTs with the cognate gene using the computer software GeneSeqer. Expression validation of maize and sorghum SR genes by RT-PCR analysis using SR gene specific primers revealed tissue specificity in mRNA extracted from etiolated roots and shoots. Intriguingly, ~35 percent of the cloned RT-PCR products of these SR genes were not represented in the available EST collection. The majority of these new transcripts represent alternatively spliced isoforms generated by differential selection of splice sites during pre-mRNA processing. Several of these transcripts utilized non-canonical splice sites. Our data indicates that alternative splicing profile of SR genes display evolutionary conservation between maize and sorghum. These findings suggest an important biological relevance of this process in gene expression.

Funding acknowledgement: National Science Foundation (NSF)

P249

Divergence time of the abnormal chromosome 10 haplotype in Zea mays

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Abnormal chromosome 10 is a rare chromosome variant of normal chromosome 10 found within the genus Zea. Ab10 is distinguished from N10 by an extrachromosomal region containing "knobs:" heterochromatic regions composed of either a 350-bp or 180-bp repeat motif. These same "knobs" are located throughout the maize genome and, in the presence of Ab10, undergo meiotic drive in a female plant to become present in a larger percentage of the progeny than predicted by Mendelian genetics.

Knobs are present in all lineages of Zea, so the origin of Ab10 likely lies within the distinct history of the plant. Recently, two more structural variants of Ab10 have been discovered, providing the opportunity to date the divergence between the haplotype and normal 10 in maize. We obtained sequence data from three genes present in Ab10, N10, and sorghum (as an outgroup). Analysis of unique and fixed SNPs between the haplotypes led to estimations of both the most recent common ancestor (MRCA) and the time of divergence between the two chromosome variants. These estimates of age were dependent on a global maize mutation rate, which for this study was 3 x 10 -8. Knowledge of Ab10's origin helps us construct a clearer picture of the history of meiotic drive in maize.

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P250

Do Maize, Rice and Sorghum share miRNA targets: Predicting mi-rna targets using more than one classifier.

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Predicting targets of miRNa in plants has been a challenge for computational biology. More so when splice variants have to be taken into account.When trained using one genome such as *Zea mays*, can classifiers predict the targets in a similar genome, such as *Oryza* spp or Sorghum?

While many of them can be predicted, splice variation continues to be a challenge. To date, the following methods have been employed to predict targets: Complementary, Thermodynamics, Statistical SVM and Bayesian.They have only predicted targets with some degree of accuracy. This may be because target prediction is further complicated by splice variation in miRNA as well as the target mRNa sequence itself. Some members of Triticae involve heterosis, hybrids and polyploids and this make predictions tougher, calling for the use of two classifiers and to eventually select predictions that are common to both classifiers. Our preliminary studies show that the biggest difference comes from using information about validated targets in the training data. This should be done in addition to using two classifiers and using results prediction by both.

P251

Dueling Genomes: Reciprocal Introgression Between Maize and its Wild Relative, *Zea mays* ssp. *mexicana*

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Maize and its wild relative, *Zea mays* ssp. *mexicana* (hereafter, *mexicana*), have long been known to hybridize in the fields of highland Mexico. Despite widespread evidence for introgression, these taxa maintain their distinct morphologies and have done so in sympatry for thousands of years. Two fascinating unanswered questions in *Zea* evolution are: 1) To what extent do genomes of maize and *mexicana* remain differentiated despite widespread evidence of hybridization? and 2) How has reciprocal gene flow contributed to the evolution of both maize and *mexicana*? Here we assess patterns of genome-wide introgression in approximately 200 individuals from nine sympatric maize-*mexicana* populations through comparison to reference allopatric populations based on genotyping of over 40,000 single nucleotide polymorphisms. While portions of maize and *mexicana* genomes remain distinct (notably near known pollen pistil compatibility loci), we detect widespread evidence for introgression between sympatric maize and *mexicana* and long haplotypes shared across the majority of highland Mexican maize and *mexicana* that are not found in the progenitor of maize, *Zea mays* ssp. *parviglumis*. Through further characterization of these regions we evaluate the hypothesis that maize, domesticated in the lowlands of West Mexico, received beneficial alleles for highland adaptation via introgression from *mexicana* during its expansion into the highlands of the Central Plateau. In turn, we consider whether introgression from maize into *mexicana* has potentially altered the ecology of this wild taxon, increasing its capacity to persist commensal to agriculture.

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

P252

Empirical observations of genotyping by sequencing in maize diverse inbreds and recombinant inbred populations

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Genotyping by sequencing (GBS) is a recently proposed protocol that can be used for discovery and mapping of SNP markers in virtually any species (Elshire et al., 2011). We utilized GBS, with the inclusion of gel-based fragment size selection, on 751 maize inbred lines from an association panel and three recombinant inbred populations. To empirically assess the distribution of fragments produced, we generated 9.28 Gb of B73 sequence using single-end reads of 100 bp. As there are approximately 3.9 million ApeK1 sites in the B73 reference genome and with two potential 100 bp reads adjacent to each cut site, 9.28 Gb should minimally provide 11.9X coverage of the target space. Due to the gel-based size selection of fragments, the expected coverage is even greater. Empirically, we observed that fragments between 70 bp and 318 bp represented 95% of the fragments that mapped to the B73 reference genome with an expected coverage of 44.1X. However, we observed a wide variation in sequence coverage across the sites; 5.7% percent of sites within the size-selected range had a read depth much greater than expected which reduced the median read depth of the majority of sites. This is highlighted by the fact that the observed median read depth for sites within the likely size range was 0, and the same for observed sites with at least one read was only 6. Some of the more repetitive sites are attributable to plastid and mitochondrial sequences mapping to insertion sites in the nuclear genome. Because of the uneven distribution of read coverage throughout the genome, the required read number needed to achieve desirable coverage is substantially greater than the simple approximation of total bases divided by targets. We will present approaches to determine the sequencing depth required to achieve coverage levels necessary for genetic studies.

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P253

First steps towards a green gene regulatory grid

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After interactomics replaced genomics as the favorite approach to potentially predict the phenotype outcome of a given genotype, massive data sets are increasingly becoming available to improve our understanding of the underlying rules governing gene regulation. In plants, genome-wide profiling of TF-DNA interactions and consequences on transcription are known for only a few TFs and exclusively in Arabidopsis (**AGRIS; http://arabidopsis.med.ohio-state.edu/REIN**). To obtain a systems-level view of transcriptional gene regulation in the grasses, we generated the first draft of a maize transcriptional gene regulatory grid (GRG). A GRG is a static reconstruction of all possible interactions TF-target in an organism, from which dynamic gene regulatory networks are manifestations. The organization of the genomic regions into the gene regulatory grid was accomplished by using ChIP-Seq data from several TFs that belong to different families, sampled in distinct tissues and inbred lines. When available, DNA-binding information was overlapped with the corresponding gene expression studies (RNA-Seq). We also analyzed the grid topological characteristics, including comparison with the Arabidopsis GRG and searched for network motif enrichment. The maize gene regulatory grid and source data (once published) will be available through **GRASSIUS (http://grassius.org/**) grid explorer and genome browser. We demonstrate the utility of a comprehensive systems approach towards generating hypotheses for maize regulatory circuitry functions. While initially compared with Arabidopsis, integration into **GRASSIUS** will facilitate the study of network motifs evolution in corn-related species such as sorghum, rice and sugarcane. This integrative effort demonstrates that the plant biology community has the resources to engage in plant ENCODE projects to allow full reconstruction of the green GRG.

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P254

Fractionation mutagenesis: Natural Promoter Bashing in Maize

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Detailed characterization of promoter elements regulating the expression patterns of individual genes remains a difficult and time consuming process. We have developed a pipeline for investigating the function of specific, conserved, cis-regulary elements. An ancient -- approximately twelve million years old -- whole genome duplication in the maize lineage created two copies of every gene, each with an initially identical complement of regulatory sequences. Following a whole genome duplication small deletions resulting from intrachromosomal recombination slowly remove duplicate copies – fractionate--of either whole genes or regulatory regions. As a result, many genes in maize possess a closely related paralog which shares some, but not all, of the same regulatory sequences. It is possible to identify putative regulatory sequences by comparing the noncoding sequence surrounding duplicate gene pairs in maize to the sequence surrounding orthologous genes in sorghum and rice and locating sequences which show unexpectedly high conservation between the three species. When a conserved noncoding sequence associated with orthologous genes in rice and sorghum is found adjacent to only one of two duplicate genes in maize, we can develop testable hypotheses about the function of this sequence by using RNA-seq data generated by the maize community to compare the expression patterns of each gene copy in a a variety of datasets. Most of the RNA-seq datasets published to date in maize have focused on measuring tissue specific expression so our initial investigations have focused on identify regulators of tissue specific expression. However, as more gene expression datasets are generated, natural promoter bashing should prove useful for investigating promoter elements involved in differential regulation in response to stimuli or differential regulation of genes between mutants vs wild type plants.

Our tool for extracting/visualizing expression data for specific genes using public RNA-seq datasets: http://www.qteller.com (hosted at UC-Berkeley)

Funding acknowledgement: National Science Foundation (NSF)

P255

GA modulates ABA-regulated gene expression during maize embryogenesis

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Developmental processes are often controlled by the interplay of positive and antagonistic, or modulating signaling pathways. During maize seed development, bioactive gibberellic acids (GA) accumulate in the embryo, and then decline prior to a wave of embryo abscisic acid (ABA) that initiates maturation phase. ABA's role in maize maturation is well-known -- ABA-deficient kernels are viviparous and desiccation-sensitive. However, maize embryos deficient in both ABA and GA exhibit the wild-type phenotypes of quiescence and desiccation tolerance, suggesting that these hormone signals jointly and antagonistically govern the maturation of cereal seeds. We compared transcription profiles of developing maize embryos that compared four genotypes: wild-type (WT), GA-deficient (GA–), ABA-deficient (ABA–), and ABA¬–GA¬– double mutants. The level of expression of maturation-phase genes was hugely reduced in ABA- mutants but was restored to almost wild-type levels in the double hormone mutant. ABA-down regulated genes were over-expressed in the ABA- background, but also restored to fairly normal expression the double mutant. The temporal pattern of expression is very different from the early, rapid change in transcript level that is seen in wild-type embryos. Instead, double mutants show a surge in transcript accumulation occurring late in seed development (after both GA and ABA have fallen to basal levels in wild-types). Also, GA synthesis must cease early in embryo development to have this effect. Together, these indicate that the double mutant phenotype is not due to direct interaction of hormone signaling pathways, and it implies that in addition to the ABA signal initiating maturation phase, there is a later cue that maintains high transcript levels in the absence of the ABA. Transcript profiles of GA– mutants show alterations in carbohydrate pathways and in the expression of chromatin factors, suggesting GA-modulated sugar signaling and/or epigenetic factors may be regulators of embryo maturation.

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P256

Genetic architecture of maize and teosinte

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Genetic architecture is the constellation of gene effects and interactions that underlie variation in a quantitative trait. Essentially, genetic architecture is the map between phenotype and genotype. Understanding variation in genetic architecture is key to understanding evolution, manipulating species for a sustainable agriculture, and preserving variation as species adapt. This NSF project (DBI 0820619) is improving our understanding of the genetic architecture of complex traits in maize and its wild relative, teosinte. Maize has a combination of life history, economic and societal value, and genetic tools that make it uniquely suited to studying genetic architecture. We are identifying genes that control domestication traits and three key agronomic traits: flowering time, plant height, and kernel quality. Genetic linkage, association, and fine mapping analyses are being performed on the largest and most diverse set of mapping families publicly available for any species. A large series of isogenic lines are being used to characterize allelic series and epistatic interactions. The genetic architecture of each of the four trait groups will be compared and contrasted, and the influence of recombination and past domestication bottlenecks on the genomic distribution of functional diversity will be examined. Finally, the ability of genetic architecture-based models to predict phenotype will be evaluated in a broad range of germplasm, including elite US hybrids. This project will take a step toward the ultimate goal of predicting phenotype from genotype.

