

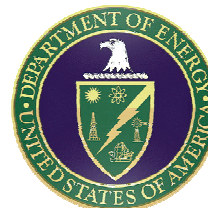
50th Annual Maize Genetics Conference
Program and Abstracts

February 27 – March 1, 2008

Marriott - Wardman Park
Washington, D.C.

This conference received financial support from:

National Science Foundation
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Dow Agrosciences
Biogemma



We thank these contributors for their generosity!

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General Information

Registration

Wednesday: 4:00-8:00 pm. Registration Room B

Thursday: 7:00-8:00 am. Salon I; 1:00-2:00 pm. Registration Room B

Friday: 1:00-2:00 pm. Registration Room B

Meals

Wednesday: Reception (hors d'oeuvres) 8:00-11:00 pm. Marriott Foyer.

Dinner on your own.

Thursday: Breakfast 7:00-8:00 am. Salon 1.

Coffee break 9:50-10:20 am. Thurgood Marshall Northeast Foyer (next to general session).

Lunch 12:00-1:00 pm. Salon 1.

Coffee break 2:00-2:30 pm. Marriott Foyer (next to posters).

Reception (hors d'oeuvres) 6:30-8:00 pm. Smithsonian Natural history Museum.

Dinner on your own.

Friday: Breakfast 7:00-8:00 am. Salon 1.

Coffee break 10:10-10:40 am. Thurgood Marshall Northeast Foyer (next to general session).

Lunch 12:20-1:00 pm. Salon 1.

Coffee break 2:00-3:00 pm. Marriott Foyer (next to posters).

Dinner 6:00-7:40 pm. Salon 1.

Saturday: Breakfast 7:00-8:00 am. Salon 1.

Coffee break 10:10-10:40 am. Thurgood Marshall Northeast Foyer (next to general session).

Lunch 12:20-1:00 pm. Salon 1.

Coffee break 2:00-3:00 pm. Marriott Foyer (next to posters).

Dinner 6:00-7:40 pm. Salon 1.

Talks and Posters

All talks will be presented in the Thurgood Marshall Ballroom Northeast. Posters will be in the Marriott Foyer and Mezzanine – they should be posted on Wednesday evening and taken down on Saturday night. During the poster sessions each day, presenters are asked to stand by odd numbered posters from 1 PM to 2 PM each day and even numbered posters from 2 PM to 3 PM each day.

Hospitality

Hospitality will be available each evening, Wednesday to Saturday, from 11:00 pm- 2:00 am, in Wilson A B C.

Dance

A dance will be held Saturday evening from 9:45 pm to 2:00 am in the Thurgood Marshall Ballroom West.

Steering Committee

Please share your suggestions and comments about the meeting with the 2008 Steering Committee:

Tom Brutnell, Chair (tpb8@cornell.edu)
Steve Moose, Co-Chair (smoose@uiuc.edu)
Pablo Rabinowicz, Local Organizer (prabinowicz@som.umaryland.edu)
Erin Irish (erin-irish@uiowa.edu)
Jorge Nieto-Sotelo (jorge@ibt.unam.mx)
Mei Guo (mei.guo@pioneer.com)
Peter Rogowsky (Peter.Rogowsky@ens-lyon.fr)
Mike Muszynski (mgmuszyn@iastate.edu)
Elizabeth Kellogg (tkellogg@umsl.edu)
Giuseppe Gavazzi (giuseppe.gavazzi@unimi.it)
Marty Sachs, local organizer, ex officio (msachs@uiuc.edu)
Karen Cone, treasurer, ex officio (ConeK@missouri.edu)
Mary Schaeffer, abstract coordinator, ex officio (Mary.Schaeffer@ars.usda.gov)
Trent Seigfried, abstract coordinator, ex officio (Trent.Seigfried@ars.usda.gov)

Acknowledgements

Many thanks go to Trent Seigfried and Mary Schaeffer for their tremendous efforts in organizing and assembling the conference program and Mike McMullen for the design of the poster. We also thank Angela Freemeyer and the team at the Missouri University Conference Center for hosting our meeting web site and for quickly implementing updates and changes to the program. Special thanks are also extended to Pablo Rabinowicz, for organizing the many receptions and events associated with this meeting and to Erin Parrish, Evangeline Wilson, Omur Tutunco and the Marriott staff for their help in organizing this conference. Thanks go to Karen Cone for her stewardship in managing the finances for this meeting and Mei Guo, Mike Muszynski, Steve Moose and Karen Cone for their efforts in securing funding to support graduate student attendance at this meeting. Finally, many thanks go to Marty Sachs for his wisdom in all things related to the Maize Meeting.

Next Maize Genetics Meeting

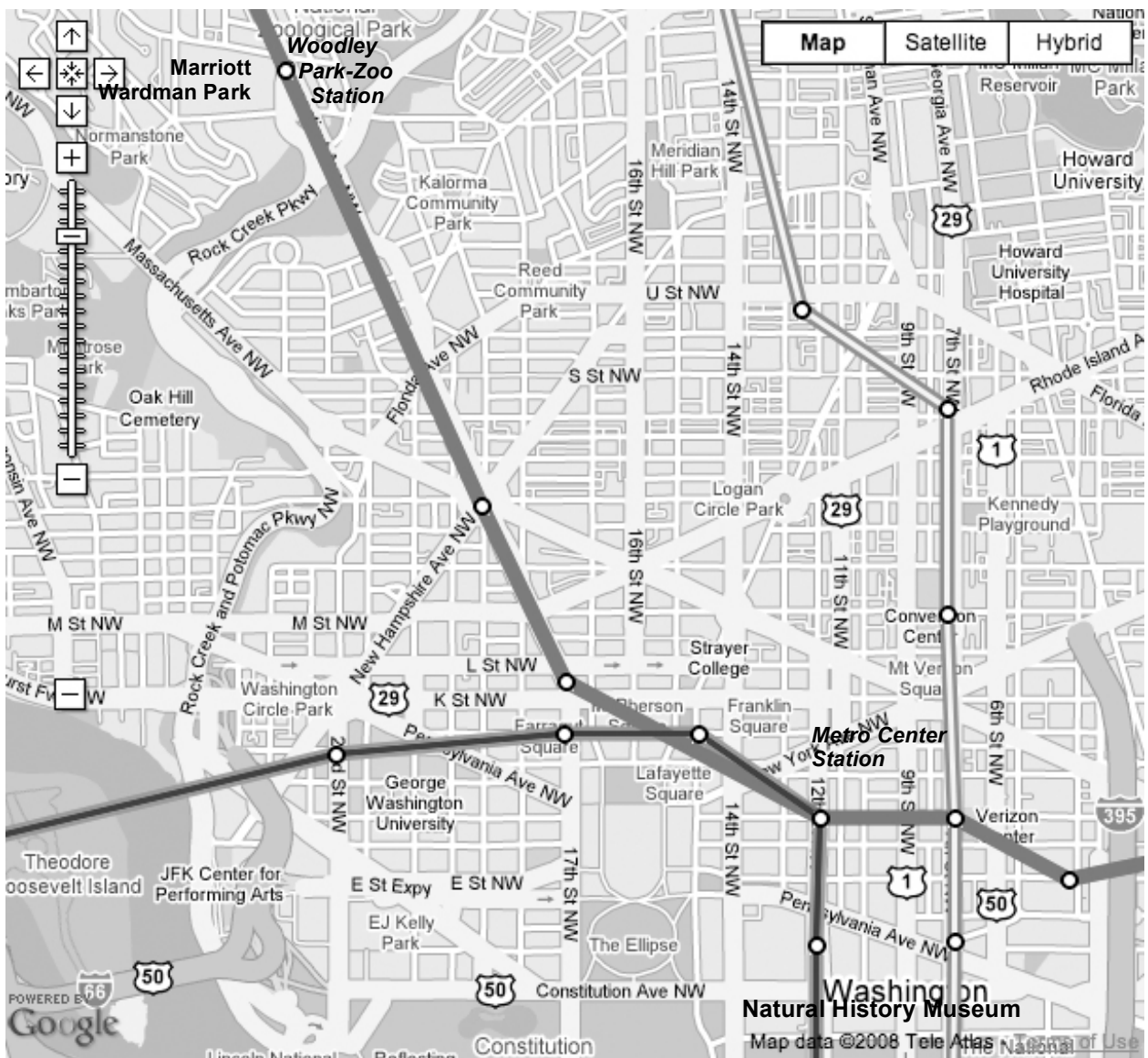
The 51th Annual Maize Genetics Conference will be held on March 12-15, 2009 at Pheasant Run in St. Charles, IL.

Directions to Smithsonian's National Museum of Natural History Thursday February 28th, 6:30 - 8:00 pm

Take the Metro Red Line in Woodley Park-Zoo station, on Connecticut Ave. NW, just by the Marriott Wardman Park Hotel. Take the train in the "Glenmont" direction and get off in Metro Center station (third station after Woodley Park-Zoo).