Funding acknowledgement: National Science Foundation (NSF)

P257

Genome Dominance in Maize and Brassica rapa

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The new subgenomes of most eukaryotic paleopolyploids delete genes from one subgenome to a greater extent than they do the other: biased fractionation. In maize [1] and in *Brassica rapa* [2], the mechanism of biased fractionation is deletion, usually involving intrachromosomal recombination. The reason the two subgenomes have different gene content is best known in maize: genes on the over-fractionated subgenome tend to be expressed to lower RNA levels than do genes on the under-fractionated subgenome, a phenomenon called genome dominance [3]. This same explanation applies to *Brassica rapa* [4]. I will present unpublished genome dominance data in *Brassica*. What is the initial cause of genome dominance and biased fractionation? I will show that genome dominance tends to be inherited through the *Brassica* phylogenetic tree, over tens of millions of years. We will implicate epigenetic marks that might alter gene expression as an original cause, and present preliminary data concerning the nature of these marks.

1. Woodhouse, M., et al. (2010) Following tetraploidy in maize, a short deletion mechanism removed genes preferentially from one of the two homeologs. *PLoS Biology* 8, 15
2. Tang, H., et al. (2012) Altered patterns of fractionation and exon deletion in Brassica rapa support a two-step model for paleohexaploidy. *Genetics* accepted
3. Schnable, J.C., et al. (2011) Differentiation of the maize subgenomes by genome dominance and both ancient and ongoing gene loss. *Proc Natl Acad Sci U S A* 108, 4069-4074
4. The *Brassica rapa* genome sequencing project consortium, X. Wang (2011) The genome of the mesohexaploid crop species Brassica rapa. *Nature Genetics* 43, 1035-1039 and Feng et al (Wang lab), submitted manuscript. Also, Pires-Freeling collaboration unpublished data.

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P258

Genome-Wide Allele Specific Expression Assays in Maize-Teosinte Hybrids

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Modification to gene expression levels by *cis*-regulator changes has been shown to be responsible for a number of domestication phenotypes in plants, including maize. This project is using strand specific mRNA sequencing to investigate allele specific expression ratios on a genome-wide scale in a collection of maize-teosinte F1 hybrids. Genes with a heavy expression bias for the maize or teosinte allele represent candidate domestication genes with putative *cis*-regulatory differences between the two alleles. Six maize and nine teosinte inbred lines were crossed to produce a collection of 31 F1 maize-teosinte hybrids. Three tissues (immature ear, v4 leaf, and v4 shoot including the meristem) were chosen for study and RNA samples were produced from the F1 hybrid tissues to allow for assessment of differential allele expression in identical cellular contexts. Using hybrids avoids the confounding issues that exist in comparisons between two parental inbred lines such as variations in developmental stage, *trans*-factors, and growing conditions. Strand specific mRNA sequencing libraries are being produced and sequenced on the Illumina HiSeq2000 platform. Following sequencing, trimmed reads will be aligned to the reference B73 maize genome. Allele specific SNPs will be identified in parental genomic sequencing data and used to assign hybrid mRNA reads to the maize or teosinte parent. The percentage of SNP depth assignable to the maize parent will then be calculated and used to infer allele specific expression for genes with sufficient coverage, SNP number, and consistent expression patterns between SNPs. After sequencing and calculation of expression ratios, the results of the mRNA sequencing experiment will be spot checked for consistency and accuracy using an alternative technique such as allele specific qPCR of select genes. Library production and sequencing is currently underway. Genes with consistently strong allelic bias across multiple maize or teosinte inbred lines are of great interest in this study and will be subjects of future experiments investigating their involvement in the domestication process.

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P259

Genomics of adaptation in natural teosinte populations

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Teosintes *Zea mays* ssp. *parviglumis* and ssp. *mexicana* occupy diverse habitats in south and western Mexico. Subspecies *mexicana* lives in cooler high elevation (~2000 m) conditions whereas *parviglumis* occupies lower (~1000 m) and warmer locations. Both subspecies consist of hundreds of distinct geographically separated populations. The combination of population structure, varying environmental conditions and genomic resources make teosinte an excellent study system for plant ecological genomics. In this study we wanted to define genetic relationships among teosinte populations in a geographical context and use this knowledge to further understand the genetic basis of adaptation at both the subspecies and population level. We used the MaizeSNP50 chip to genotype 250 teosintes from 21 populations sampled across their natural distribution. Individuals formed clear genetic clusters at several hierarchical levels. The two subspecies were genetically distinct except for one high elevation *parviglumis* population that showed strong evidence for admixture with *mexicana*. Altitude was an important covariate in the distribution of genetic variation between *mexicana* and *parviglumis*, but not within subspecies where stochastic events rather than geographic distance dominate the among-population structure. We also identified SNPs and segments of the genome that showed excess differentiation among subspecies and populations, as well as SNPs that were correlated with various environmental and soil variables. We present analysis of the function and expression patterns of these candidate loci. Finally, our data provides evidence for potentially adaptive gene flow between the subspecies.

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P260

Global gene expression profiling of maize copy number variation (CNV) with phenotypic impact

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Recent comparisons of different maize inbred lines via array-based comparative genomic hybridization (Springer NM et al. 2009) and sequence-based whole genome re-sequencing (Lai J et al. 2010) has begun to reveal significant levels of copy number variation (CNV) and presence/absence variation (PAV). However, the question of how structural variation contributes to phenotypic diversity has remained unanswered. Here, we analyzed lines that vary in copy number of a specific segment of chromosome 1 due to duplication caused by alternative Ac transposition. We have shown previously that directly-oriented Ac 3' and 5' termini can generate paired segmental deletions and duplications by Sister Chromatid Transposition (Zhang and Peterson, 1999, 2005). We isolated a number of such duplications and deletions, and conducted expression analysis on one case (p1-ww714), which contains an inverted duplication on chromosome 1S. The region duplicated in p1-ww714 is 14.7 Mb in size and is predicted to contain approximately 300 gene models according to Maize GDB. Plants homozygous for p1-ww714 (i.e., four copies of the duplicated region) are significantly shorter and have smaller ears than normal siblings. Whereas, heterozygous plants (p1-ww714/normal; three copies of the duplicated region) are intermediate in height and ear size, suggesting that the segmental duplication in p1-ww714 exerts a CNV effect on phenotype. We implemented both GeneChip (new Affymetrix Maize WT 100K array) and high throughput sequencing (mRNA-Seq) approaches to study the relationship between CNV, and transcript accumulation. Preliminary results show that most genes within the duplicated segment exhibit dosage compensation, while some genes (~15%) exhibit dosage-dependent expression. Some genes outside the duplicated segment are differentially expressed and may represent the trans-effects of the duplicated genes. Genes within the segment are clearly overrepresented among all of the differentially expressed genes detected. These results provide insight into the transcriptional expression and phenotypic effect of a specific maize CNV.

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P261

Gramene: a resource for comparative plant genomics

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The Gramene database (http://www.gramene.org) is a curated data resource for comparative genome and functional analysis in over 20 plant species, including *Zea mays*. Through integration of genomic sequence, genes, proteins, genetic and physical maps, germplasm, genetic and phenotypic diversity, and biochemical pathways, we provide an unparalleled framework for carrying out maize research in the context of other crops. To index and combine data from multiple plant species, Gramene uses ontologies (controlled vocabularies), including those for plant structures and growth stages, traits and phenotypes, gene function, biological processes, cellular components, and environments. All data in Gramene is publicly available, and all code is open source. Online tutorials and help documents provide users with an overview on how to conduct a wide variety of analyses on the database, and an interactive helpdesk supports individual queries. Release 34b (January 2012) includes complete genome sequences of six monocots (*Oryza sativa* subspp, *O. glaberrima*, sorghum, *Brachypodium*, and maize), five dicots (*Arabidopsis* spp, soybean, grape, and poplar), two algae (*Chlamydomonas* and *Cyanidioschyzon*), *Physcomitrella* (moss), and *Selaginella* (spikemoss), and partial genomes of several wild rice species. We have updated our genome browser to Ensembl version 65. New data includes annotations, gene predictions, phylogenetic trees, and genetic diversity data supplied by contributing rice, Arabidopsis, and maize databases. New features include multi-species views, synteny maps based on phylogenetically-determined orthologs, and multiple whole-genome alignments. Data and features are fully integrated with other Gramene resources, including gene and protein-level annotations, ontologies, genome browsers, diversity data, and pathways. Together with MaizeGDB, we launched the MaizeCyc collection of metabolic pathways, and independently provide a common portal for comparative pathway analysis. We also provide links to external genetic analysis and/or visualization tools, such as TASSEL and Flapjack. Gramene is supported by an NSF grant (IOS-0703908) and works alongside Ensembl Genomes.

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P262

Improving the B73 reference genome via genotyping by sequencing (GBS)

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Genotyping by sequencing (GBS) provides a robust and cost-effective means to genotype large numbers of individuals at high density by targeting sequence adjacent to restriction enzyme cut sites. We genetically mapped (binomial p-value <0.001) 589,002 segregating 64-base ApeKI GBS tags as presence/absence markers in the maize IBM population relative to a new, high density map of 239 IBM lines based on the Illumina MaizeSNP50 Genotyping BeadChip. Using a high stringency (p<10-7) subset of these genetically mapped tags, we found that, of the GBS tags that segregated with B73, only 0.4% genetically mapped to a chromosome different from the one to which they physically align on B73 RefGen_v2. In contrast, for tags that segregated with Mo17, the comparable proportion was 9.3%. This difference likely results from structural variation combined with the ancient polyploidization that occurred in maize. We have also used the GBS tag segregation data to genetically map (1) contigs from chromosome 0 of B73 RefGen_v2, (2) contigs from de novo 454 sequencing of B73, and (3) full length cDNAs. We were able to genetically map 7 of the 17 chromosome 0 contigs, 3408 novel 454 contigs and 407 FLcDNAs that are not represented in B73 RefGen_v2. Segments of the B73 reference genome to which elevated proportions of novel 454 contigs genetically map are prime targets for future improvement. To improve resolution, we are currently mapping de novo, misplaced and unplaced contigs using GBS data from the maize NAM population in which B73 is the common parent.

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P263

Inferences on Maize Biodiversity from Genotyping-by-Sequencing (GBS) American Landraces

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Developing maize for a future defined by a rapidly changing and unpredictable climate may require greater input from locally- and, in many cases, drought-adapted landrace germplasm. However, at the present it is unclear how much of a landrace's adaptive advantage comes from unique combinations of widely shared genes and how much comes from unique allelic variation. Due to advances in and the increasingly lower cost of sequencing technology, researchers have well characterized the diversity contained within the inbred maize lines. However, research is only now beginning to focus on understanding genomic diversity uniquely present in heterozygous open-pollinated varieties and landraces.

I apply genotyping-by-sequencing (GBS) technology to 95 landrace samples from across the Americas in order to estimate the degree of genomic diversity not captured in inbred lines and to identify candidates landraces with unique haplotypes for further analysis using whole-genome sequencing. Candidate landrace accessions carrying novel haplotypes are identified through a range of approaches now possible with an open genotyping platform.

P264

Investigating tissue specific and genotype specific alternative splicing in maize

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Alternative Splicing (AS) produces multiple isoforms from a single pre-mRNA through selective use of splice sites. AS can influence protein diversity or affect protein levels by regulating message processing. AS is known to play roles during plant development, stress response, and flowering, but the extent of AS in plants is not well understood. We are examining RNA-Seq data to investigate tissue specific AS within endosperm and embryo in B73 and Mo17 maize. We anticipate that the identification of AS specific to, or differentially regulated between, these tissues will help us to further understand the cross talk between endosperm and embryo during seed development and embryogenesis. By comparing AS between B73 and Mo17 we can test for the existence of genotype-specific AS. Ultimately we will examine AS in Mo17/B73 hybrids and IBM RILs to identify genome-wide patterns of regulation of isoform abundance. Our analysis centers on the creation of a Mo17 pseudo reference sequence, RNA-Seq read alignment, alternate isoform detection and identification of allelic gene structures and isoforms. Our preliminary results have identified differences in transcript isoform abundance between embryo and endosperm, as well as some evidence for genotype-specific AS. Our strategy, pipeline and results will be discussed.

P265

Large-scale proteomic & phospho-proteomic analyses of growing maize leaves

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As part of a large-scale proteomics project to develop a maize protein atlas, we analyzed developing maize leaves. During leaf development, a series of symmetric generative divisions are followed by several asymmetric and defined symmetric divisions to yield differentiated cells. Unexpanded maize leaves were divided into 3 sections: the basal 0-1.25 cm, composed of primarily symmetrically dividing cells and those undergoing early stages of differentiation; 1.5-2.75 cm from the base, composed of dividing and differentiating cells, including those undergoing asymmetric stomatal generative divisions; and 3.5-5.5 cm from the base, composed of primarily of rapidly expanding cells. Additionally, the entire leaf blade of leaf 8 was analyzed as a control. At least four biological replicates were performed from each tissue, lending statistical power not commonly seen in proteomic approaches. Over 65 000 peptides representing at least 12 000 unmodified proteins were identified; in addition 15 000 phosphopeptides, representing at least 3557 phosphorylated proteins were identified from across these four tissues. Parallel analyses of these two proteomes allows for identification of tissue-specific protein enrichment, as well as tissue-specific phosphorylation changes that are not simply a result of change in protein abundance. Functional analyses show mature leaf tissue was enriched in proteins involved in photosynthesis and basic metabolism; expanding tissues show enrichment in proteins involved in cell wall biosynthesis and lipid metabolism; and dividing tissues are enriched in proteins involved in hormone biosynthesis, transcription and signaling. Examples of tissue-specific phosphorylations, as well as previously unidentified phosphorylation sites will be shown. Identifications of proteins and phosphorylations enriched within different regions of the expanding maize leaf provided in this dataset will aid in future studies of maize leaf differentiation and growth.

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P266

Mapping microarray probe data to gene models reveals a low level of conservation between probes and maize gene models

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Microarrays have been standard means of measuring transcript abundances over the past decade in *Zea mays* (maize) and other species. As genomes are sequenced and annotated, it is important to transfer data from these experiments to the shared vocabulary of gene models, and to keep probe and gene model associations up to date as gene models improve. We developed a protocol that identifies gene models that unambiguously correspond to microarray probes. The method accounts both for redundancy, when multiple array elements hybridize with a single transcript, and for non-specificity, when an array element interrogates transcripts from different genes. As an example, we map probes of the Arizona Maize Oligonucleotide Array, using RefGen_v2 of the B73 maize inbred line. A large number of probes map to unannotated genomic regions or do not map to the genome. Microarray probe sequences may enhance the annotation of the genome and highlight the degree to which genic content varies among maize genotypes.

P267

miRpp - A Plant microRNA Precursor Prediction Tool for Small RNA Reads on Genome Sequences and its application for 46k maize oligonucleotide array annotation

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microRNAs (miRNAs) are small single-stranded noncoding RNAs that are processed from precursor transcripts (pre-miRNAs) with characteristic stem-loop structures. They are involved in the negative regulation of gene expression at the post-transcriptional level by transcript degradation or translation inhibition. Recently discovered long-miRNAs are involved in transcriptional gene regulation by guiding de novo DNA-methylation. For an efficient and systematic experimental identification of pre-miRNAs a preliminary computational prediction is required. Due to the heterogeneity of plant pre-miRNAs the prediction does not allow the application of available animal pre-miRNA prediction tools. We developed miRpp, a plant microRNA precursor prediction tool, which predicts pre-miRNAs in genomic sequences. The method initially detects sequence regions that contain a small RNA read and are able to fold into a stable stem-loop structure. Overlapping precursor candidates are clustered, filtered by characteristics obtained from sequence and secondary structure analysis and finally classified by a support vector machine.

We compare miRpp to available pre-miRNA prediction tools and show its application for the annotation of the 46k maize oligonucleotide array. Furthermore we updated the GO-annotation of the array using Blast2GO resulting in twice as much genes with functional information. We further link expression profiles and functional annotation to genome loci and sequence by mapping the oligonucleotides to the maize genome.

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P268

MotifView: A comparative genomics tool for analyzing motifs and their genes

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MotifView (http://genomevolution.org/CoGe/MotifView.pl) is a newly-released tool within the CoGe platform (http://coge.iplantcollaborative.org) that allows the user to dynamically examine genomic regions on the scale of motifs and surrounding areas. Users can select sequences from the 30+ plant genomes available in CoGe, automatically download sequences from NCBI, or submit their own sequences. Sequence alignment algorithms, genome viewing tools, and genome comparison tools are integrated through CoGe so data can be analyzed in several levels of organization. MotifView analyzes nucleotide sequence for user-selected motifs visually presented with the option of customizable results parameters and graphical modifications. Modifications include viewing additional annotations (CNSs, methlyation data, ChIP-seq sites etc., all when available) and user-selected DNA binding motifs. Written and video tutorials provide users examples of how to navigate, choose the best genomic regions, genomes, search parameters, and motifs. Motifs are reduced to regular expressions. 417 plant motifs, some overlapping, were curated with their reference from publicly available motif databases, including 101 motifs from AGRIS, 80 from DATF, 14 from Jasper, 132 from PLACE., and a few of our own or from the literature. Our motif selection page provides some ability to condense and organizes motifs as to "function," family of known binding protein, or common name (like "G-box."). All results are downloadable from the main viewing page: FASTA sequences, GAF annotation files, image files, alignment reports and motif results.

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P269

Parallel adaptation of maize landraces to highland environments

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After its initial domestication, maize spread quickly across the Americas, rapidly adapting to a number of novel environments. From its origin in the Balsas River Basin, maize spread to the highlands of central Mexico, as well as through the lowlands of Central America to the high altitudes of the Andes. Previous genetic data show that adaptation to high altitude in Mexico and South America occurred independently from locally-adapted lowland maize. Here, we present preliminary population genetic analyses of parallel adaptation to high elevation using dense SNP data from the Illumina 50K SNP chip to genotype 94 maize lines from low and high elevation in Mexico and South America. We find numerous SNPs that are differentiated between lowland and highland populations, including many shared between Mexico and South America. We also present results of an association experiment using a subset of lines from the maize 282 association panel to identify correlations between putatively adaptive SNPs and germination and growth under high and low temperature treatments.

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P270

Please Tell Us How You Think! or, Steps to Inferring Networks

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We all want to know the "wiring diagram" of maize. However, inferring a network from high through-put molecular data is extremely difficult because of fundamental mathematical and computational issues. We are investigating a different approach to network inference that explores the relationships between phenotypic expression, the genotypes, and the network that connects them.

We begin with close observation of phenotypes, separating each into its distinctive components and representing it as a high-dimensional vector for each plant. We want to understand the relationships between these phenotypic vectors (which form a mathematical space) and the genotypes(which form a different space). We are implementing a novel mathematical and statistical modeling procedure that will map a high dimensional phenotypic vector to a high dimensional genotypic vector. This identifies the number of factors in one space necessary to reach a point in the other, identifying the simple and more complex interactions directly related to biological processes of interest. Loosely, these mappings demarcate low-resolution, interacting "regions" of the network: more precisely, they are probabilistic tilings of the spaces. Each tile in network space subsumes several possible hypotheses about the classes of molecular mechanisms in that region. To computationally generate and winnow these into mutually consistent groups, we will implement the reasoning of geneticists, present and past. The winnowed hypotheses would be tested with "wet-bench" experiments.

We are studying the lesion mimic mutants, a system of at least 200 genes and their entailed reactions. This is large enough to be a good test and small enough so we can figure out our mistakes. We are starting with kernel colors, especially anthocyanins.

We want to understand how YOU think about genes, pathways, and mechanisms when you see novel phenotypes. Please talk with Amy or Toni about your favorite phenotypes and what and how you infer their mechanisms!

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P271

Protein dynamics of the developing maize seed

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Genome-wide, quantitative proteomics makes possible the creation of a protein atlas that catalogs where, when, and how much of a given protein is present. We are in the process of building a comprehensive protein atlas for maize by sampling over 43 different tissues and developmental stages, subcellular compartments, and responses to environmental stimuli. To highlight our results, we will describe dynamic changes in protein abundance and phosphorylation levels in the developing maize seed. From ~99 million tandem mass spectra we were able to quantify the abundance of proteins from more than 13,000 genes and localize 12,770 sites of phosphorylation. Several proteins with known distributions were used to validate our data. All of the endosperm proteins known to participate in starch biosynthesis were measured and their sites of phosphorylation were mapped, creating the possibility of making site-directed mutations that regulate starch synthase assembly. Additionally, we have used the mass spectra, for proteogenomics, to discover new protein coding genes and to refine existing gene models. These results will enhance genome-enabled maize research and breeding by increasing the completeness and accuracy of maize genome annotation. Investigations of maize physiology will benefit from the maize protein atlas.

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P272

Rapid Evolution of the Maize Centromere Repeat CentC

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We set out to investigate the evolution of repetitive DNA in centromeres of the *Zea mays* genome. Maize centromeres are comprised of a number of repeats, including arrays of a 156-bp centromere-specific repeat called CentC that has been shown to be important in kinetochore formation. CentC may comprise as much as ~1% of the maize genome. We investigated CentC abundance in a diverse panel of inbred and landrace lines as well as multiple populations of two subspecies of the wild relative teosinte from different regions of Mexico. We first constructed a database of CentC variants across each chromosome from the reference B73 sequence. We then mapped Illumina short read sequence from 82 lines from the maize HapMap version 2 and our own low-coverage Illumina sequence from 288 lines against this reference. Read mapping was used to estimate abundance of CentC repeats in each line. Genome size was also estimated for a subset of 151 lines in the diversity panel. Findings suggest a genome-wide reduction in CentC abundance as a result of the domestication bottleneck, while subsequent improvement has not caused significant changes in CentC abundance. In highland landrace maize populations, we observed a higher relative abundance of CentC that corresponds to an overall smaller genome size. We correlate fluctuations of CentC abundance with SNP and genome size data from populations of teosinte to compare rates of evolution of structural elements to nucleotide substitutions within the genome.

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P273

Re-annotation of the Agilent maize microarray based on the B73 genome sequence

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Microarrays are a mature technology that can be applied for high-throughput transcriptomics in maize, since analysis software is readily available and many of the statistical approaches are now standardized. One platform is the Agilent-016047 maize "whole genome" microarray made up of 42,034 maize reporters. However, these 60-mer reporters were designed from ESTs from different maize lines prior to release of the B73 genome sequence. We developed a set of rules for re-annotation of the reporters after BLAST similarity searching against the maize B73 RefGen v2 "Working Gene Set" (WGS) predicted transcripts and the genome sequence. Our results showed that the reporters could be grouped into six categories: (i) "annotation by sense gene model" (56% of the reporters), (ii) "annotation by antisense gene model" (10%); (iii) "annotation by gDNA" without a WGS transcript hit (4%); (iv) "annotation by EST", in which case the EST from which the reporter was designed, but not the reporter itself, has a WGS transcript hit (8%); (v) "ambiguous annotation" (6%); and (vi) "inconclusive annotation" (15%). This information and functional annotations based on BLASTX and Blast2GO have been made available in a Maize Microarray Annotation Database (http://MaizeArrayAnnot.bi.up.ac.za/), as well as a MaizeGDB annotation track.

Funding acknowledgement: Technology Innovation Agency (TIA) South Africa, National Research Foundation (NRF) South Africa

P274

RNA-Seq Analysis of Maize Gametophytic Transcriptomes

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Both male and female gametophytes play central roles in sexual plant reproduction. Furthermore, a hallmark of the plant life cycle is that gene expression is required in the haploid gametophytes, as many mutant phenotypes are expressed in this phase, affecting transmission of the mutant allele. However, the relative inaccessibility of the female gametophyte has limited the availability of genome-scale data on this structure. We have taken advantage of the relatively large size of the maize female gametophyte to sequence RNA-Seq libraries using the Illumina platform from dissected B73 embryo sacs, comparator ovules, mature pollen, and seedlings. The transcriptomes of the two gametophytes appear fairly distinct, as only ~5% of the loci enriched in at least one of the gametophytic samples are enriched in both. Large-scale validation of embryo sac specific gene expression is being performed using SOLiD sequencing of embryo sac and comparator ovules from a second inbred, W23. Genome wide analysis of the available *Ds* and Uniform *Mu* insertion collections shows a statistically significant deficit in insertions in gametophyte-expressed genes and validates RNA-Seq as a method for identifying gametophyte-essential genes. Validation of a subset of gametophyte-expressed genes is being performed using *in situ* hybridization and mutant analysis. Gene annotation and ontology terms are being analyzed for biological processes and molecular functions enriched in gametophyte cells.