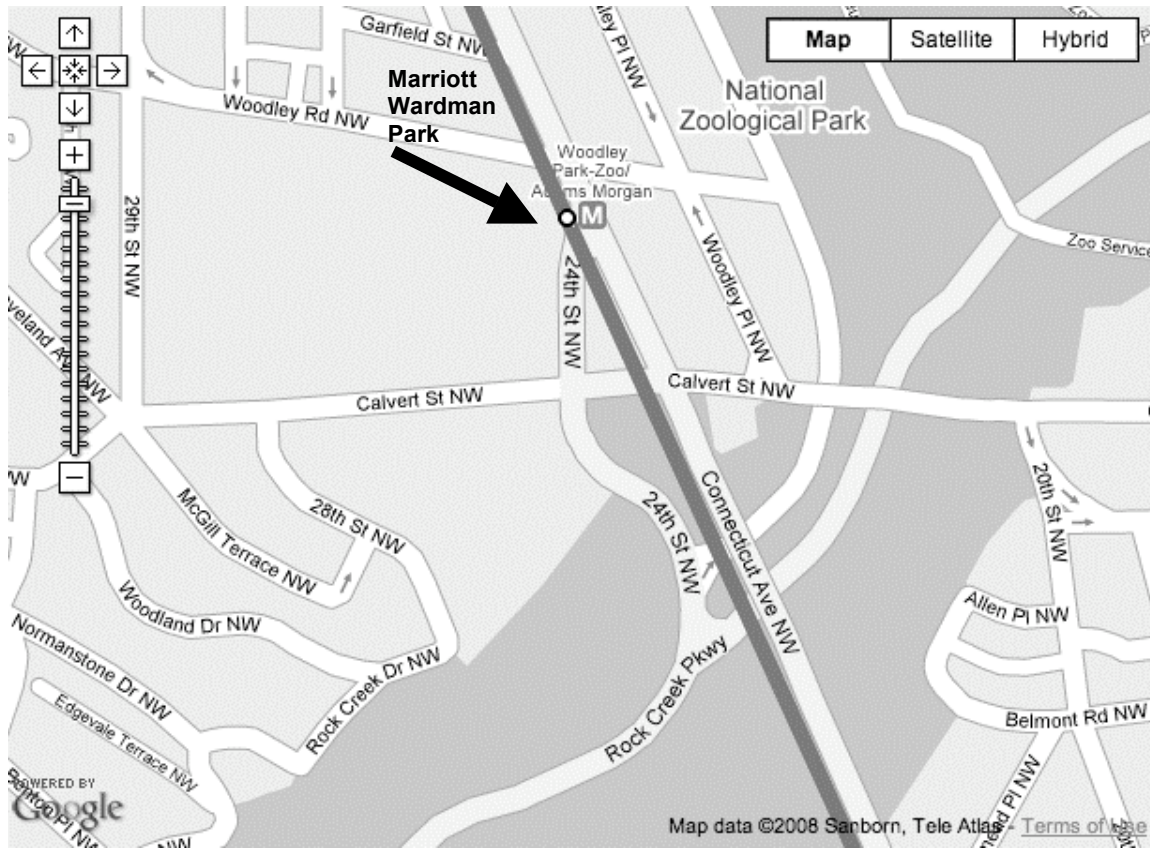
Metro Center station is on G St. NW between 11 St. NW and 12 St. NW. As you exit the escalators walk East by G St. NW and make a right on 10 St. NW. Walk South on 10 St. NW until Constitution Ave. NW. The Museum's entrance is on Constitution Ave. NW in the intersection with 10 St. NW.

Metro Red Line to the Natural History Museum:



Detailed maps:

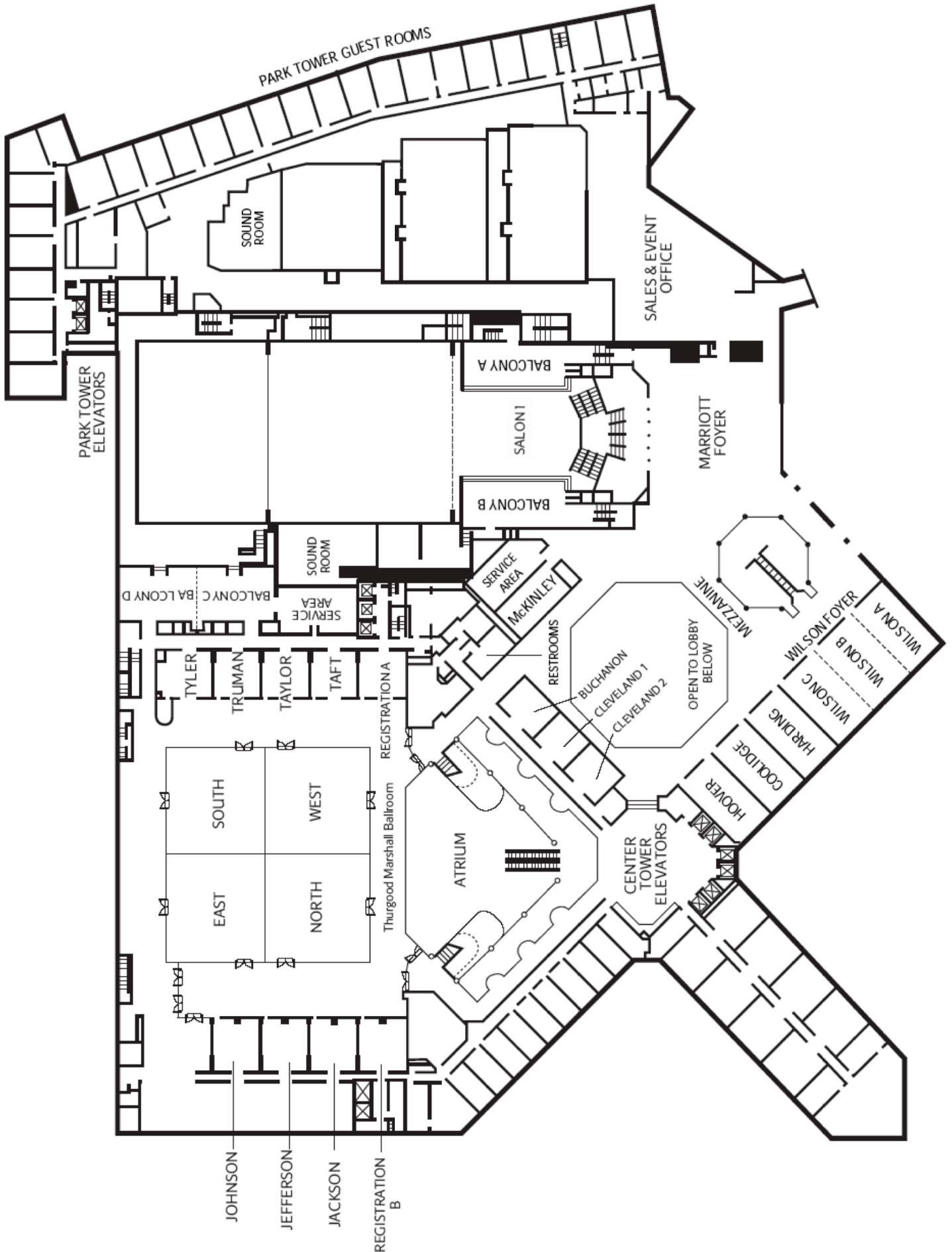
Woodley Park Zoo station:



Metro Center station:



Marriott Floor Plan (Mezzanine level)



Schedule of Events

Wednesday, February 27

- 3:00 PM – HOTEL CHECK-IN
- 4:00 PM – 8:00 PM MEETING REGISTRATION
- NO SCHEDULED DINNER
- 7:00 PM – 11:00 PM POSTER HANGING
- 8:00 PM – 11:00 PM RECEPTION (Hors d'oeuvres)/INFORMAL POSTER VIEWING
- 11:00 PM – 2:00 AM HOSPITALITY

Thursday, February 28

- 7:00 AM – 8:00 AM BREAKFAST/REGISTRATION
- 8:00 AM – 8:10 AM ANNOUNCEMENTS
- 8:10 AM – 9:00 AM **SESSION 1 – HISTORICAL PERSPECTIVES** Chair: Tom Brutnell
- 8:10 AM – 8:30 AM **William Sheridan, University of North Dakota**
Origin and evolution of the annual Maize Genetics Conference
- 8:40 AM – 9:00 AM **Lee Kass, Cornell University**
Will the real Barbara McClintock please stand up
- 9:00 AM – 12:00 PM **SESSION 2 – TRANSLATING GENOMICS TO MAIZE IMPROVEMENT** Chair: Steve Moose
- 9:00 AM **Steve Moose, University of Illinois at Urbana-Champaign**
Session introduction
- 9:10 AM **Geoff Graham, Pioneer Hi-Bred Int. Inc.**
The development and use of genetic information in a breeding program
- 9:30 AM **Nathan Springer, University of Minnesota**
Using functional genomics approaches to further understand heterosis
- 9:50 AM – 10:20 AM **COFFEE BREAK**
- 10:20 AM **Mei Guo, Pioneer Hi-Bred Int. Inc.**
Genome-wide allele-specific expression analysis of a maize hybrid and inbred parents reveals different modes of gene regulation and their roles in heterosis
- 10:40 AM **Thomas Greene, Dow AgroSciences**
Leveraging maize biochemical genetics to drive agronomic improvements in elite hybrids
- 11:00 AM **Paul Chomet, Monsanto**
Transgenic approaches to improving drought stress tolerance in maize

11:20 AM	Ed Buckler, U.S. Plant, Soil and Nutrition Lab, Cornell University <i>QTL analysis of flowering time using the maize nested association mapping panel</i>
11:40 AM	Jianbing Yan, International Maize and Wheat Improvement Center, Mexico <i>HYDB1 and its interaction with LCYE influence beta-carotene synthesis and improve provitamin A content in maize</i>
12:00 PM – 1:00 PM	LUNCH
1:00 PM – 3:00 PM	POSTER SESSION (COFFEE BREAK 2:00 PM)
3:00 PM – 5:00 PM	TRANSLATING GENOMICS POSTER SESSION
5:00 PM – 6:30 PM	CLOSE OF SESSION, TRAVEL TO SMITHSONIAN via METRO/TAXI
6:30 PM – 8:00 PM	SMITHSONIAN RECEPTION (Hors d'oeuvres)

NO EVENTS PLANNED FOR THURSDAY EVENING (POSTERS ACCESSIBLE)

Friday, February 29

7:00 AM – 8:00 AM	BREAKFAST
8:00 AM – 8:10 AM	ANNOUNCEMENTS
8:10 AM – 9:50 AM	SESSION 3 – DEVELOPMENTAL GENETICS Chair: Giuseppe Gavazzi
8:10 AM	Masaharu Suzuki, University of Florida <i>Viviparous8 encodes a putative membrane localized peptidase required for regulation of ABA accumulation as well as key embryonic regulators in maize seed development</i>
8:30 AM	Fabio Nogueira, Cold Spring Harbor Laboratory <i>Complex regulation of small RNA accumulation in the maize shoot apex</i>
8:50 AM	Paula McSteen, Penn State University <i>Integration of auxin transport and response during maize inflorescence development</i>
9:10 AM	Nathalie Bolduc, University of California, Berkeley <i>KNOTTED1 and the regulation of GA in maize meristems</i>
9:30 AM	Clinton Whipple, Cold Spring Harbor Laboratory <i>The tassel sheath loci control bract suppression during grass inflorescence development</i>
9:50 AM	Andrea Gallavotti, University of California, San Diego <i>The repression of indeterminate growth of primary axillary meristems in maize inflorescences by the rel2 gene</i>
10:10 AM – 10:40 AM	COFFEE BREAK