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P275

Systems approaches in maize inflorescence architecture

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Branching patterns of grass inflorescences are determined by developmental fate of stem cell populations called axillary meristems. Genetic control of branching in maize has clear relevance to grain yield and harvesting ability. In this work, we took a systems approach, integrating genetics and genomics methods, to investigate transcriptional networks controlling axillary meristem determinacy. We used mRNA-seq to generate genome-wide transcript profiles capturing spatiotemporal expression changes during development in ear and tassel primordia, and those resulting from genetic perturbation. The latter includes loss-of-function mutations in the *RAMOSA* (*RA*) genes, key regulators of a pathway controlling branching. *RA1* and *RA2*, which encode transcription factors (TFs), and *RA3*, a sugar metabolic enzyme, are essential for repressing branches. Expression signatures from developmentally staged ears of *ra* branching mutants were compared with those of maize tassels and sorghum inflorescences, all of which show distinct patterns and degrees of branching, revealing novel candidates in the modulation of inflorescence architecture.
The mRNA-seq datasets were integrated to resolve co-expression networks of TFs and their potential targets in relation to the RA pathway. We identified classes of TFs as putative candidates in the regulation of branching, and which intersect multiple hormone pathways as well as distinct signaling networks. To expand the co-expression network, we performed ChIP-seq using maize transgenic RA1-tagged lines, and are integrating genome-wide DNA occupancy profiles for RA1 in relation to expression differences and enrichment of regulatory elements. These data also revealed the potential for combinatorial binding of developmentally-regulated TFs at RA1-bound loci. Collectively, these data provide a framework for elucidating key components of a developmental program with significant agronomic importance. Funding by the NSF-PGRP and USDA-ARS is gratefully acknowledged.

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P276

The Maize Genome Project, an Update

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Work continues on refining the assembly and annotation of the B73 reference sequence. The primary objective is to place genes that are currently missing or incomplete. A whole genome shotgun library was sequenced to 14X coverage using the 454 Titanium method and resulting reads were assembled using Abyss. To define genes we used the FLcDNA collection, aligning both contig and RefGen_v2 sequences. A graph-based algorithm was developed to construct novel gene-scaffolds having complete coding sequence coverage. The gene-scaffolds were genetically mapped by a previously described genotype by sequencing (GBS) method, which was applied both to the 454 contigs and to the FLcDNA sequences. Final incorporation of gene-scaffolds into the reference sequence was refined using DNA-based and gene-based synteny to rice and sorghum. The resulting new assembly contains an additional ~1.3 Mb of sequence. Approximately 500 genes were added or improved with new annotation. The GBS results, along with synteny, were also used to anchor a number of unknown (chr0) physical map contigs. Release of the new reference assembly will proceed following its acceptance by GenBank. This work was funded by the NSF/DOE/USDA "Sequencing The Maize Genome" project (NSF #0527192 and #0910642).

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P277

The Maize Transcription Factor ORFeome (TFome) Project

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Gene regulatory networks are central to all cellular processes. In plants they also help link molecular targets with agronomic traits of functional value including biofuel/biomass production, biomaterials, and nutritional health. An emerging theme is the identification of these regulatory networks in which TFs participate. TFs represent ~7% of the maize genome, consisting of ~3500 genes. In order to dissect the gene regulatory network that regulate metabolism of maize phenolic compounds, we initiated The Grass Transcription Factor ORFeome Project (TFome). As part of this effort to clone the entire maize TFome, we previously generated ~200 maize TFs. Full-length ORFs or cDNAs (flcDNAs) are being amplified from existing flcDNAs or by RT-PCR, and rare transcripts are custom synthesized. Full-length ORFs are amplified and cloned into Gateway® Entry vectors that permits recombination into plasmids for expression in plants or microorganisms. The entire collection will be recombined into yeast two hybrid vectors aimed at finding TFs regulatory partners, combinations of TFs responsible for target regulation of any gene of interest. This approach will contribute to the understanding of metabolic pathways in plants providing a comprehensive ORFeome collection. Clones for these TFs will be publicly available to researchers through Addgene (www.addgene.com) and later through the Arizona Genomics Institute. Information on available clones is being posted at the GRASSIUS (www.grassius.org) web resource. Researchers that have cloned novel or rare maize TFs are invited to send their full length clones to Dr. John Gray. As part of the database development we have proposed a set of rules for naming TF proteins in the grasses Plant Physiology 2009 149(1) p4-6. This project is funded by NSF grants DBI-0701405 and IOS-1125620.

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P278

Tissue-Specific Nucleosome Occupancy in the Promoter/TSS Region of 400 Classical Maize Genes.

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We have developed a novel platform for chromatin fine structure mapping using formaldehyde-fixed, purified nuclei, nuclease digestion, and recovery of mononucleosome-sized DNA fragments. These target DNA fragments are hybridized to NimbleGen tiling arrays with probes centered around the transcription start site (TSS) of ~400 maize genes defined as "classical" on the basis of literature citations (courtesy J. Schnable, UC Berkeley). We isolated nuclei from multiple different tissues, including 10 day-old seedling (aerial tissues), normal ear shoots (3-5 cm), high or low-nitrogen treatment earshoots, mature leaf, and immature tassels. For each tissue, hybridizations were carried out using isolated mononucleosomal-sized DNA fragments, quantified relative to signals from total maize (genotype B73) DNA. Using a 12-plex array designed to enable multiple rapid experiments, we have determined the sequence enrichment profile (nucleosome occupancy) across 400 genes in different tissues. To facilitate data analysis and share these findings with the public, we have established a new high-speed B73 genome browser (GENOMAIZE.org) using the UCSC Genome Browser platform. We used this to compare empirical to predicted nucleosome occupancy. We found good general agreement between the two and between the empirical occupancy profiles across various tissues. Intriguing exceptions included the detection of nucleosome-occupied areas (peaks) or nucleosome-free areas (valleys) without a corresponding peak or valley in the sequence-based prediction. In addition, we found several genes for which a strong nucleosome occupancy signal was seen in a single tissue source, seedling. This may reflect a unique promoter architecture associated with the stem cell-like state of seedling tissues. This project illustrates the power of our cost-efficient chromatin structure mapping methods, while uncovering core promoter architectural features suitable for further gene-specific investigation. Future experiments are in preparation using a similar array design, but with the TSS regions from 17,000 maize genes (project web site maizenucleosome.org).

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P279

Using Empirical Maize Chromatin Data to Train a Support Vector Machine to Predict Nucleosome Occupancy Likelihood (NOL)

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Understanding the functional organization of the genome remains one of the biggest challenges in biology. Eukaryotic DNA is packaged in chromatin whose fundamental subunit is the nucleosome: ~150bp of DNA wrapped around a histone octamer. The position and density of nucleosomes plays a key regulatory role and is controlled by both chromatin regulatory complexes and by features intrinsic to DNA sequence. Recent work has described nucleosome occupancy in animals, yet this information is essentially nonexistent in plants. We hypothesized that information from human-based computational models of nucleosome occupancy may be used to identify regulatory elements in the recently sequenced maize genome. Nucleosome mapping predictions in maize were made using support vector machine (SVM) software that was trained on human chromatin. Following, a novel SVM was trained using maize microarray data. Nucleosome Occupancy Likelihood (NOL) scores were generated across maize, sorghum and rice using both human and maize trained SVMs. NOL plots were able to identify canonical chromatin structural features at multiple scales of resolution. Viewed at the single gene scale the NOL plots reveal distinct signatures, as well as recently described signals at exon boundaries. At the larger scales, NOL plots are ideal for genome annotation and visual scanning, as well as pinpointing the location of genes and mobile repetitive elements. The training of a new SVM using nucleosome occupancy data from the Maize genome, allows for comparative studies between it and the Human trained model. This research is expected to lead to useful annotation of the maize genome, uncover fundamental attributes of plant chromatin structure, create testable models of nuclear architecture, and establish a new paradigm for understanding the structure of the maize genome.

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P280

Using the *Corngrass1* gene to identify a grass specific juvenile transcriptome

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Plants undergo a series of development stages over time in response to variety of stimuli, both external and internal. Each phase displays unique morphological characters, and the switch from one stage to another is called a transition. Our analysis of the dominant Corngrass1 (Cg1) mutant in maize has directed us to a group of temporally regulated microRNA genes that controls the juvenile to adult transition. Cg1 mutant plants are fixed in the juvenile phase of development and increase biomass of vegetative shoots by continuously initiating extra axillary meristems and leaves.

Since the target genes of this microRNA are highly conserved in many plant species, we hypothesized that it should be possible to transfer the juvenile properties of the maize Cg1 mutant into any crop of choice simply by overexpressing the Cg1 cDNA. This was tested in the model grass system Brachypodium, and the biofuel crop plant Panicum virgatum (switchgrass), both of which replicated the juvenile specific phenotypes observed in maize.

In order to identify a common juvenile transcriptome for maize, Brachypodium and switchgrass, Illumina sequencing was done on three biological replicates of wildtype and Cg1 expressing shoots from each species at two developmental stages. Differentially expressed genes specific to each species, and common to two or more species were identified. Common down-regulated genes include SPL transcription factors that are targeted by miR156, as well as AP1 related MADS box genes necessary for flowering. Interestingly, known genes responsible for juvenile leaf development were ectopically expressed in Cg1 mutants of either maize or brachypodium, but not both. This may indicate that juvenile developmental pathways have diverged among different grasses.

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P281

DNA binding properties of the ZmCENH3 nucleosome

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As the fundamental units in eukaryote chromosome, the nucleosomes are composed of a histone octamer (two copies of each H3, H4, H2A and H2B) and a ~147 bp DNA fragment, which are further linked by H1 histone protein. In centromeric nucleosomes, the centromere-specific H3 variant CENH3 replaces canonical H3 protein. It is well known that the centromere regions are defined epigenetically by the deposition of CENH3 protein. However, how the underlying DNA sequences affect the deposition of CENH3 and centromere identity remains elusive. Here, using in vitro nucleosome reconstitution technique, we show for the first time that the maize CENH3 octamer has a stronger DNA-binding affinity to the centromeric DNA repeats than does the canonical H3 octamer. In particular, we observed higher affinity binding of CENH3 nucleosomes to sequences derived from centromeric retrotransposon of maize (CRM) and the maize centromere-specific satellite repeat CentC, but not to several non-centromeric DNA regions from maize chromosome. The preferential in vitro nucleosome formation of ZmCENH3 on maize genomic regions is under investigation.

P282

The role and evolution of maize centromere repeats

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Maize centromeres contain different amounts of centromere-specific tandem satellite repeats and retrotransposons, whose roles in centromere function have not yet been determined. Recent *in vitro* evidence indicating that these repeats may bind centromeric (CENH3) nucleosomes better than canonical nucleosomes suggests that they have evolved to this end. Here we present a unifying model that explains the fluctuation of centromeric repeats over evolutionary time and accounts for most of the observations that have been made with respect to these repeats: 1) Centromeric retrotransposons have the ability to target their own insertion to centromeres and also attract CENH3 nucleosomes. 2) Initially, short regions of tandem repeats are derived from retrotransposon DNA with increased CENH3 nucleosome binding capacity. 3) These tandem repeats are multiplied by rolling circle amplification to produce large blocks of centromeric tandem repeat arrays. 4) Tandem repeat arrays shrink due to intra-strand recombination to repair double strand breaks. 5) Allelic diversity of centromeric retrotransposons is increased by horizontal transfer and/or wide hybridization, allowing the formation of recombinant retrotransposons that can adapt to evolving CENH3 proteins and potentially even replace the tandem repeats as outlined in steps 1-3. This model not only accounts for the centromere composition observed in different plants, but also predicts that transformation with centromeric repeats should facilitate the *de novo* formation of centromeres.