10:40 AM – 12:20 PM	SESSION 4 – CELL BIOLOGY Chair: Peter Rogowsky
10:40 AM	Amanda Wright, University of California, San Diego <i>dcd1 and add are needed for correct cell wall placement and encode PP2A regulatory phosphatase subunits</i>
11:00 AM	Arnaud Ronceret, Cornell University <i>POOR HOMOLOGOUS SYNAPSIS 1 acts in the cytoplasm to control homologous chromosome pairing by a novel mechanism</i>
11:20 AM	Anne Sylvester, University of Wyoming <i>Vesicle trafficking during cell wall expansion: Identifying cellular compartments by live cell imaging of tagged RAB2A1 in maize leaf cells</i>
11:40 AM	Zac Cande, University of California, Berkeley <i>Regulation of meiotic chromosome architecture by ameiotic 1</i>
12:00 PM	Michael Scanlon, Cornell University <i>Genomic analyses of functional domains in the maize shoot apical meristem</i>
12:20 PM – 1:00 PM	LUNCH
1:00 PM – 3:00 PM	POSTER SESSION (COFFEE BREAK 2:00 PM)
3:00 PM – 5:00 PM	SESSION 5 – QUANTITATIVE GENETICS Chair: Toby Kellogg
3:00 PM	Nicholas Carpita, Purdue University <i>Maize as a genetic model for improvement of wall biogenesis in bioenergy grasses</i>
3:20 PM	Bo Shen, Pioneer Hi-Bred Int. Inc. <i>A phenylalanine in DGAT is a major determinant of oil content and composition in maize</i>
3:40 PM	Michael Chandler, University of Wisconsin, Madison <i>Endogenous variation revealed by selection in su1 maize</i>
4:00 PM	Lewis Lukens, University of Guelph, Canada <i>Genomic survey of gene expression diversity in Zea mays roots in response to water stress</i>
4:20 PM	Michael McMullen, USDA-ARS, University of Missouri-Columbia <i>The nested association mapping (NAM) genetic map</i>
4:40 PM	James Holland, USDA-ARS, North Carolina University <i>Maize phenomics: massively parallel phenotyping of the nested association mapping population</i>
6:00 PM – 7:40 PM	DINNER

7:45 PM – 10:00 PM	SESSION 6 – PLENARY TALKS Chair: Tom Brutnell
7:45 PM	Susan Wessler, University of Georgia <i>Transposable Elements: From Genetics to Genomics</i>
8:35 PM	Rick Wilson, Washington University in St. Louis <i>Sequence and Assembly of The B73 Genome</i>
9:25 PM – 12:00 AM	INFORMAL POSTER VIEWING
12:00 AM – 2:00 AM	HOSPITALITY

Saturday, March 1

7:00 AM – 8:00 AM	BREAKFAST
8:00 AM – 8:10 AM	ANNOUNCEMENTS
8:10 AM – 9:50 AM	SESSION 7 – BIOCHEMICAL GENETICS Chair: Jorge Nieto-Sotelo
8:10 AM	David Braun, Penn State University <i>tie-dyed1 leaves contain increased cellulose: biofuel applicability and underlying mechanism</i>
8:30 AM	Jun Zhao, Chinese Academy of Agricultural Sciences <i>ABP9, a stress- and ABA-inducible bZIP transcription factor of maize, mediates ABA signaling and ROS scavenging and enhances tolerance to multiple environmental stresses in transgenic Arabidopsis</i>
8:50 AM	Jeff Church, University of Illinois, Urbana <i>Coordinated control of the maize carbon/nitrogen balance</i>
9:10 AM	Li Li, University of Florida, Gainesville <i>Multiple non-redundant roles for plastidic 6-phosphogluconate dehydrogenase in maize</i>
9:30 AM	Maureen Hanson, Cornell University <i>Regulation of maize chloroplast gene expression by RNA editing</i>
9:50 AM	Eleanore Wurtzel, Lehman College, City University of New York <i>The revised carotenoid biosynthetic pathway in plants</i>
10:10 AM – 10:40 AM	COFFEE BREAK
10:40 AM – 12:20 PM	SESSION 8 – GENOMICS/EPIGENETICS Chair: Mei Guo
10:40 AM	Stefan Scholten, University of Hamburg, Germany <i>Immediate paternal genome activation and enhanced trans-regulatory interactions in early maize F1 hybrid embryos</i>
11:00 AM	Karl Erhard, University of California, Berkeley <i>A maize RNA Polymerase IV large subunit is required to maintain trans-generational epigenetic regulatory states</i>

11:20 AM	Philippe Herve, IRRI, Philippines <i>Gene silencing by artificial microRNAs in monocots</i>
11:40 AM	Mihai Miclaus, Rutgers University <i>Short and long-distance placement of gene copies in the maize genome</i>
12:00 PM	Vipula Shukla, Dow AgroSciences <i>Zinc finger nuclease-mediated gene targeting in maize</i>
12:20 PM – 1:00 PM	Lunch BREAK
1:00 PM – 3:00 PM	POSTER SESSION (COFFEE BREAK 2:00 PM)
3:00 PM – 5:00 PM	SESSION 9 – THE MAIZE GENOME Chair: Pablo Rabinowicz
3:00 PM	Jean-Philippe Vielle-Calzada, National Laboratory of Genomics for Biodiversity, Mexico <i>The codifying genome of the Palomero Toluqueno Mexican landrace</i>
3:30 PM	Richard McCombie, The Maize Sequencing Consortium, Cold Spring Harbor Laboratory <i>Detailed sequence analysis of a 22 MB region of the maize genome</i>
3:42 PM	Fusheng Wei, The Maize Sequencing Consortium, University of Arizona, Tucson <i>In-depth analysis of the maize genome: case studies in a 22-Mb region and two orthologous regions totaling 14 Mb</i>
3:54 PM	Chengzhi Liang, The Maize Sequencing Consortium, Cold Spring Harbor Laboratory <i>Evidence-based gene builds in plant genomes</i>
4:06 PM	Jon Duvick, Iowa State University <i>Maize full-length gene identification, annotation, and comparison with model species</i>
4:18 PM	Eric Lyons, University of California, Berkeley <i>Maize syntenic analyses using all grass genomes as outgroups</i>
4:30 PM	Shawn Carlson, Chromatin, Inc., Chicago <i>In vitro assembled autonomous maize mini-chromosomes</i>
4:42 PM	Sergei Svitashv, Pioneer Hi-Bred Int'l, Johnston IA <i>Artificial chromosome construction in <i>Zea mays</i> L.</i>
5:00 PM	Rich Jorgensen, University of Arizona, Tucson <i>The iPlant collaborative: Empowering a new plant biology</i>
6:00 PM – 7:40 PM	DINNER

7:45 PM – 10:00 PM	SESSION 6 – PLENARY TALKS Chair: Mike Muszynski
7:45 PM	Vicki Chandler, University of Arizona <i>Epigenetic Silencing Across Generations</i>
8:35 PM	John Doebley, University of Wisconsin <i>Unraveling a Developmental Pathway Involved in Maize Domestication</i>
9:25 PM	MEETING ADJOURNMENT
9:40 PM – 12:00 AM	INFORMAL POSTER VIEWING & DANCE
12:00 AM – 2:00 AM	HOSPITALITY/POSTER REMOVAL

Posters

Biochemical Genetics

- P1 **Yi Ma**
<yum105@psu.edu>
Tie-dyed1 and sucrose transporters are expressed in the same tissues
- P2 **Christopher Diehl**
<cdiehl@vt.edu>
A jacalin-related chimeric lectin is responsible for beta glucosidase null phenotype in maize
- P3 **Federico Martin**
<fmartin@ufl.edu>
A knockout mutation of a Zea mays U2AF35 related protein alters splicing in a subset of maize genes
- P4 **Sylvia Morais de Sousa**
<smsousa@ufl.edu>
A sorbitol dehydrogenase deficiency increases sugar levels during maize development
- P5 **Andrew Funk**
<andyfunk@ufl.edu>
Altered ethylene levels and ethylene-related transcripts are seen in developing seeds of two sugar mutants in maize
- P6 **Andrew Hauck**
<ahauck@uiuc.edu>
Biomass composition diversity in maize
- P7 **Regina Dick**
<regina.dick@wzv.tum.de>
Biosynthesis of DIMBOA in maize is solved
- P8 **Yaqing Du**
<yadu@plantbio.uga.edu>
Centromere Protein C (CENP-C) targets centromeres by a novel DNA/RNA interaction
- P9 **Qiaohui Lin**
<qhlin@iastate.edu>
Characterization of dull1 plants transformed with a mutagenized Du1 coding sequence containing an altered 14-3-3 binding site
- P10 **Tracie Hennen-Bierwagen**
<tabier@iastate.edu>
Characterization of two high-molecular weight complexes from developing maize endosperm containing multiple starch biosynthetic enzymes
- P11 **Andrew Burt**
<aburt@uoguelph.ca>
Complementary sources of a high-lutein phenotype in yellow dent inbred lines
- P12 **Wojtek Majeran**
<wm48@cornell.edu>
Consequences of C4 differentiation for chloroplast membrane proteomes in maize mesophyll and bundle sheath cells
- P13 **Victor Sokolov**
<sokolov@bionet.nsc.ru>
Effect of mutagens on imprinting expression in apomictic maize-Tripsacum hybrids
- P14 **Nick Georgelis**
<gnick@ufl.edu>
Evolution and functional divergence of ADP-glucose pyrophosphorylase subunits in angiosperms
- P15 **Karen Cone**
<ConeK@missouri.edu>
Functional genomic analysis of maize chromatin genes
- P16 **Ling Bai**
<lb226@cornell.edu>
Genetic and molecular regulation of cyclic carotenoid biosynthesis in maize seeds
- P17 **Catherine Kandianis**
<cbermude@uiuc.edu>
Genetic control of provitamin A synthesis, modification and degradation in maize grain
- P18 **Cagla Altun**
<caltun@purdue.edu>
Maize has two Mre11 genes: one is novel and both appear to be developmentally regulated
- P19 **Eric Lyons**
<elyons@nature.berkeley.edu>
Maize syntenic analyses using all grass genomes as outgroups
- P20 **Taijoon Chung**
<tchung2@wisc.edu>
Molecular analysis of autophagic pathway in maize
- P21 **Maureen Hanson**
<mrh5@cornell.edu>
Molecular studies of maize photosynthetic mutants with transposon insertions in nuclear genes encoding chloroplast targeted pentatricopeptide repeat (PPR) proteins