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P283

A two transgene system to induce DNA methylation and modify gene expression in maize.

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In maize and other organisms, transcriptional gene silencing can be mediated by siRNA-dependent, epigenetic pathways frequently correlated with differential DNA methylation. Promoters of silenced genes are often hypermethylated compared to promoters of expressed genes. Transgenes have also been demonstrated to be regulated by these pathways, and can be designed to study the mechanisms underlying regulation of gene expression. We have developed a two-transgene system to study the relationship between DNA modifications and the regulation of gene expression. The first transgene encodes for a maize DNA methyltransferase translationally fused to a heterologous protein sequence. The second transgene includes a reporter gene immediately adjacent to an operator sequence designed to interact with the translational fusion protein. Expression of this reporter gene results in pigmented plant tissue and serves as a visual marker of gene expression. Independent transgenic lines were created for each of these constructs, and plants containing the transgenes were crossed to create segregating populations of plants with either one or both transgenes. The progeny were genotyped and analyzed for gene expression and promoter methylation. In a segregating population, plants with both transgenic constructs exhibit hypermethylation in the promoter of the reporter gene and reduced gene expression compared to plants transgenic for the reporter construct only. These results are consistent with tethering of the DNA methyltransferase to the reporter gene loci, and a concomitant change in the expression level of the reporter gene. This system represents a useful tool for studying the establishment of gene silencing in maize, and could be used to isolate the signal sufficient for the establishment of silencing.

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P284

Ac Alternative Transposition Generates Segmental Duplications and New Chimeric Genes at the Maize p1 Locus

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Maize Ac/Ds transposable elements can generate genome rearrangements by alternative transposition reactions that involve the 5' end of one Ac/Ds element and the 3' end of a different, nearby element. Ac/Ds termini in reversed orientation can undergo transposition reactions resulting in inversions, deletions and translocations (Zhang and Peterson, 2004; Zhang et al., 2006, 2009). In addition, duplications and gene fusions can be generated if transposition occurs during DNA replication and the excised transposon ends insert into the sister chromatid. Here, we studied 13 independent cases in which alternative transposition generated new chimeric genes formed by fusion of the paralogous p1 and p2 genes. The fusion breakpoints were identified by PCR and confirmed by genomic Southern blot. Transcript analysis by RT-PCR indicates that some p1/p2 fusion allele transcripts are alternatively spliced, but the amino acid sequences are not changed due to the near-identity of the p1 and p2 coding sequences. The new fusion alleles impart varying levels of pericarp pigmentation, indicating that these new chimeric genes are functional. Our study demonstrates that Ac/Ds alternative transposition can generate new chimeric genes that are expressed and functional. These results indicate that alternative transposition can produce gene duplications, exon shuffling and formation of novel genes. Combination of exons from different types of genes may result in the formation of new chimeric genes with the potential to impart novel phenotypes.

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P285

Alternative transposition during DNA replication: a route to genome expansion?

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Every DNA segment in a eukaryotic genome normally replicates once and only once per cell cycle to maintain genome stability. Our recent results show how this restriction can be bypassed through alternative Ac/Ds transposition, a reaction involving the termini of two different transposons. Starting from maize p1 alleles containing Ac and fAc (fractured Ac) elements in configurations capable of undergoing alternative transposition, we isolated a series of direct and inverted segmental duplications ranging in size from several kb to over 14 Mb in length. The endpoints of some duplications also had complex macrotransposon-like insertions containing varying lengths of maize genomic DNA flanked by Ac elements. We propose that these duplications and complex insertions are generated by alternative transposition events that occur during DNA replication, and that the excised transposons and their flanking sequences can replicate twice in a single cell cycle if the excised Ac ends insert into an unreplicated site. Preliminary data suggest that the second round of DNA replication can spontaneously abort to generate Double-Strand Breaks (DSB) which can be repaired by either Homologous Recombination (HR) or Non-Homologous End Joining (NHEJ). These results show how alternative transposition coupled with DNA replication and repair can significantly alter genome structure and may have contributed to rapid genome evolution in maize.

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P286

An *Ac* transposon system based on maize chromosome 4S for isolating long distance transposed *Ac* tags in maize genome

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Transposon tagging is an important tool for gene isolation and functional studies. In maize, several transposon-tagging systems have been developed, mostly using *Activator/Dissociation* (*Ac/Ds*) and *Mutator* systems. Here, we establish another *Ac*-based transposon system with the donor *Ac* tightly linked with *sugary1* (*su1*) on maize chromosome 4S. Newly transposed *Acs* (*tr-Acs*) were detected based on a negative dosage effect, and long-distance-transposed *Ac* events were identified and isolated from the donor *Ac* by a simple backcross scheme. In this study, we identified 1507 independent long-distance-transposed *Ac* lines. Sixty flanking sequences of these *tr-Acs* were isolated and localized in the maize genome. As found in previous studies, the *tr-Acs* preferentially inserted into genic sequences. Our system is complementary to other *Ac*-based regional-mutagenesis systems in maize, and the combined use of these systems will achieve an even and high-density distribution of *Ac* elements throughout the maize genome for functional genomics studies.

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P287

An *Ac/Ds*-based reverse genetics resource for maize in the post-genomic era

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A sequence-indexed reverse genetics resource is essential to fully exploit the maize genome sequence. Our NSF-PGRP-funded project will generate and sequence-index a collection of *Ac* and *Ds* insertions using a cost-effective method that takes advantage of next-generation sequencing technologies. Specifically, we propose to: (1) Sequence-index an existing collection of over 1000 *Ac* transposants from *wx-m7(Ac)*; (2) Complete a set of 120 roughly equidistant transgenic *Ds** launching platforms carrying markers that allow simple visual selection of element transposition from any region of the genome and, thus, enable researchers to generate regional gene knock-out collections; (3) Sequence-index several thousand *Ds** insertion sites from model platforms using a method generalizable to any collection of insertions; and (4) Develop a web-searchable database of insertion site sequences cross-referenced to stocks available from the Maize Genetics Co-op.

The following is a summary of our current progress. (1) Taking advantage of deep sequencing technologies, we have mapped over 300 *tac (trAc)* or *tds (trDs)* sites to the maize genome. (2) Using a *C1* (colored seed) marker interrupted by a *GFP*-tagged *Ds** element, more than 200 *c1-m* transgenic lines with *Ds** transposition activity have been generated and half of the platforms have been mapped to the reference B73 genome. (3) More than 10,000 C' revertants bearing a *trDs** have been selected from lines with a high reversion frequency. In a test of 5,000 *C′* and *GFP* (purple, green fluorescent) selections, >93% were heritable, showing that the system is extremely efficient for recovering *Ds** transposition. A subset of the *trDs** target sites has been mapped to the reference genome using next-generation DNA sequencing techniques and others are presently being mappped. (4) All the lines generated in this project are listed in our web-searchable database (http://www.acdsinsertions.org/) and, upon seed increase, will be sent to the Maize Genetics Stock Center for distribution.

Funding acknowledgement: National Science Foundation (NSF)

P288

Bisulfite-based methylation analysis at the maize flowering time locus *Vgt1*

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One of the major quantitative trait locus (QTL) for flowering time in maize, the *Vegetative to generative transition 1 (Vgt1)* locus, corresponds to an upstream (70 kb) non-coding regulatory element of *ZmRap2.7*, a repressor of flowering. Among the polymorphisms that distinguish late and early *Vgt1* alleles, the insertion of a MITE transposon was found to be highly associated with flowering time in independent studies. As cytosine methylation is known to influence gene expression, we aimed to determine if methylation might be involved in the relationship existing between *Vgt1* and *Rap2.7*. The methylation state at *Vgt1* was initially assayed using an approach that combines digestion with McrBc (an endonuclease that acts upon methylated DNA), and quantitative PCR. The analyses were performed on genomic DNA from leaves of six different maize lines at four stages of development. The results showed a trend of reduction of methylation from the first to the last stage with the exception of a short genomic region flanking the MITE insertion, which showed a constant and very dense methylation throughout leaf development and for both alleles. Bisulfite sequencing of *Vgt1* is in progress and has already revealed differential cytosine methylation between the two alleles.

P289

Characterization of an Ac transposon system based on *apt1-m1 (Ac)* on long arm of chromosome 9 in maize

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Activator/Dissociation (*Ac/Ds*) have been used in insertion mutagenesis as a complementary to *Mutator* (*Mu*) in maize. To further improve the efficiency of the *Ac/Ds* mutagenesis system, in this study, we adopted *apt1-m1* (*Ac*) on 9L as donor *Ac* to create *Ac* insertion library. This system based on the negative selection pressure against the donor *Ac*, and was highly efficient for isolating new transposition events. We obtained 9,625 transposition events from 1083 F1 ears with an average 8.66% transposition rate ranged from 1.11%~29.73%. We also adopted a modified PCR-based genome walking strategy to improve the efficiency of new transposon flanking sequence isolation. This method is more efficient than the Southern-based method that was used in previous studies. A validation step was developed to distinguish transposon tags derived from newly transposed *Ac* or *Ds* element. Using this PCR-base method, we had isolated 67 inheritable flanking sequences from the *apt1-m1* (*Ac*) transposition library, and 51 of them were confirmed as tr-*Ac* flanking sequences and 11 of them were tr-*Ds* flanking sequences. The *apt1-m1* (*Ac*) system also exhibited the similar transposition behavior like other *Ac* systems based on *Ac* elements from different loci. This study demonstrated a further improvement of the utilization of *Ac* mutagenesis systems in maize for gene isolation and functional genomics study.

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P290

Characterization of the *maternal rough endosperm* (*mre*) mutant, *mre*-1014*

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Resource allocation from the maternal parent to offspring is pivotal for the development and competitiveness of progeny. In plants, maternal influence on the seed is significant and can impact yield or seed quality. One mechanism by which resource allocation is thought to be influenced is through genetic imprinting, epigenetic regulation of gene expression based on the parent-of-origin of the allele. We are screening for mutations in maternal-effect genes are expected to show a phenotype only when transmitted via the maternal gamete. We identified four maternal-effect seed mutants with a rough, etched, or pitted endosperm surface from the University of Florida, UniformMu transposon-tagging population. We named this class of mutants: *maternal rough endosperm* (*mre*). The *mre*-1014* isolate shows a maternal-effect in the W22 inbred background with full transmission through pollen. Mutant seeds are significantly lighter than normal but can germinate to produce fertile plants. This isolate was mapped to the long arm of chromosome 3 using molecular markers. Interestingly, when *mre*-1014* is crossed to the Mo17 inbred, it behaves as a recessive locus in F2 populations suggesting the maternal-effect has a genetic background dependence. Fine mapping and sequencing of transposon flanking sequence tags will be used to identify the likely *mre*-1014* gene.

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P291

Creating novel alleles of target genes using the transposable element Dissociation

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A collection of nearly 2,000 *Dissociation (Ds)* elements has been created as a resource for forward and reverse genetics in maize. As non-autonomous elements, the stability of Ds insertion events can be easily controlled using *Activator* to create new insertion alleles of genetically linked genes. Using *Ac-immobilized (Ac-im)* as a stable source of transposase (Conrad and Brutnell, 2005), we have been able to generate insertion alleles in several target genes.

Here we present eight examples where donor *Ds* elements were successfully used to mutagenize tightly linked genes. A forward genetics approach was used to determine the efficacy of mobilizing several *Ds* elements genetically linked to the *pr1* locus. A reverse genetic approach was used to create new alleles of *Lph1*, PPDK, CA, ZCN8, an EIN3-like, and MED12A. A study of intragenic transposition of a *Ds* element inserted in Pho1;2b also was undertaken. Many of these new *Ds*-induced alleles will serve as donor loci for future mutagenesis screens, while others will be used to create both stable and unstable allelic variants of target genes through remobilization to create new excision alleles and additional insertion alleles.