- P22 **Ratnakar Vallabhaneni** [<ratnakarvallabhaneni@yahoo.com>](mailto:ratnakarvallabhaneni@yahoo.com) *Natural genetic diversity as a tool towards developing metabolic engineering strategies to improve or modulate endosperm carotenogenesis*
- P23 **Robert Baker** [<rbl11@psu.edu>](mailto:rbl11@psu.edu) *Oh tie-dyed, where art thou?*
- P24 **Xiang Yang** [<yangx@iastate.edu>](mailto:yangx@iastate.edu) *Regulation of hormone pathways by the ramosa genes in maize inflorescence development*
- P25 **Hugh Young** [<hyoung@purdue.edu>](mailto:hyoung@purdue.edu) *Role of HC-toxin in disease susceptibility: redefining the paradigm*
- P26 **Marna Yandea-Nelson** [<mdn3@psu.edu>](mailto:mdn3@psu.edu) *Starch branching enzyme (SBE) IIa is required for diurnal cycling of starch within the maize leaf*
- P27 **Clifford Weil** [<cweil@purdue.edu>](mailto:cweil@purdue.edu) *Sucrose hyperaccumulation and ionic changes in maize leaves over development*
- P28 **Mingshu Huang** [<muh147@psu.edu>](mailto:muh147@psu.edu) *The camouflage1 mutant is defective in tetrapyrrole synthesis and displays nonclonal sectors in its leaves*
- P29 **Thomas L. Slewinski** [<tls315@psu.edu>](mailto:tls315@psu.edu) *Tie-dyed1 localizes to the endoplasmic reticulum and co-localizes with sucrose transporter1*
- P30 **George Chuck** [<gchuck@nature.berkeley.edu>](mailto:gchuck@nature.berkeley.edu) *Using the Corngrass1 gene as a tool for biofuel improvement*

Bioinformatics

- P31 **James Estill** [<jestill@plantbio.uga.edu>](mailto:jestill@plantbio.uga.edu) *A RepMiner analysis of maize LTR Retrotransposons reveals a previously unrecognized split in the Huck family*
- P32 **Zhiwu Zhang** [<zz9@cornell.edu>](mailto:zz9@cornell.edu) *An efficient algorithm to strengthen the power of nested association mapping experimental design*
- P33 **Lisa Harper** [<ligule@nature.berkeley.edu>](mailto:ligule@nature.berkeley.edu) *Ask for a MaizeGDB outreach visit to your institution!*
- P34 **Erich Grotewold** [<grotewold.1@osu.edu>](mailto:grotewold.1@osu.edu) *GRASSIUS: a blueprint for comparative regulatory genomics across the grasses*
- P35 **Yujun Han** [<yhan@plantbio.uga.edu>](mailto:yhan@plantbio.uga.edu) *Identifying active transposable elements candidates: faster and simpler*
- P36 **Nick Lauter** [<nick.lauter@ars.usda.gov>](mailto:nick.lauter@ars.usda.gov) *Maize Microarray Platform Translator, a web-based tool at PLEXdb to enhance meta-analysis of gene expression profiling data*
- P37 **Matthew Krakowsky** [<Matt.Krakowsky@ars.usda.gov>](mailto:Matt.Krakowsky@ars.usda.gov) *Maize allelic diversity project*
- P38 **Brad Barbazuk** [<bbarbazuk@danforthcenter.org>](mailto:bbarbazuk@danforthcenter.org) *Maize trained TWINSCAN and ab initio gene finding in maize*
- P39 **Mary Schaeffer (Polacco)** [<Mary.Schaeffer@ars.usda.gov>](mailto:Mary.Schaeffer@ars.usda.gov) *MaizeGDB as chromosome walking companion*
- P40 **Taner Sen** [<Taner.Sen@ars.usda.gov>](mailto:Taner.Sen@ars.usda.gov) *MaizeGDB's new genome browser project*
- P41 **Pierre Montalent** [<montalen@moulon.inra.fr>](mailto:montalen@moulon.inra.fr) *Structural annotation of maize genes: training and tuning of the Eugene combiner*
- P42 **Apurva Narechania** [<apurva@cshl.edu>](mailto:apurva@cshl.edu) *Toward a better understanding of cereal genome evolution through Ensembl Compara*
- P43 **Jason Green** [<jason@diglib1.cecs.missouri.edu>](mailto:jason@diglib1.cecs.missouri.edu) *Using association rules in the QTL mapping of complex quantitative traits*
- P44 **Shiran Pasternak** [<shiran@cshl.edu>](mailto:shiran@cshl.edu) *What's new at MaizeSequence.org*

Cell Biology

- P45 **Christopher Bozza**
<cgb25@cornell.edu> *dsyCS and segII: unique mutants help understand homologous pairing*
- P46 **Matthew Hudson**
<mhudson@uiuc.edu> *A conserved role for bHLH transcription factors in maize light signaling*
- P47 **John Humphries**
<jhumphries@ucsd.edu> *A role for pan1 in cell polarization during maize stomata development*
- P48 **Janelle Jung**
<jkj4@cornell.edu> *Anatomical differences in the bundle sheath and mesophyll cells of maize seedlings across a leaf developmental gradient*
- P49 **Amber Brown**
<brown@bio.fsu.edu> *Genetic analysis of telomere length regulation*
- P50 **Brent O'Brien**
<bob2373@ufl.edu> *Identification and characterization of Mu-inserts in genes potentially affecting cell wall biosynthesis*
- P51 **Xuexian Li**
<xli@plantbio.uga.edu> *Identification and characterization of maize inner kinetochore protein MIS12*
- P52 **Terry L. Kamps**
<kampsufl@yahoo.com> *Mutations in nuclear genes alter post-transcriptional regulation of mitochondrial genes*
- P53 **Christine Chase**
<ctdc@ifas.ufl.edu> *Pollen development in male-fertile and S male-sterile maize*
- P54 **Prem Chourey**
<pschourey@ifas.ufl.edu> *The Miniature-1 (Mn1) gene product, cell wall invertase-2 (INCW2), is associated with wall-in-growths (WIGs) in basal endosperm transfer cells (BETCs) in developing seeds of maize*
- P55 **Christopher Topp**
<ctopp@plantbio.uga.edu> *xChIP combined with deep sequencing to reconcile the functional maize centromere with its DNA sequence*

Cytogenetics

- P56 **Moira Sheehan**
<mjs224@cornell.edu> *Dissecting the formation and function of the meiotic telomere bouquet using the plural abnormalities of meiosis1 (pam1) mutant of maize*
- P57 **Rachel Wang**
<rachelcjw@berkeley.edu> *Homologous synapsis revealed by ultrahigh resolution structured illumination (SI) microscopy*
- P58 **Rick Masonbrink**
<remkv6@mizzou.edu> *Increasing the copy number of minichromosomes derived from the B chromosome*
- P59 **Ashley Lough**
<anl6d9@mizzou.edu> *Investigation of the mitochondrial DNA insertion site on maize chromosome 9L*
- P60 **Inna Golubovskaya**
<innagol@berkeley.edu> *Maize meiosis and meiotic genes*
- P61 **William F. Sheridan**
<bill.sheridan@und.edu> *Maize nonhomologous chromosome segments likely interact throughout the genome in a dosage sensitive fashion to affect plant development*
- P62 **Tatiana Danilova**
<danilovat@missouri.edu> *Primed in situ labeling (PRINS) of maize somatic chromosomes*
- P63 **Fangpu Han**
<hanf@missouri.edu> *Reactivation of inactive centromeres in maize*
- P64 **Shaun Murphy**
<murphy@sb.fsu.edu> *Towards the molecular cloning of meiotic telomere behavior mutants in maize*

Developmental Genetics

- P65 **Solmaz Barazesh**
<sxb944@psu.edu> *sparse inflorescence 1 encodes an auxin biosynthesis gene which functions in axillary meristem initiation in the inflorescence*
- P66 **Peter Bommert**
<bommert@cshl.edu> *Analysis of meristem size regulation in maize*
- P67 **Christine Majer**
<christine.majer@zmbp.uni-tuebingen.de> *Analysis of molecular interactions during shoot-borne root formation in maize*
- P68 **Cristian Forestan**
<cristian_forestan@unipd.it> *Auxins & pin1 proteins: Role during maize kernel development and membrane targeting analysis*
- P69 **Candice Hansey**
<cnhansev@wisc.edu> *Axillary meristem development of a variable penetrance maize mutant, grassy tillers1*
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- P126 **Brandi Sigmon**
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<ibe@ufl.edu> *Reverse genetics for cell-wall mutants in the UniformMu maize population*
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<sonali.gandhi@syngenta.com> *Syngenta maize allelic diversity platform*
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<jkpz2@missouri.edu> *A survey of diverse Zea mays germplasm identifies new sources of resistance to Western corn rootworm (Diabrotica virgifera virgifera LeConte)*
- P172 **Nick Lauter**
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- P175 **Raja Khanal**
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Association and linkage mapping of nitrogen metabolism enzyme activities in maize
- P177 **Elliot Heffner**
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Association mapping and genome-wide breeding value estimation for marker assisted selection of parental and elite inbred lines
- P178 **Patrick Brown**
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- P184 **Nick Lauter**
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- P185 **Elhan Ersoz**
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Genetic modulation of harvest index for improved biofuel feedstocks
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- P189 **Junyun Yang**
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- P190 **Thanda Dhliwayo**
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High precision growth and high-content phenotyping of complete corn plants
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- P193 **Devin Nichols**
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- P194 **Maria Mayor**
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Impact of lignin on resistance to Aspergillus flavus infection in maize

- P196 **Xiaohong Yang**
<redvx@163.com> *Major and minor QTL as well as epistasis contribute to fatty acid composition and oil concentration in high-oil maize*
- P197 **Chia-Lin Chung**
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<garci191@umn.edu> *Mapping quantitative trait loci for kernel oil production in Korean High Oil (KHO) corn*
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<upadyayu@uiuc.edu> *Nested association mapping analysis of tassel architecture*
- P200 **Kendra Meade**
<kameade@iastate.edu> *Phenotypic analysis of kernel water relations in the Mo17xH99 RIL population*
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<wangtianyu@263.net> *Phenotypic diversity of flowering-related traits of maize landraces from the core collection preserved in Chinese National Genebank*
- P202 **Andrea Chambers**
<aarmst03@uoguelph.ca> *Physiological mechanisms underlying grain yield QTLs*
- P203 **Ramneek Kooner**
<rzk126@psu.edu> *Proteomic analysis of maize leaf tissues from inbred lines resistant and susceptible to fall armyworm, *Spodoptera frugiperda* (J.E. Smith)*
- P204 **Bryan Penning**
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- P205 **Patrice G. Dubois**
<pgd7@cornell.edu> *QTL analysis of the shade avoidance syndrome in maize*
- P206 **Eric Riedeman**
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- P209 **Zhonghui Tang**
<zhonghui.tang@gmail.com> *Single segment introgression lines based cloning a QTL for plant high in maize*
- P210 **Valeriy Rotarencu**
<rotarencu@mail.md> *Some aspects of haploid induction in maize*
- P211 **Maria Cinta Romay**
<cromay@mbg.cesga.es> *Study of *Vgt1* using joint linkage and association mapping*
- P212 **Hanneke Witsenboer**
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- P213 **Peter Balint-Kurti**
<peter_balintkurti@ncsu.edu> *The analysis of quantitative resistance to foliar diseases of maize*
- P214 **Martin Bohn**
<mbohn@uiuc.edu> *The genetic analysis of maize root complexity*
- P215 **Peter Balint-Kurti**
<Peter.Balint-Kurti@ars.usda.gov> *The genetic architecture of multiple disease resistance in maize*
- P216 **Kristen Kump**
<klkump@ncsu.edu> *The genetic architecture of southern leaf blight resistance revealed by nested association mapping*
- P217 **Travis Coleman**
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- P218 **Michael Gerau**
<mjgf36@mizzou.edu> *Traits associated with brace root characters implicate light and hormonal signaling pathways*
- P219 **Juan Salerno**
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- P220 **Jesse Poland**
<jap226@cornell.edu> *Uncovering molecular mechanisms of quantitative disease resistance using nested association mapping*
- P221 **Feng Tian**
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- P222 **Allison Krill**
<amk72@cornell.edu> *Using nested association mapping to study aluminum tolerance in maize*
- P223 **John Fowler**
<fowlerj@science.oregonstate.edu> *Variation in pollen competitive ability in diverse maize lines*

Transposable Elements

- P224 **Chuanhe Yu**
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- P225 **Erik Vollbrecht**
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- P226 **Kazuhiro Kikuchi**
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- P227 **Damon Lisch**
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- P228 **R. Keith Slotkin**
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- P229 **Lixing Yang**
<lxyang@uga.edu> *Helitron discovery and description across the plant kingdom*
- P230 **Clementine Vitte**
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- P231 **Charles Hunter**
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- P232 **David Skibbe**
<skibbe@stanford.edu> *Mu transposition alters the transcriptome and proteome of developing anthers*
- P233 **Charles Nathan Hancock**
<cnhancock@plantbio.uga.edu> *Regulation of the Ping transposase proteins*
- P234 **Wusirika Ramakrishna**
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- P235 **Regina Baucom**
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- P236 **Jun Huang**
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- P237 **Michael Adedotun**
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- P239 **Natalie Fredette**
<nf04d@fsu.edu> *The Maize-10-Maze project, an educational public chromosome map garden featuring the magnificent mutants of maize*
- P240 **Anne Sylvester**
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- P241 **Olga Danilevskaya**
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Plenary Talk Abstracts

Plen1

Understanding the other big bang: how TEs amplify throughout genomes

(presented by Sue Wessler <suew@plantbio.uga.edu>)

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The results of genome sequencing projects have revealed that the genes and genomes of higher plants and animals are littered with transposable elements (TEs). However, virtually nothing is known about how some TEs in some genomes overcome host repression and scatter like buckshot throughout the chromosomes of a population. While the impact of TEs on mammalian genome evolution has begun to receive considerable attention, TE analysis has been central to plant genetics and genomics. Most characterized plant genomes have rich collections of TEs including many active elements. In particular, members of the grass clade, including maize and rice, are in an epoch of TE-mediated genome diversification and, as such, are ideal for analyzing the earliest stages of TE amplification.

Our laboratory has employed computational methods to determine the TE content of plant genomes and to identify potentially active elements. These studies led to the discovery of MITEs, short, nonautonomous DNA transposons that are the prevalent TE in and near plant genes. In this talk I will focus on the ability of MITEs to rapidly increase their copy number from one or a few elements to thousands of insertions in genic regions. For one rice MITE family, mPing, we have caught the element in the act of bursting from 1 to over 1000 elements and have documented the impact of insertion at these early stages. For another rice MITE family called Stowaway, successful transposition in yeast has facilitated the isolation of a hyperactive MITE and the identification of features necessary for its efficient transposition. I will also discuss how knowledge of the TE landscape of the relatively stable rice genome is informing our studies into the extremely dynamic genome of maize.

Plen2

Sequence and assembly of the maize B73 genome

(presented by Richard Wilson <rwilson@wustl.edu>)

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The Maize Genome Sequencing Consortium was launched with a three-year grant from NSF to produce a complete sequence of the maize (B73) genome. We currently are at the beginning of the third year of the project, and recently have completed the sequencing of 15,200 BAC clones that correspond to an initial minimal tiling path for the genome. Assembled together, these clones represent a high-quality draft sequence of the maize genome, comprised of approximately 2 billion non-redundant bases. This draft sequence, accessible via GenBank and, of most relevance to the maize geneticist, via the Gramene Genome Browser (maizesequence.org), provides a very exciting first look at the entire maize genome. In this presentation, we will describe the maize genome draft sequence and the work that was required to produce it. We also will discuss the efforts required during the third year of the project to improve upon the draft sequence and develop the associated annotation.

Plen3

Epigenetic silencing across generations

(presented by Vicki Chandler <chandler@Ag.arizona.edu>)

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Paramutation is the fascinating ability of specific DNA sequences to communicate in trans to establish meiotically heritable expression states. Originally described at the r1 locus in maize, additional paramutation examples have been studied in maize and in other species. Paramutation at the maize b1 locus is mediated by seven unique noncoding tandem repeats of 853 bp. Experiments will be presented demonstrating that these repeats are both necessary and sufficient to communicate in trans to establish and maintain the meiotically heritable expression and distinct chromatin states associated with b1 paramutation. Several genes required for paramutation have been identified through genetic screens. These mediator of paramutation (mop) genes are required for paramutation at multiple loci, Mutator transposon silencing, and reactivation of transcriptionally silent transgenes. Several mutants exhibit pleiotropic developmental phenotypes. Map-based cloning of mop1 has revealed it encodes an RNA dependent RNA polymerase gene (RDR), most similar to RDR2, the RDR in Arabidopsis that is associated with production of siRNA (short interfering RNA) molecules targeting heterochromatin. Nuclear run-on assays reveal that the tandem repeats mediating b1 paramutation are transcribed from both strands, and the presence of tandem repeat siRNAs depends on mop1. However, the tandem repeats are transcribed and siRNAs produced from them in all b1 alleles, even those which do not undergo paramutation and have a single copy of the sequence. These data suggest siRNAs are involved, but are not sufficient. Models for b1 paramutation incorporating published and unpublished data will be discussed.

Plen4

Unraveling a developmental pathway involved in maize domestication

(presented by John Doebley <jdoebley@wisc.edu>)

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Maize is a domesticated form of a wild Mexican grass called teosinte. The domestication of maize from teosinte occurred about 8,000 years ago. As a result of human (artificial) selection during the domestication process, dramatic changes in morphology arose such that maize no longer closely resembles its teosinte ancestor in ear and plant architecture. Quantitative trait locus (QTL) mapping has shown that many genes contributed to the differences between maize and teosinte, but among these are several of very large effect. We have cloned and analyzed two of these large-effect genes. teosinte branched (tb1) is largely responsible for the difference between the long branches of teosinte versus the short branches of maize. tb1 encodes a transcriptional regulator that functions as a repressor of branch elongation. Gene expression analysis indicates that the product of the teosinte allele of tb1 accumulates at about half the level of the maize allele. Fine-mapping experiments show that the differences in phenotype and gene expression are controlled by an enhancer that is 65 kb upstream of the ORF. teosinte glume architecture (tga1) is largely responsible for the formation of a casing that surrounds teosinte seeds but is lacking in maize. tga1 also encodes a transcriptional regulator, however in this case a single amino acid change represents the functional difference between maize and teosinte. This single amino acid change appears to convert the maize allele into a transcriptional repressor of target genes. Analysis of the interactions between tb1, tga1 and other domestication genes indicates that they form a cascade of transcriptional regulators that were a target of human selection during the domestication process.

Short Talk Abstracts

T1

Origin and evolution of the annual Maize Genetics Conference

(submitted by William Sheridan <bill.sheridan@und.nodak.edu>)

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The first Maize Genetics Conference was initiated by a letter sent out from the University of Illinois by Professor John Laughnan. The letter was an invitation to other maize geneticists at Indiana, Iowa State, Minnesota, Missouri, Purdue, and Wisconsin to meet at the Allerton House in January 1959. At that meeting the 22 participants decided that the meeting in 1960 would be held in March and would be open to maize geneticists at other institutions. Throughout the ensuing years the attendance at the meeting gradually increased until there were over 100 participants that overflowed the meeting room. Brief talks were presented and Professor Earl Patterson oversaw the scheduling during the meetings. At the 25th Conference in 1983 the first Steering Committee was organized, and it recommended that the meeting be relocated. The 26th Conference was held at the Ramada Inn in Champaign and titles of talks were solicited by postcards prior to the meeting, resulting in prior scheduling of talks. The 27th Conference was held at Delavan, WI and the program was printed and distributed. The number of participants continued to increase and the Conference was eventually relocated to other sites in Wisconsin and Illinois. During this period the submission of abstracts and their printing was started, and the presentation of posters was introduced. In addition, evening sessions with invited speakers began. Because of the growth of maize genetics, interest developed in meeting outside the Midwest. In 1995 the 37th Conference was held in Pacific Grove, CA. The number of participants continued to increase to about 500, and the Conference has been held at several sites outside the Midwest, including Mexico City.