To facilitate the adoption of this resource by the community, we conducted a *Ds* tagging workshop June 20-24, 2011 that included lectures by Erik Vollbrecht, Tom Brutnell and Jon Duvick on the genetics of *Ac/Ds* and bioinformatics resources related to *Ds* tagging. We also discussed strategies to tag genes using forward and reverse genetic techniques. Practical lab experiments included conducting high throughput DNA extractions, PCR amplifications and pool deconvolution to identify *Ds* insertions in target genes. A second workshop will be hosted this year at Iowa State University in June of 2012

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P292

Defining the chromatin domain organization of the maize genome

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Although the maize genome is comparable in size to that of the human genome, its linear gene distribution differs greatly. Human chromosomes are largely organized into megabase-scale gene islands and gene deserts. Human gene islands are mostly organized into active chromatin domains marked by contiguous regions of active histone marks, a depletion of lamin interactions, early replication timing, increased chromatin accessibility, and higher transcription rates. In maize, gene islands are smaller and their chromatin landscapes are poorly understood, raising questions about the organization of chromatin domains in maize. Matrix attachment regions (MARs) are believed to play an important role in nuclear organization and have been found to influence the stability of transgene expression, but their role as widespread chromatin boundaries or insulators in maize has yet to be analyzed. This work aims to address the question of how the maize genome is organized in the context of three measurable features of chromatin domain organization: chromatin accessibility, matrix interactions, and transcriptional activity. Through the use of high-density microarrays, this work will provide the first high-resolution maps of chromatin accessibility and matrix attachment regions in maize, while characterizing the domain organization of the maize genome and its relationship to gene expression.

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P293

Differential modes of transposon regulation revealed by analysis of cytosine methylation

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Mechanisms that control transposon activity also have the potential to impact both nearby gene activity and large-scale chromatin structure.
Recent advances in bisulfite sequencing have made possible genome-wide, highly precise, and quantifiable measurements of distinct DNA methylation pathways (characterized in part by sequence context—CG, CHG, and CHH). We are using bisulfite sequencing in order to accomplish two broad goals: 1) to understand regulatory pathways that control transposon expression, and 2) to understand what the consequences of transposon regulation are on the epigenome. We have found a genome-wide inverse association between transposon methylation in the CHH sequence context and methylation in both CG and CHG contexts, suggesting two distinct modes of transposon regulation. In particular, class II transposons exhibit high levels of methylation in the CHH context, while class I transposons exhibit low levels of methylation in CHH context but high in CG and CHG. We have also found evidence that the level of methylation within transposons can depend on distance to the nearest gene. Our current work is directed at distinguishing gene effects from transpons-intrinsic effects on methylation (both within and flanking the transposons).

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P294

Epigenetic mechanisms and environmental stresses in maize: a multiple approach to study epiallele formation and inheritance

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Plant epigenetics is gaining more interest as subject of both basic research and new source of traits for breeding. DNA methylation and histone modifications play indeed a primary role in regulating gene expression throughout the formation of epigenetic gene variants (epialleles) that can be propagated mitotically and transmitted to the progeny remaining stable for several generations. In particular, environmental cues activate specific epigenetic mechanisms that contribute to transcriptional and post-transcriptional regulation of gene expression during stress responses. These epigenetic states could be passed to the progeny as a kind of memory of the stress. In this respect, environmentally triggered formation of epialleles and their maintenance represent an important, yet unexplored, adaptive power that can contribute to crop plant improvement.
In the framework of the European project AENEAS we are exploring the mechanisms underlying environmentally-induced epigenetic changes, investigating the detailed mechanisms of epiallele formation and their heritable maintenance in maize.
AENEAS Consortium is investigating the effects of a cold stress on transcriptome, at genome-wide level through mRNA-Seq, sRNA-Seq, BS-Seq and CHIP-Seq. In parallel we are analyzing the effects on transcriptional regulation, epigenetic marks and transposon activity of salt and drought stresses on B73 inbred and epiregulator mutants. Stressed young leaves were collected after stress application and at two recovery stages. Expression analysis of stress markers (kindly provided by Biogemma) were used to monitor the plant stress responses. Stressed plant were also self-pollinated and seed harvested for transgenerational analysis.
The results obtained by these different approaches aim to identify a robust list of sequences target of epigenetic regulation (epitargets) belonging to three main epigenetic pathways (autonomous, small RNA and CpG methylation) for which we are currently introgressing in B73 new insertional mutants. Once identified the targets, their trans-generational inheritance will be analyzed in stressed maize wt and mutants for the three pathways.

P295

Epigenetics controls seed development

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We are exploring dosage-sensitive genetic mechanisms responsible for maize endosperm development. In double fertilization, the single nucleate haploid egg cell is fertilized by a haploid sperm to produce a diploid embryo while two nuclei from the central cell and a haploid sperm fuse to produce the triploid endosperm. In interploidy crosses, crosses with differing number of genome copies, this balance of two maternal and one paternal genomes is disrupted. It is known that providing two maternal and one paternal genomes to the endosperm is necessary as disruption of this endosperm genome balance results in seed lethality. We are using both quantitative genetic variation and mutants to identify genetic mechansims responsible for the integration of parental genomes at fertilization. We have identified lines with differences in interploidy cross survival rates, and attempted a QTL study with the IBM RIL (recombinant inbred line). Surprisingly, little direct genetic effects were observed in the RIL and a grandparental effect was detected in three separate replicates of B73 x Mo17 F1 ear by tetraploid pollen. We propose that epigenetics control seed development and plays a critical role in genomic balance in endosperm. Small RNA (sRNA) have emerged as determinants of gametophyte development, disease resistance, gene expression and genome protection from transposable elements (TEs). To examine the effect of sRNA on seed development, mutants in sRNA biogenesis, MOP1 and MOP2, were used in interploidy crosses. We have observed a genotypic affect on seed survival. The role of sRNA on seed survival and as a modulator of genomic balance will be discussed.

P296

Evolution and diversity of Mutator transposable elements

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Despite the importance of Mutator (Mu) transposons in maize biology and research, their evolutionary history and modes of action remain poorly understood. A bioinformatic analysis of the B73 genome revealed over 160 potentially-active Mu elements with intact terminal-inverted repeats (TIRs). Examination of internal sequences of these elements showed high diversity among them, and common ancestry between many individual Mu elements. About 20% contain sequences derived from the autonomous MuDR-transposase genes (*mudra* and *mudrb*). A small number (at least four) of these MuDR-like elements contained full, intact copies of both *mudra* and *mudrb* genes. The presence of these sequences in the "stable" B73 genome opens the possibility of multiple autonomous elements controlling distinct or overlapping sets of non-autonomous elements. To better clarify relationships within the Mu transposon class in maize, the highly-conserved TIR sequences were analyzed phylogenetically. Three major clades emerged within the Mutator family, with the canonical Mu's (Mu1 through Mu9) forming only part of one clade. Recent transposition was indicated for members of all three major clades in the Mu family by the presence of multiple copies in the B73 genome. The non-canonical Mu elements showed similarity to previously-described, active Mu elements (Mu10, Mu11, and Mu12), further supporting their functionality in modern maize lines. Heteromorphic Mu elements (those with two TIRs from different clades) were highly abundant and their transposability was supported by the phylogenetic positions of nearly-identical copies. The Mu family in maize is thus considerably larger than generally recognized, includes more active transposons, more putative transposases, and numerous, apparently-transposing elements with heteromorphic TIRs. The latter suggests that mechanisms of transposition may extend beyond current models that rely on complementary TIRs.

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P297

Examining epigenetic aspects of centromere specification in maize

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Eukaryotic centromeres are specified by a suite of poorly understood epigenetic marks; specific DNA sequences are neither required nor sufficient to recruit a kinetochore and form a functional centromere. A dicentric maize (*Zea mays*) chromosome containing a translocated, intact, and inactived B centromere on the short arm of chromosome 9 (9Bic-1) was previously generated. The supernumery B chromosome centromere provides an excellent tool to study centromere biology because it possesses a B-specific repeat that can be easily tracked. The B centromere of 9Bic-1 originated from the B centromere in the B-A translocation stock TB-9Sb. When canonical B chromosomes are present, the inactive B centromere of 9Bic-1 may undergo non-disjunction in the second pollen mitosis. Occasionally, non-disjunction results in the breakage of 9Bic-1, which can free the inactive B centromere from the active A centromere of chromosome 9. Once released, the inactive B centromere may reactivate and create a heritable minichromosome. Approximately 1400 kernels exhibiting signs of chromosome breakage were screened for reactivated B centromeres using fluorescence *in situ* hybridization (FISH). Nine independently-derived, heritable minichromosomes have been recovered to date. The recovered minichromosomes may contain reactivated B centromeres released from 9Bic-1 or potentially they may be breakage products of canonical B chromosomes. FISH probes specific to 9S reveal that at least two of the recovered minichromosomes originated from 9Bic-1. Therefore, it is possible to compare an active B centromere (TB-9Sb), its inactive descendant (9Bic-1), and its reactivated form. Because these B centromeres possess the same DNA sequence, epigenetic changes between active, inactive, and reactivated centromeres can be evaluated. The reactivated centromeres illustrate that despite the epigenetic nature of centromere function, the re-establishment of functionality, albeit at low frequency, suggests that the sequence does foster the incorporation of centromeric histones at that site unless there is an unknown epigenetic mark that has been maintained without detection over several generations.

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P298

Extensive genetic imprinting in the developing endosperm of maize

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Genetic imprinting refers to a special epigenetic phenomenon, whereas the differential allelic expressions of a subset of genes are dependant on their parent-of-origin. Genetic imprinting plays important roles in seed development of higher plants. Although genetic imprinting was first discovered in maize 40 years ago, its exact extent in the triploid endosperm remains unknown. Here, we have analyzed global patterns of allelic gene expression in 10 DAP maize endosperms from reciprocal crosses between inbred B73 and Mo17. We found that at least 179 genes (1.6% of protein-coding genes) expressed in the endosperm are imprinted, with 68 of them showing maternal preferential expression and 111 paternal preferential expression. Additionally, 38 long non-coding RNAs were imprinted. The latter are transcribed in either sense or antisense orientation from intronic regions of normal protein-coding genes or from intergenic regions. Imprinted genes show a clear pattern of clustering around the genome, with a number of imprinted genes being adjacent to each other. Besides, from the sequencing of maize 12 DAP endosperm small RNAs, we also obtained a number of imprinted small RNAs. Bisulfite sequencing of reciprocal crosses of B73 and Mo17 12 DAP endosperm allows us to identify hundreds of DMRs. All DMRs identified are uniformly hypomethylated in maternal alleles while hypermethylated in paternal alleles. Our study indicates highly extensive and complex regulation of genetic imprinting and DNA methylation in maize endosperm.

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P299

Half-leaf chimeric mutants in maize: can they be the works of gene silencing?

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It is not uncommon to find mutants exhibiting half leaf chimeras (HLCs) following EMS mutagenesis in maize. However, the mechanism by which these HLC phenotypes originate remains unknown. An opportunity to explore their molecular basis was presented to us in two targeted mutagenesis projects in the lab. One was seeking to generate weak alleles of *Hm1*, a disease resistance gene conferring protection from a leaf blight pathogen— *Cochliobolus carbonum*. Two plants were found in the M1 progeny that had half leaf chimeras of both resistant and susceptible tissue. The *Hm1* gene was cloned and sequenced from both the mutant and wild-type sectors of both chimeric mutants. However, no difference in their sequence could be detected, tempting us to think that their phenotypic differences were caused by a mechanism other than simple genetic mutation. In the second project, the target gene was *Rp1-D21*, an autoimmune R gene causing HR lesions to form spontaneously. Six plants were identified in the M1 progeny that contained large sectors (HLCs) of both mutant (*Rp1-D21*) and WT sectors side by side. RT-PCR was used to compare levels of *Rp1-D21* mRNA in the two sectors, which were found to be high in the *Rp1-D21* sector but undetectable in the wild-type sector. These results suggest that transcriptional gene silencing, possibly in the form of DNA methylation, could be responsible for the HLC phenotypes of *Rp1-D21* mutants. DNA methylation is a common epigenetic mechanism in plants that is used to silence transposons and to control development. The gene methylation status of the different sectors will be compared for both of these mutants using bisulfite sequencing and can be confirmed using methylation-sensitive restriction enzymes combined with Southern Blot analysis. Determining whether the EMS-induced HLC phenotypes involve differences in epigenetic regulation will be a step in understanding the mechanism leading to these phenotypes.