T2

Will the real Barbara McClintock please stand up

(submitted by Lee B Kass <lbk7@cornell.edu>)

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Popular accounts of Barbara McClintock, Nobel Prize winning geneticist for discovery of transposable elements in maize, have portrayed her life as isolated and secluded and her work as unappreciated and rejected. These stories are founded on memories and recollections far removed from contemporaneous events. As Nina Fedoroff reminded us in the DYNAMIC GENOME McClintock's early contributions to maize genetics were "greater than any of her peers - had she done no more, McClintock would have become a major figure in the history of genetics." I will briefly present McClintock's early contributions to the maize genetics and cytogenetics community, which prepared the way for the spirit of cooperation in maize cytogenetics and for which she was widely recognized.

T3

The development and use of genetic information in a breeding program

(submitted by Geoff Graham <geoff.graham@pioneer.com>)

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Pioneer Hi-Bred Int'l has been investigating genetic markers and their applications to breeding programs since the late 1980s. Today, genetic information is routinely generated and used in all components of a breeding program from choosing breeding crosses to predicting specific hybrid performance. Another consequence of the identification of QTL has been a rich supply of targets for further characterization to understand the underlying genetic causes of phenotypic variation and how this impacts selection. In this talk we will discuss how genetic information is extracted from the breeding program and its downstream uses.

T4

Using functional genomics approaches to further understand heterosis

(submitted by Nathan Springer <springer@umn.edu>)

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Heterosis describes the superior performance of an F1 hybrid individual relative to the inbred parents. Maize hybrids exhibit high levels of heterosis for a variety of phenotypic traits. While we have made extensive use of heterosis to increase agricultural production of maize, we have a limited understanding of the molecular basis for heterosis. The recent availability of functional genomics approaches has provided opportunities to further study gene expression in hybrids. Several groups have used expression profiling approaches to characterize the levels of transcriptome variation between maize inbreds. In addition, these expression profiling approaches have been used to study expression levels in hybrids relative to the inbred parental lines. In general, most genes are expressed at additive levels in maize hybrids. However, some genes exhibit non-additive expression and a small number of these genes can exhibit expression levels outside the parental range. A recent study suggests that maize hybrids with higher levels of heterosis exhibit similar proportions more transcriptional diversity than maize hybrids with lower levels of heterosis but that the proportions of additive and non-additive expression are similar in these hybrids. Together, these studies have provided a much clearer understanding of gene expression patterns in maize hybrids. While these studies have not provided a detailed mechanistic understanding of heterosis, they have helped to further shape the discussion of the mechanisms of heterosis.

T5

Genome-wide allele-specific expression analysis of a maize hybrid and inbred parents reveals different modes of gene regulation and their roles in heterosis

(submitted by Mei Guo <mei.guo@pioneer.com>)

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Most of the efforts in understanding the molecular basis of heterosis have been focusing on total transcript level in the hybrid and inbred parents without discriminating parental alleles. Heterosis results from the combination of two parental alleles, presumably altered expression of the alleles in the hybrid. In this study we address hybrid gene regulation at the allele-specific level. We developed a new methodology for genome-wide allele-specific expression analysis by applying Massively Parallel Signature Sequencing (MPSS), an open ended and sequencing based mRNA profiling technology. Analysis of a maize hybrid and its inbred parents during meristem development reveals that ~60% of the genes exhibited differential allelic expression in the hybrid. Because both alleles are regulated by the same trans-acting factors in the common hybrid, the wide-spread allelic expression diversity suggests the roles of allelic variation in cis-regulatory factors. Such allelic diversity is positively associated with hybrid yield and heterosis and has been favored in breeding improvement. Comparison of the same allele expressed in the hybrid relative to its inbred parent showed that ~35% of the genes were differentially expressed, presumably due to different trans-acting effects in the two genotypes. Such trans-acting effects may result in non-additive gene expression in the hybrid. The study reveals various modes of gene regulation at the allele-specific level, and provides a new level of understanding the molecular basis of heterosis.

T6

Leveraging maize biochemical genetics to drive agronomic improvements in elite hybrids

(submitted by Thomas Greene <twgreene@dow.com>)

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Characterization of maize mutants has provided critical biological information leading to a greater understanding of biochemical pathways. The role of biochemical genetics is perhaps best exemplified by starch endosperm mutants. Analysis of these mutants has helped reveal the roles and impact of the genes that catalyze key metabolic steps in the starch biosynthetic pathway. Mutants of *shrunken2* and *brittle2* clearly show that ADPglucose pyrophosphorylase (AGP) catalyzes a major flux control point in this pathway. This has been further substantiated by a growing body of work defining AGP's role in starch biosynthesis and seed fill. That AGP has such a prominent role in this pathway clearly identifies it as a target for manipulating carbon flux in the seed and potentially yield in higher plants. Because the endogenous AGP enzyme is heat labile, we have developed elite corn lines expressing AGP stability variants to enhance the agronomic performance of our elite hybrids. Initial field data indicate that enhanced stability of AGP may stabilize yield under high temperature field conditions. Development of the lead transgenic events and initial field results will be discussed.

T7

Transgenic approaches to improving drought stress tolerance in maize

(submitted by Paul Chomet <paul.chomet@Monsanto.com>)

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Efficient use of water in agricultural production will be one of the great challenges during the 21st Century, with agriculture currently being responsible for ~70% of freshwater withdrawal. As such, yield improvement through tolerance to water deficits that occur routinely in the Central Corn Belt and frequently in western states are an important challenge in the coming decade. Benefits of improving water utilization efficiency, in addition to higher yield, are expected to include reduced water consumption and environmental sustainability. Genetic approaches using model systems are adding to our understanding of plant pathways that are important to water stress tolerance. Transgenic approaches in model systems such as *Arabidopsis* have identified genes that effectively confer drought tolerance in both *Arabidopsis* and crops such as soybean and corn. This presentation will illustrate our ability to uncover novel drought protection mechanisms using genomics data and transgenic screening and will highlight data from *Arabidopsis*, and corn transgenics that demonstrate the enormous opportunity that exists for the application of genomics to product development in crops.

T8

QTL analysis of flowering time using the maize nested association mapping panel

(submitted by Edward Buckler <esb33@cornell.edu>)

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The maize genome is a source of tremendous phenotypic and molecular diversity. Such abundant variation was first used by Native Americans to adapt maize from the tropics to the Andes to Canada. The long-term objective of our group is to understand how a complex trait like flowering time is controlled and evolved. To study complex traits, we have developed a genetic design that integrates both linkage and association approaches to provide high resolution and statistical power. The maize nested association mapping (NAM) panel is the product of crossing 25 highly diverse maize lines to B73 and deriving 5000 recombinant inbred lines, which were subsequently phenotyped, and genotyped. This is the largest complex trait study of its kind, and it provides important insights into the nature of the genetic architecture for a species. Over 50 QTL were identified, the majority are shared among populations. Despite tremendous power to detect B73 specific QTL, they appear to be very rare. This QTL distribution differs from the SNP distribution. There were no large effect QTL across the species the vast majority of QTL had effects under two days. Additionally, we find evidence that the majority of QTL have an allelic series. Many QTL correlate with population structure but none define it. We are currently testing the limits of linkage versus association mapping on this population. Through NAM, Vgt1 is validated. Additionally, we have found evidence for a QTL near Rap2.7, a locus regulated by Vgt1. Progress on fine mapping of major QTL on chromosomes 1 and 10 will be presented. The full power of this system will be enabled with the ongoing sequencing of the parental lines by next generation sequencing technology and further high density genotyping. Large multi-family germplasm, association analysis, and genomic tools will likely revolutionize the study of complex traits.

T9

HYDB1 and its interaction with LCYE influence beta-carotene synthesis and improve provitamin A content in maize

(submitted by Jianbing Yan <j.yan@cgiar.org>)

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In developing countries, nearly one quarter of all pre-school children (127 million) suffer from vitamin A deficiency (VAD). Globally, approximately 4.4 million pre-school aged children have developed visible eye damage due to VAD. Enhancing the micronutrient content of staple food crops is one economical alternative to improve human nutrition and health. Here, we report a gene underlying a quantitative trait locus, beta-hydroxylase (*hydb1*), is associated with provitamin A content in maize kernels. Three independent association mapping panels, three independent linkage mapping populations, qRT-PCR expression analysis and E.coli functional assays were used to confirm these results. Natural variation was identified within *hydb1* that explains 23% and 37% of the phenotypic variation for beta-carotene content and the ratio of beta-carotene over total carotenoids, respectively. Strong epistatic interactions were found between *hydb1* and lycopene epsilon cyclase gene (*lcy-beta*), another recently identified major gene controlling provitamin A content in maize. Combined analysis of the two genes indicate that 43% and 56% of the phenotypic variation for beta-carotene content and the ratio of beta-carotene over total carotenoids respectively are accounted for. The frequencies of the beneficial alleles of the two genes are very different and contrasting in temperate and tropical lines, indicating that major advances in beta-carotene improvement are likely if alleles from these two different germplasm sources are combined. To facilitate this task, we have created PCR-based markers and present the allelic composition of global maize germplasm that should be very useful for producing high level provitamin A maize in the future, especially in developing countries.

T10

***Viviparous8* encodes a putative membrane localized peptidase required for regulation of ABA accumulation as well as key embryonic regulators in maize seed development**

(submitted by Masaharu Suzuki <masaharu@ufl.edu>)

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The regulatory network comprised of interacting LEC1 and B3 transcription factors, respectively, plays a central role in the developmental program for seed formation in plants. A subset of these factors interacts with specific hormone signaling pathways to enable integration of the developmental processes. In maize, mutants that cause ABA deficiency or loss of ABA sensitivity typically have a viviparous seed phenotype. Hence, most of the molecularly characterized viviparous mutants of maize have either been implicated in the ABA biosynthesis or ABA signaling. An intriguing exception is the pleiotropic *viviparous-8* mutant. The *Vp8* gene, which we have recently cloned, encodes a putative membrane localized peptidase. In addition to the viviparous phenotype, *vp8* causes a heterochronic shift in vegetative development that is not attributable to ABA biosynthesis/signaling (Evans and Poethig, 1997). In order to gain insight on function of *Vp8* in regulation of hormone accumulation and signaling during seed development, we conducted extensive characterization of *vp8* seed development. Consistent with the early onset vivipary, expression of a subset of LEC1/B3 domain transcription factors, was decreased in the *vp8* embryo. Moreover, reduced ABA content of *vp8* embryo was correlated with increased expression of a key gene in the ABA catabolic pathway. Interestingly, the *vp8* phenotype was remarkably different in three genetic backgrounds that we tested. The phenotypic differences are due at least in part to presence of a partially dominant suppressor of *vp8* in a certain background. In W22 inbred where the suppressor is not present, the mutant seeds have aborted embryos and altered patterning of aleurone differentiation in endosperm. Consistent with the severe phenotype, profound changes in gene expression related to ABA accumulation and the embryonic regulatory network were detected. We will discuss on function of *Vp8* in regulation of meristem development as well as maturation processes during maize seed development.

T11

Complex regulation of small RNA accumulation in the maize shoot apex

(submitted by Fabio Nogueira <nogueira@cshl.edu>)

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Establishment of adaxial-abaxial (upper-lower) polarity is essential for the outgrowth and patterning of leaves. We have recently shown that organ polarity in maize is specified through the opposing activity of two distinct small RNAs. The trans-acting siRNA, tasiR-ARF, defines the adaxial side of the leaf by restricting the expression domain of the microRNA miR166, which in turn delineates the abaxial side by restricting expression of adaxial determinants. Biogenesis of tasiR-ARF further requires the activity of miR390. We have found that miR390 is expressed on the adaxial side of incipient and developing leaf primordia. Polarized miR390 expression persists even in the abaxialized leaves of *leafbladeless1* (*lbl1*) mutants, suggesting this small RNA is an upstream component in the adaxial-abaxial patterning network. To further dissect the mechanisms underlying the polarized accumulation of these polarizing small RNAs, we used laser-capture microdissection (LCM) coupled to RT-PCR to determine the expression domains of their precursor transcripts in distinct domains of the SAM and developing leaves. Our data reveals that: 1) the pattern of miR166 accumulation results in part from the intricate transcriptional regulation of its precursors; 2) four miR166 genes are expressed in or immediately below the incipient primordium and contribute to its adaxial-abaxial patterning; 3) miRNA accumulation is regulated at the level of biogenesis or stability, because miR166 and miR390 precursors are expressed at the tip of the SAM where the mature miRNAs do not accumulate but their targets are abundantly expressed; 4) both miR390 precursors are expressed in the epidermal layer of the SAM, whereas the mature miR390 accumulates in both epidermal and sub-epidermal layers of the incipient leaf. This incongruence in expression patterns suggests the intriguing possibility that miRNAs may be able to traffic from the epidermis into the underlying tissues and function as positional signals in development.

T12

Integration of auxin transport and response during maize inflorescence development

(submitted by Paula McSteen <pcm11@psu.edu>)

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Auxin plays a fundamental role in organogenesis. We are taking a genetic approach to understanding the role of auxin in inflorescence development through characterization of the barren inflorescence class of mutants. These mutants have fewer branches, spikelets, florets and floral organs in the tassel and often have no ear shoot indicative of specific defects in the initiation of lateral meristems and organs. This approach has led to the identification of genes required for auxin biosynthesis, transport and response. *barren inflorescence2*, *bif2*, encodes a maize co-ortholog of the serine/threonine protein kinase, PINOID, which regulates auxin transport in Arabidopsis. *bif2* is expressed in lateral meristems and organs during vegetative and reproductive development. Translation fusion of BIF2 with GFP shows that BIF2 is subcellularly localized at the cell periphery and in the nucleus. *barren stalk1*, *ba1*, which encodes an atypical bHLH expressed as lateral meristems initiate, acts downstream of auxin transport. Here, we report that BA1 is a direct phosphorylation target of BIF2. We propose a model in which BIF2 acts both at the cell membrane and in the nucleus, providing a potentially direct mechanism for the transmission of the auxin signal during lateral meristem initiation.

T13

KNOTTED1 and the regulation of GA in maize meristems

(submitted by Nathalie Bolduc <nathaliebolduc@berkeley.edu>)

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The maize homeobox protein KNOTTED1 (KN1) and its orthologs KN1-like homeobox (KNOX) in various monocot and dicot species are involved in the establishment and maintenance of vegetative and floral meristems. In species with simple leaves such as maize, KN1 and other KNOX proteins are strictly excluded from developing leaves. Dominant mutations leading to ectopic KN1 expression in maize leaves induce cellular proliferations or knots on the leaf blade, while recessive mutant alleles have severe inflorescence and floral defects. Although the role of KNOX genes in plant development has been extensively documented, their functions at the molecular level are poorly understood. It is currently believed that KNOX proteins regulate activities of vegetative meristems by simultaneously promoting cytokinin and repressing gibberellin (GA) biosynthesis.

Our goal is to get a broader understanding of KN1 function and to identify direct and indirect downstream target. Our analysis focused on *ga2ox1*, a gene up-regulated in the immature leaves of the semi-dominant *Kn1-N* mutant. GA-2oxidases are involved in inactivation of GA. Chromatin immunoprecipitation experiments showed that KN1 binds in vivo to an intronic region of this gene. Furthermore, gel shift experiments demonstrated that the KN1-DNA interaction occurs through a double binding site, the second site being potentially bound by a BEL1-like protein partner such as KIP. Finally, in situ hybridization experiments showed that *kn1* and *ga2ox1* expression patterns overlap in the vegetative meristem. Taken together, our data support a model where KN1 modulates the accumulation of GA through the control of *ga2ox1* transcription.

T14

The *tassel sheath* loci control bract suppression during grass inflorescence development

(submitted by Clinton Whipple <whipple@cshl.edu>)

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The transition from vegetative to reproductive development in maize is characterized by a number of dramatic changes. One of the most striking of these changes is the complete suppression of bract (i.e. inflorescence leaf) development that would normally subtend tassel branches and spikelet pairs in both the tassel and ear. Undertaking a genetic approach to understand the mechanism of bract suppression in maize, we have identified mutants representing four loci that fail to suppress bract development. These *tassel sheath* (*tsh*) mutants have been designated *tsh1-tsh4*. By positional cloning we have identified *Tsh1* as an ortholog of the *Arabidopsis* gene *HANABA TARANU* (*HAN*). While *HAN* is expressed in boundary domains of *Arabidopsis* meristems, *Tsh1* is primarily expressed in the region of the suppressed bract in maize. This is consistent with the pleiotropic floral and meristematic phenotypes of *han* mutants as opposed to the very specific phenotype of *tsh1*, which is limited to bract de-repression and reduced branching. The divergent phenotypes and expression patterns of *Tsh1* and *HAN* are consistent with the independent evolution of bract suppression in maize and *Arabidopsis*. We also present evidence that *Tsh4* is *Squamosa Promoter Binding Protein6* (*SBP6*), a target of the microRNA *Corngrass1*, and acts upstream of *Tsh1* to inhibit bract growth. The similar phenotypes and syntenous genomic locations of the rice *neck leaf1* (*n1l*) and barley *third outer glume* mutants with *tsh1* suggests that the grasses have inherited a conserved mechanism of bract suppression. Sequencing of *n1l* alleles reveals deletions, confirming *n1l* as the rice *tsh1* ortholog. Our results suggest that *Tsh1* was recruited to a novel role in bract suppression in a common ancestor of the grasses.

T15

The repression of indeterminate growth of primary axillary meristems in maize inflorescences by the *rel2* gene

(submitted by Andrea Gallavotti <agallavotti@ucsd.edu>)

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In maize axillary meristems can choose between a determinate or indeterminate growth. This is particularly evident in the tassel where the first basal axillary meristems elongate to produce the conspicuous long branches, whereas the subsequent axillary meristems assume a determinate fate. In the *ramosa* class of mutants this difference is no longer evident, and both tassel and ear show a proliferation of branches due to the lack of determinacy of most primary axillary meristems. Three *ramosa* mutants have been characterized so far, *ramosa1* (*ra1*; Vollbrecht et al. 2006), *ramosa2* (*ra2*; Bortiri et al. 2006) and *ramosa3* (*ra3*, Satoh-Nagasawa et al. 2006), but the mechanism by which an axillary meristem chooses between a determinate or indeterminate state is still unknown. To more thoroughly investigate this pathway we undertook a mutagenesis program aimed at the identification of enhancer and suppressor loci of a weak *ra1* mutant. Here we present a modifier of the *ramosa1* mutant, the *ramosa* enhancer locus2 (*rel2*). The *rel2* mutation strongly enhances the branching of both ear and tassel in the background of weak *ra1* alleles. *rel2* mutants also show a striking upright tassel phenotype reminiscent of another *ramosa* mutant, *ra2*. We cloned *rel2* by map-based cloning and showed that it encodes a large protein with several WD40 repeats, with homology to the *Arabidopsis* transcriptional corepressor TOPLESS (Long et al. 2006). Genetic data and in vitro and in vivo interaction assays show that REL2 and RA1 can interact, suggesting that they are part of a repressor complex that determines the fate of most primary axillary meristems in both tassel and ear. The specific interaction of REL2 with RA1 and the relationship with the other members of the *ramosa* pathway will be discussed. This work was funded by the National Science Foundation.

T16

***dcd1* and *add* are needed for correct cell wall placement and encode PP2A regulatory phosphatase subunits**

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In plants, cell wall placement at cytokinesis is determined by the position of the preprophase band (PPB) and the subsequent expansion of the phragmoplast, which deposits the new cell wall, to the cortical site delineated by the PPB. New cell walls are often incorrectly orientated during asymmetric cell divisions in the leaf epidermis of *discordial* (*dcd1*) maize mutants. Previous work showed that PPBs form normally in *dcd1* mutants and defects in phragmoplast guidance are the cause of the *dcd1* phenotype (Gallagher and Smith, 1999). Cloning of *dcd1* showed that it encodes an orthologue of the *Arabidopsis fass/ton2* gene, a putative B" regulatory subunit that targets the serine/threonine phosphatase PP2A to appropriate substrates. *Arabidopsis fass* mutants completely lack PPBs, resulting in a random placement of cell walls and subsequent tissue disorganization (Camilleri et al., 2002). This phenotype is more severe than that observed in the maize *dcd1* mutants. We identified an additional gene, *alternative discordial* (*add1*), which encodes a protein 96% identical to DCD1. Loss of *add1* function alone does not produce a noticeable phenotype. To determine the phenotype of plants lacking both genes, RNAi was used to reduce *dcd1* and *add1* transcript levels. These RNAi plants display a range of leaf epidermal phenotypes with some plants showing defects in symmetric as well as asymmetric cell divisions. Microtubule staining of RNAi plants with severe phenotypes revealed a significant percentage of preprophase cells lacking PPBs. We raised an antibody that recognizes both DCD1 and ADD1 and found that these proteins localize to PPBs, spindles, and phragmoplasts. While the RNAi results indicate that FASS and DCD1/ADD1 have evolutionarily conserved functions in PPB formation, the weaker phenotype seen in the maize *dcd1* mutant suggests an additional role for FASS/DCD1 in phragmoplast function not revealed by analysis of the stronger *Arabidopsis fass* mutant.