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P300

HelitronScanner: A general Helitron detection tool

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As a new class of transposable elements, *Helitrons* feature remarkably the ability to capture gene sequences, which makes them of considerable potential evolutionary importance. Unlike other DNA transposable elements, *Helitrons* possess low conservation in terms of sequence or structure features. Known *Helitrons* were mainly reported to exist in plant genomes. So far there is no effective way to detect *Helitrons* in all species with general patterns. Here we propose a Local Combinational Variable (LCV) approach for developing the HelitronScanner program, aiming to scan whole genomes of all species available and find *Helitrons* within them. First, LCVs are generated from high quality *Helitron* sequences from maize genome. These LCVs as well as some biology characteristics of *Helitron* are employed to develop the HelitronScanner program. Pair ends are adopted in our program, where a possible *Helitron* end should have a matching score above the empirical threshold while also meet the biological requirements. To validate prediction quality, we compared detected *Helitrons* in maize genome by both HelitronFinder (one of our previous version for *Helitron* detection) and HelitronScanner. The comparison is conducted by matching flanking sequences (combine together 100 bps from both) of putative *Helitrons* against maize genome data of 6 inbred lines from our collaborator lab. The comparison feedback is then used to improve the HelitronScanner. Analysis of putative *Helitrons* from other available species (i.e. sorghum, poplar, rice, *Arobadopsis*, soybean) is also expected to boost performance of HelitronScanner. For *Helitrons* detected in maize genome by both HelitronFinder and HelitronScanner, comparison is conducted by matching flanking sequences (combine together 100 bps from both ends) against maize genome data of 6 inbred lines from our collaborator lab. Further wet lab verification is also planned to conclude the effectiveness of HelitronScanner.

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P301

Maize waxy gene and *Ds* mobile element secondary structure polymorphism

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One of the key enzymes of starch synthesis is granule-bound starch synthase I (GBSSI) enzymes, that controlled amylose/amylopectin ratio. Gene encodes GBSSI exist in single copy in almost all plants studied so far. In maize GBSSI molecular weight of 58,000 Daltons is encoded by dominant gene *Wx (waxy),* localized on the short arm of chromosome 9 (bin 9.03). *Wx* gene sequence contains 14 exons, 13 introns, three regions with microsatellite repeats phi022, phi027, phi061. In normal (non mutant) maize GBSSI is highly active and its products (amylose) can not be completely transformed into amylopectin by starch branching enzymes. Recessive gene *wx* is mutated *Wx* gene through insertion of transposon elements *Ac, Ds, En / Spm, Mu* and other sites in different exons, which changes the structure of GBSS protein and inhibits the synthesis of amylose. GBSS activity decreases to 95 %, all amylose is transformed into amylopectin by starch branching enzymes. Our research aim was devoted to the problems of *Ds* mobile element secondary structure prediction, in particular, to investigate Ds mobile element secondary structure polymorphism.

23 maize *Ds* mobile element nucleotide sequences from NCBI were folded using RNAstructure software, as well as with RNAfold web server. Minimum free energy and thermodynamic ensemble methods for optimal structure prediction were used. Predicted structures free energy value does not exceeded 800 kkal/mol.

Optimal structures for all *Ds* mobile element nucleotide sequences were predicted. *Ds* mobile element sequences structural variations were descripted.

P302

Mutator-like elements with multiple long terminal inverted repeats (TIR) in plants

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Mutator-like transposable elements (MULEs) are widespread in plants and the majority have long terminal inverted repeats (TIRs), which distinguish them from other DNA transposons. It is known that the long TIRs of *Mutator* elements harbor transposase binding sites and promoters for transcription, indicating that the TIR sequence is critical for transposition and for expression of sequences between the TIRs. Here we report the presence of MULEs with multiple TIRs mostly located in tandem. These elements are detected in the genomes of maize, tomato, rice, and *Arabidopsis*. Some of these elements are present in multiple copies, suggesting their mobility. For those elements that have amplified, sequence conservation was observed for both of the tandem TIRs. For one MULE family carrying a gene fragment, the elements with tandem TIRs are more prevalent than their counterparts with a single TIR. The successful amplification of this particular MULE demonstrates that MULEs with tandem TIRs are functional in both transposition and duplication of gene sequences.

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P303

Natural Variation at the teosinte branched1 (tb1) Locus in Teosinte

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Maize (*Zea mays* ssp. *mays*) was domesticated from teosinte roughly 10,000 B.P. ago in Southwest Mexico, and many morphological changes are associated with this event. Of these changes, differences in plant architecture are perhaps the most obvious; domesticated maize is characterized by a central stalk with few to no branches (tillers) with terminal ears of grain, while teosinte is highly branched and and bears tassels at the ends of branches. The teosinte branched1 (*tb1*) gene, a repressor of organ growth, was identified as a major QTL involved in branching differences between maize and teosinte, and further studies have shown that the insertion of a transposable element (Hopscotch) in the upstream control region of *tb1* leads to increased expression of this gene, causing the reduction in branching observed in domesticated maize. Dating of this element has suggested that its insertion predates the domestication of maize, leading to the hypothesis that it would be segregating in natural populations of teosinte. Here we use a combination of phenotyping, genotyping and sequencing to investigate the role of *tb1* in teosinte. Our results suggest that the Hopscotch has an effect on tillering in teosinte and that it is segregating at a higher than expected frequency in a number of populations of teosinte. We find it at highest frequency in the central Jalisco region (ssp. *parviglumis*) and in the northern Michoacan region (ssp. *mexicana*); however, LD analysis between the Hopscotch and variation in surrounding regions does not support a hypothesis of recent introgression, and we find no correlation between allele frequency and environmental variables. We argue that crop mimicry and selection may be playing an important role in the persistence of this element in populations of teosinte.

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P304

Paramutation at the maize *pl1* locus requires a pioneer protein encoded by *required to maintain repression2*

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Meiotically-heritable epigenetic changes in gene regulation known as paramutations are facilitated by poorly-understood *trans*-homologue interactions. Mutations interfering with paramutations in maize identify components required for accumulation of 24nt RNA species. Some of these components have *Arabidopsis thaliana* orthologues that constitute part of an RNA-directed DNA Methylation (RdDM) pathway. It remains unclear if small RNAs actually facilitate paramutations and whether the maize-specific molecules identified to date define a mechanism distinct from RdDM. Here we identify a novel protein required for paramutation at the *purple plant1* locus. This REQUIRED TO MAINTAIN REPRESSION2 protein defines the founding member of a plant-specific clade of hypothetical proteins. We show that RMR2 is required for transcriptional repression at the *Pl1-Rhoades* haplotype, accumulation of 24nt RNA species, and for maintenance of specific 5meC patterns distinct from those maintained by RNA Polymerase IV. Genetic tests indicate that RMR2 is not required for paramutation occuring at the *red1* locus. These results identify a diversity of paramutation-type mechanisms occurring at distinct haplotypes that share components of an RdDM-like pathway but that are distinguished by a specific RMR2 protein. The RMR2 clade of proteins provides a new entry point for understanding epigenomic control in higher plants.

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P305

Repeat associated small RNAs vary among parents and following hybridization in maize

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Small RNAs (sRNAs) are hypothesized to contribute to hybrid vigor because they maintain genome integrity, contribute to genetic diversity, and control gene expression. We used Illumina sequencing to assess how sRNA populations vary between two maize inbred lines (B73, Mo17) and their hybrid. We sampled sRNAs from the seedling shoot apex and the developing ear, two rapidly growing tissues that program the greater growth of maize hybrids. We found that parental differences in siRNAs primarily originate from repeat regions. Although the maize genome contains greater number and complexity of repeats compared to *Arabidopsis* or rice, we confirmed that like these simpler plant genomes, 24-nt siRNAs whose abundance differs between maize parents also show a trend of downregulation following hybridization. Surprisingly, hybrid vigor is fully maintained when 24-nt siRNAs are globally reduced by mutation of the RNA-dependent RNA polymerase2 (RDR2) encoded by *modifier of paramutation1* (*mop1*). We also discovered that 21-22nt siRNAs derived from a number of distinct retrotransposon families differentially accumulate between B73 and Mo17 as well as their hybrid. We found that significant variation exists for 21-22-nt siRNA accumulation for these families among a larger set of diverse maize inbred lines. Thus, maize possesses a novel source of genetic variation for regulating both transposons and genes at a genomic scale, which may contribute to its high degree of observed heterosis.

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P306

RNAseq based discovery of novel imprinted genes in maize

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Imprinting, the biased expression of alleles based on their parent of origin, is an epigenetic phenomenon that likely plays an important role in the regulation of seed development. The development of RNAseq technologies has provided an opportunity to study the extent and variation for imprinting in maize. Deep sequencing of RNAs from B73xMo17 and Mo17xB73 endosperm collected 14 days after pollination was used to assess allelic expression among a set of 12,000 genes, which contained single nucleotide polymorphisms between the two inbred lines. The analysis of parent-of-origin patterns of expression resulted in the identification of 97 novel putative imprinted genes in maize endosperm including 54 maternally expressed genes (MEGs) and 46 paternally expressed genes (PEGs). Genome wide methylation analysis identified 19 differentially methylated regions (DMRs) in association with some of the imprinted genes. Another recent study (Zhang et al., 2011 PNAS 108) that used a slightly different development stage identified an overlapping set of novel imprinted maize genes. We have also found a number of genes that exhibit allele-specific imprinting such that imprinting is observed for some haplotypes but not others. The analysis of this large set of imprinted genes is likely to reveal the functional roles of imprinting and shed light on the mechanisms that underlie imprinting.

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P307

Small RNA Profiling in Maize Hybrids and Their Parents with Reduced RNA-Dependent RNA Polymerase2 Function

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Heterosis refers to the phenomenon where hybrids show better performance than either parent. Heterosis has long been exploited in agricultural production, but its genetic basis remains elusive. Factors that regulate gene expression are prime candidates for influencing heterosis, including small RNAs. Among maize tissues, analysis of the developing ear is perhaps most important to practical understanding of heterosis, because grain yield is significantly increased in hybrids. However, prior studies showed that 24-nt siRNAs dominate small RNA populations in maize earshoots, which may obscure the ability to observe variation in other classes of small RNAs. Furthermore, our lab has found that *mop1* hybrids defective in RNA-DEPENDENT RNA POLYMERASE2 (RDR2) required for 24-nt siRNA amplification show equivalent heterosis to normal hybrids, suggested that RDR2-independent small RNAs may be more relevant to heterosis. To explore how hybridization impacts RDR2-independent small RNAs, we profiled small RNAs from earshoots of two maize inbred lines (B73 and Mo17) and their reciprocal hybrids, in comparisons of near-isogenic *mop1* mutant and normal genotypes. As expected, the proportions of 21-nt and 22-nt small RNAs were dramatically increased in the *mop1* mutant genotypes due to reductions in 24-nt small RNAs. Irrespective of the difference in small RNA size profiles between the normal and *mop1* mutants, some small RNAs (including both miRNAs and siRNAs) were found to be differentially expressed among parents and their hybrids. Further investigation of these RDR2-independent changes in small RNAs may provide insights into the potential contributions of small RNAs to heterosis.

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P308

The impact of *Mutator*-like transposable elements (MULEs) on the Illinois Long-Term Selection maize strains

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Mutator-like elements (MULEs) belong to a highly mutagenic and active DNA transposon family, and have the propensity to insert into genic regions in several plant genomes, which influences the expression of adjacent genes. The Illinois Long-Term Selection Experiment (ILTSE) maize strains are elite experimental materials for a variety of studies, especially studies on the effects of selection on the kernel chemical composition (oil or protein). However, little is known about the molecular mechanism underlying the selection. Particularly, it is not clear whether transposons have played any role in the artificial selection. To this end, the MULE insertions that are co-segregating with either high or low protein maize strains were studied. So far, over 200 polymorphic insertions were detected and >80% of which are located in low-copy genomic regions (less than 3 BLASTN hits in B73 RefGen_v2), which is in agreement with previous findings. To test whether these polymorphic insertions play any role in the selection process, we first determined their impact on the expression levels of their adjacent genes. Among the genes tested, ~24% showed reduced and ~14% showed enhanced gene expression in maize strains with MULE insertion. The remainder exhibited little or marginal changes in their expression levels. To obtain further evidence for the correlation between MULE insertions and the kernel chemical composition, maize mutants with mutations in genes exhibiting differential gene expression will be analyzed for their kernel chemical content. Furthermore, we tested these confirmed MULE insertions in the Reverse High (RHP) and Reverse Low Protein (RLP) maize strains. The RHP strains were formed by selection for low protein content in High Protein strains and vice versa. Interestingly, some MULE insertions that present in High Protein strain but absent in the Low Protein strains were detected in the RLP strains, suggesting the recurrent acquisition of the relevant insertion during the selection. Similar phenomenon was observed with the RHP strains. The determination of the function of genes with MULE insertions will facilitate our understanding of the role of transposons in the long-term artificial selection.

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P309

The many facets of *nfc102* function during maize development

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The maize *nfc102* gene encodes a WD-repeat protein belonging to the Multicopy suppressor of *IRA* (*MSI*) family, originally identified in yeast. In maize, five genes of the *MSI* family have been identified and named *nfc*, because they display homology with one of the NURF complex component, where NURF is the Nucleosome Remodeling Factor: a multi-proteins complex that regulates transcription by catalyzing nucleosome sliding. Among the maize *MSI* family members, *nfc102* displays the highest sequence homology with Arabidopsis *FVE*, which is a component of the autonomous flowering pathway and that regulates epigenetic changes in many genes and repeats.

Our previous findings indicated that nfc102 protein interacts with histones and histone deacetylases and is associated with transcription repression. In this study we have characterized maize mutants exhibiting down-regulation of *nfc102* and, to a lower extent, of its closest homologous *nfc101*. Phenotypic analysis showed that alteration of *nfc102* expression is associated with various developmental defects, suggesting a pleiotropic *nfc102* effect. Accordingly, *nfc102* mutants exhibit changes in the RNA level of important regulators of flowering, including the maize homologous of the Arabidopsis florigen *FT* gene (*ZCN8*). In particular, our results suggest that *nfc102* is involved in the regulation of *ZCN8* sense and antisense RNA processing, by mediating chromatin modifications. Additional *nfc102* targets are transposable elements (TEs). Consistently with an *nfc102* involvement in the epigenetic-mediated silencing of repeats, *nfc102* mutants display a reduction of the TEs RNA level and histone modification changes related to transcription repression. Altogether, these observations support our previous hypothesis that nfc102 is part of different chromatin remodeling complexes, involved in distinct pathways, where it may act as a scaffold to facilitate and stabilize interactions between others complex components. Possible models of *nfc102* function in regulating expression of its different targets will be illustrated.

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P310

trans-generational epigenetic variation defined by maize Pol IV

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In maize, the largest subunit of a plant-specific RNA polymerase, (Pol IV) is required for small RNA biogenesis, paramutations, and for canalizing normal plant development. Here we show that specific alleles of the *purple plant1* (*pl1*) gene, encoding an anthocyanin pigment regulator, retain an expanded domain of expression following transmission from small RNA biogenesis mutants. This conditioned *pl1* expression pattern is heritable even after restoration of Pol IV function and is not dependent on distal sequences required for paramutation. A mutant survey of diverse inbreds identified distinct sources of cryptic epigenetic variation affecting specific developmental traits. These results indicate that *trans*-generational action of Pol IV, acting on attendant transposons, defines canalized expression domains of specific maize alleles.

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P311

Transposition Fate of Seven Maize Activator (Ac) Elements Located on the Short Arm of Chromosome 1.

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Transposition rates of seven Activator (Ac) transposable elements mapped to the maize chromosome arm 1S were previously reported. Transposition events were scored as the low frequency near-colorless kernels among the mostly coarsely spotted kernels on ears borne on plants homozygous for the Ac element that were crossed by a homozygous r1-scm3 tester. The Ac elements and the r1-scm3 were all in near isogenic color converted W22 inbred lines. The transposition frequency was observed to differ to a large extent amongst the seven Ac elements. The near-colorless kernels were sorted into two groups, the near-colorless fine spotted kernels and the nonspotted kernels. Whereas the fine spots on the fine-spotted kernels demonstrated the presence of an Ac element at an increased dosage, the nonspotted kernels could manifest either the presence of Ac elements at a high dosage, or the absence of an Ac element. Transposition of an Ac element may occur prior to its duplication and therefore may not be transmitted through meiosis. In order to distinguish the nonspotted kernels with a high dosage of Ac from those lacking an Ac element we tested 199 plants grown from the nonspotted kernels from the seven Ac stocks. The plants were crossed either onto or by the r1-scm3 tester. The Ac was present in 76 (38.2%) of the plants but not present in 123 (61.8%) of the plants. The relative frequency of nonspotted kernels where Ac was not present differed significantly among the seven Ac lines that were assessed. Furthermore, there was no apparent relationship between the relative frequencies of fine spotted kernels versus nonspotted kernels when compared with the relative frequency of nonspotted kernels containing Ac versus nonspotted kernels lacking Ac. The frequency of loss of Ac upon transposition was not related to the overall frequency of Ac transposition of that element.

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Utilizing the Maize Ac/Ds Transposons for saturation mutagenesis of important QTLs in Barley

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The maize Activator/Dissociation (Ac/Ds) system has been extensively used in heterologous species for insertional mutagenesis. In barley, the Ac/Ds transposon-based approach offers great potential due to a large genome size and limited success of genetic transformation. The bias of the Ac/Ds system towards genic regions and its tendency for localized transpositions can greatly enhance gene discovery in large genome cereals. Barley is a key ingredient in malting and brewing industry; therefore, gene discovery in relation to malting quality has an industrial perspective. Malting quality is a complex and quantitatively inherited trait and two major quantitative trait loci (QTLs) affecting malting quality traits have been located on chromosome 4H. In this study, Ds was reactivated from parent transposition (TNP) lines, TNP- 29 and TNP-79, in which the Ds was previously mapped in the vicinity of important malting QTLs. Reactivation of Ds was carried out both by conventional breeding and a novel in vitro approach. We observed a threefold increase in reactivation frequency through the in vitro approach as compared to the conventional technique. In vitro reactivation of Ds enabled the development of a new TNP lines for dissection of malting quality QTL in barley. Identification of unique flanking sequences, using high-efficiency thermal asymmetric interlaced PCR and inverse PCR confirmed Ds insertion in genes potentially associated with malting quality such as β-GAL1, β-amylase-like and ABC transporter. In addition, we have identified mutants exhibiting male sterility and increased seed dormancy. This effort of saturation mutagenesis with Ds transposons will lead to a better understanding of malting quality traits and candidate genes that display quantitative variation.

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Late Submissions

Biochemical and Molecular Genetics

P313

Developing Maize Circadian Clock Mutants by Identifying Transposon Insertion Alleles

(submitted by Juan Mendoza <juan_miguel_mendoza@yahoo.com>)
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One of the main questions the Harmon lab addresses is how the circadian clock in maize contributes to overall plant fitness. The circadian clock is the molecular timing mechanism that operates to synchronize cellular signaling activity with environmental cues like light and temperature. To identify important genes for circadian rhythms in maize, our research has directed us towards the identification and study of mutants in four genes suspected to be important to the general functioning of the maize circadian clock: *timing of CAB expression 1 (toc1), zeitlupe (ztl), gigantea of zea mays1a (gigz1a),* and *gigz1b*. GIGZ1a is an ortholog of Arabidopsis GIGANTEA (GI). GI is a component of the core circadian oscillator, as well as a key factor in regulation of flowering time according to photoperiod. ZTL is a clock-specific photoreceptor protein that is involved in targeting the core oscillator component TOC1 for degradation by the 26S proteasome. TOC1 is a transcription factor that negatively regulates expression of other core clock genes, which contribute to the transcriptional-translational feedback-loop at the core of the oscillator. The rhythms of this clock are set by cues from the environment, which prepares the plant for repeated, daily changes in the environment. We have identified maize *Mutator (Mu)* transposable element insertions in *gigz1a/b*, *toc1*, and *ztl*. Sequencing of the *Mu* elements confirms that for each gene at least one of these elements interrupts an exon in the target gene. Furthermore, analysis of transcript levels in plants with the *Mu* alleles confirms each exon-located insertion reduces gene expression. These mutants will be useful tools to study the contribution of the maize circadian clock to important agronomic traits, like flowering time, drought tolerance, and pathogen resistance.

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P314

OS1,a RWP-RK family transcription factor,is involved in both maize seed development and drought tolerance

(submitted by Weibin Song <songwb@cau.edu.cn>)
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Recessive *os1* (opaque endosperm-small germ) mutant is located on the short arm of chromosome 2. We have cloned the os1 gene through map-based approach. Additional allele and genetic complementation via transformation maize had verified that the OS1 gene encodes a RWP-RK domain containing transcription factor. Tissue-specific expression using semi-quantitative analysis showed that the OS1 transcripts can be detected in maize root, leaves, silk, anther, cob, tassel, shoot, kernel (16DAP), embryo (16DAP) and endosperm (16DAP), but expressed highest in the cob. The expression pattern was also studied among the seed after different DAP (day after pollination), which showed that the OS1 expressed higher after pollination, the peak of which was 10 DAP. We also found out that OS1 mutants are more tolerant to the drought stress in the seedlings stage than the wild-type plants.

Funding acknowledgement: 973 program of China

Quantitative Genetics & Breeding

P315

A conceptual solution to the heterosis problem

(submitted by Michael Freeling <freeling@berkeley.edu>)
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This is my labs explanation of hybrid vigor. Heterosis has been so difficult to understand because the vigor cannot be captured by a best genotype (allelic combination) as if heterosis were an emergent property of the hybrid *per se*, a hybrid luxuriance. In our studies of genome dominance-- where one subgenome postpaleopolyploid is expressed on average more than the other-- we noted a similarity to inbreeding depression. We reasoned that the unique hybrid soma could be "acting-out" until monitored as small RNA and governed in the germ going forward. Then we read an inspired paper by the Brandon Gaut lab: Hollister, J.D and Gaut, B.S. (2009) Epigenetic silencing of transposable elements: a trade-off between reduced transposition and deleterious effects on neighboring gene expression. Genome Res 19, 1419-1428. Extrapolating from the Gaut labs Trade-off Model, we explained genome dominance,inbreeding depression and, thus, heterosis. We present our solution as a cartoon with 13 embedded citations. This is an excerpt from Freeling, M., Woodhouse, M.R., Subramanium, S., Turco, G., Lisch, D. and Schnable, J.C. (2012) Current Opinions in Plant Biology 15:1-9. Enjoy.

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Computational and Large-Scale Biology

P316

Understanding the effect of drought stress responses on maize transcriptional networks

(submitted by Zhixin Zhao <zxzhao@stanford.edu>)
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Drought causes major yield losses in maize field. To understand the molecular mechanisms that underlie drought response, we used the next-generation sequencing technologies to monitor global changes in gene expression in maize plants under drought stress conditions. We compared the genome-wide Pol II binding sites in maize under normal and drought conditions at day 1, 4 and 7. In general, 70-200 million reads were obtained for each ChIP experiment, of which 63%-72% were uniquely mappable to the maize genome. Model-based analysis of ChIP-Seq (MACS) showed that ~10,000 binding sites were identified for each condition. Deseq analysis showed that 1, 53 and 135 binding sites were significantly affected by drought at day1, 4 and 7, respectively. These results reveal a progressive pattern in gene expression during drought response. Functional analysis of some of these candidates are currently under investigation.

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