T17

POOR HOMOLOGOUS SYNAPSIS 1 acts in the cytoplasm to control homologous chromosome pairing by a novel mechanism

(submitted by Arnaud Ronceret <ar346@cornell.edu>)

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During the zygotene stage of meiosis, homologous chromosomes recognize each other among all the chromosomes in the nucleus, closely align, and finally synapse. Despite decades of research, the mechanism underlying pairing is unknown. Maize poor homologous synapsis1 (*psh1*) gene is providing new insight into this poorly understood process. *psh1* plays an essential role in ensuring that pairing occurs exclusively between homologous chromosomes. The *psh1* gene encodes a novel plant-specific protein with two relatively short conserved regions but with a low overall level of evolutionary conservation. We identified a TILLING mutation in conserved region 1, which showed an intermediate mutant phenotype. This suggests that this conserved region represents a functional protein domain. To test whether PHS1 acts by preventing ectopic pairing interactions between repetitive regions in the genome, we knocked down the *psh1* homolog in *Arabidopsis*, which has a 20-fold smaller genome and around 100-fold less repetitive DNA. However, the *Arabidopsis* knockdowns showed a pairing defect similar to the one in the maize mutant, suggesting that PHS1 most likely acts on euchromatin. We developed an antibody against PHS1 and used it to localize the protein with 3-dimensional deconvolution microscopy. Surprisingly, at the time it acts in meiosis, PHS1 mainly localizes to the surface of plastid-like compartments in the cytoplasm of meiocytes instead of the meiotic nucleus. Plastid surfaces are known to serve as MTOCs in lower plants but their involvement in meiosis in higher plants is unknown. We are currently testing the role of PHS1 in the organization of the meiocyte cytoskeleton. We speculate that PHS1 acts in a cytoplasmic pathway to control the general movement of zygotene chromosomes, which is essential for homologous pairing. We also developed a proteomic strategy of TAP-tagging maize PHS1 to purify and identify proteins interacting with PHS1.

T18

Vesicle trafficking during cell wall expansion: Identifying cellular compartments by live cell imaging of tagged RAB2A1 in maize leaf cells

(submitted by Anne Sylvester <annesyl@uwyo.edu>)

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Maize leaf cells grow by oriented cell division and directional cell expansion in a developmental gradient. The mutant *warty1-0* disrupts this organized pattern by causing cells to expand abnormally, resulting in a wart-like texture of mutant leaves. *Wty1* was cloned by transposon tagging, and confirmed by transposon-induced sector analysis to encode *ZmRAB2A1*, a highly conserved small GTPase associated with vesicle trafficking. RAB proteins share 100% identity in the GTP binding site, which activates trafficking, but unique domains specify cellular location: in mammalian systems RAB1 and RAB2 traffic vesicles exclusively within the perinuclear ER/Golgi compartment. *ZmRAB2* may also function in similar compartments; however, to explain the phenotypes of *wty1* alleles, we propose that RABs have acquired new functions in plants, consistent with secretory processes associated with extensive cell wall growth compared with non-walled organisms. To test the hypothesis, and to define intracellular compartments in maize, we generated stable lines transformed with *ZmRAB2A1(WTY1)* tagged with fluorescent markers YFP and CFP. Complementation analysis with *wty1-0* mutants provides functional confirmation of the transgenic lines. Live cell confocal imaging of developing leaf primordia revealed movement of fluorescently tagged vesicles adjacent to the nuclear compartment, as expected for RAB2 from mammalian and yeast systems. Unique to maize, however, we observed extensive unidirectional movement of tagged vesicles traversing the cytoplasm from the perinuclear compartment towards the cell periphery, as confirmed by FRAP experiments. Time-lapse high-resolution imaging suggests secretion of the transported vesicles to the wall space. Intracellular RAB2A1 movements differ in cells within the expansion gradient of the leaf, further supporting the hypothesis that RAB2A1 traffics in a manner unique to plants during cell wall expansion. We propose that cell compartmentation in plants is specialized to accommodate requirements for vesicle transport associated with cell wall expansion.

T19

Regulation of meiotic chromosome architecture by ameiotic 1

(submitted by Zac Cande <Zcande@berkeley.edu>)

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Meiosis is a dynamic process in which the architecture and behavior of meiotic chromosomes undergo a series of changes to produce haploid gametes. The specialized architecture of the leptotene chromosome is required for downstream processes such as homologous pairing, recombination, and synapsis. At the leptotene-zygotene (lepto-zygo) transition chromosomes undergo a transient global remodeling which is essential for subsequent events. In maize, initiation of meiosis is mediated by the protein AMEIOTIC1 (AM1), and AM1 is required to transit a lepto-zygo checkpoint. In most *am1* alleles, male meiocytes undergo mitosis, instead of meiosis. Surprisingly, in *am1-pra1*, the meiocytes enter meiosis and arrest at the lepto-zygo transition. AM1 first accumulates in nuclei during pre-meiotic interphase. It is bound to the chromatin in leptotene but largely disappears by late-zygotene. However, AM1 remains diffuse in *am1-pra1* nuclei but never loads onto chromosomes implying that the chromosome association of AM1 is important for meiotic progression. *mtm99-25* shows abnormal heterochromatin morphology and defects in homologous pairing. In *mtm99-25* knobs persist as elongated structures from leptotene to late-pachytene, whereas knobs in wild type nuclei are only elongated at the lepto-zygo transition and then recondense. We have used ultra high resolution structured illumination light microscopy (with a resolution of less than 100nm) to look at chromomere and axial element organization, using antibodies against different histone modifications as markers for heterochromatin and euchromatin and an axial element protein, AFD1. Histone H3K9 di-methylated chromatin (heterochromatin) formed compact fibers and is distributed near the axis of the pachytene chromosomes, similar to the DAPI stained chromomere pattern. H3K4 di-methylation (euchromatic marker) staining is located in thin fibers that extend further from the chromosome axis than the DAPI stained fibers and as islands in the DAPI stained chromatin. These observations are consistent with a loop domain model of chromatin organization with loops extending out from the axial elements.

T20

Genomic analyses of functional domains in the maize shoot apical meristem

(submitted by Michael Scanlon <mjs298@cornell.edu>)

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The maize shoot apical meristem (SAM) is a complex signaling network of distinct domains whose structure correlates with its function. Leaves arise from the peripheral zone (PZ) whereas the central zone (CZ) is comprised of more slowly dividing meristem initials that replenish cells lost during leaf initiation. Deciphering the genetic mechanisms that maintain the equilibrium between the dual functions of SAM maintenance and organogenesis is a central problem in plant development. Laser microdissection microarray technology is a powerful tool for global transcriptional analyses of discrete organ and tissue domains. For use in functional genomic analyses of leaf initiation and meristem maintenance in maize, SAM microdomains comprising the initiating maize leaf (P0/P1) and the SAM proper were laser-microdissected and sampled for microarray analyses. Six biological replicate comparisons were performed, each sample comprised of SAM domains dissected from 10 apices each. 526 genes were identified as differentially expressed out of > 21,000 unique genes contained on customized maize gene chips. Differential expression of control marker genes in the P0/P1 and in the SAM proper verified the precision of our laser-microdissections; identification of 63 genes of unknown function reveals the utility of laser-microdissection technology for new gene discovery. In situ hybridization analyses of selected differentially-expressed genes identified novel expression patterns. Seven genes upregulated in the P0/P1 were also differentially-expressed in auxin transport-arrested SAMs that fail to initiate new leaves. Genes identified in both these experiments are especially implicated to function during early events in leaf initiation. Reverse genetic analysis of a maize *D-type cyclin* gene (*lcd1*) upregulated in the P0/P1 (6.6 fold) identified two independent *Mutator*-transposon insertional alleles that confer novel, recessive mutant phenotypes affecting maize shoot development. Our analyses have generated a unique dataset of SAM domain-specific transcripts and reveal the power of laser microdissection to identify new gene functions in maize.

T21

Maize as a genetic model for improvement of wall biogenesis in bioenergy grasses

(submitted by Nicholas Carpita <carpita@purdue.edu>)

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Perennial grasses offer great potential as sustainable and renewable resources of biomass for the production of biofuels. The cell walls of grass species are distinct from all other flowering plants. Achieving projected targets of biofuel production will require a deep understanding of the molecular architectures of grass walls and of the genes that encode the cellular machinery to synthesize and modify the wall's component molecules. Maize and other grass species provide more appropriate genetic models fitting the types of wall that bioenergy grasses produce. Plants devote about 10% of their genomes to cell wall biogenesis. We annotated and classified over 1400 maize genes involved in wall biogenesis, and using comparative genomics with Arabidopsis and rice genes, we show numerous examples where grasses have evolved new genes related to synthesis of their unique wall composition and architecture. We are exploiting the genetic diversity in recombinant inbred lines to identify QTL that impact biomass quantity and quality. With a century of genetic investigations and breeding success, a soon-to-be-complete genome sequence, a wealth of genetic tools, well-characterized cell wall compositions, a rapidly expanding collection of mutants and diverse lines, and its close evolutionary relationship to future bioenergy grasses, maize is a key model system for gene discovery relating to yield and biomass quality.

T22

A phenylalanine in DGAT is a major determinant of oil content and composition in maize

(submitted by Bo Shen <Bo.shen@pioneer.com>)

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Plant oil is an important renewable energy source for human consumption and industrial use, especially for biodiesel production. Maize kernel oil content is controlled by multiple quantitative trait loci (QTLs). The molecular basis of oil QTL, however, remains unknown. Here we report that the qHO6 gene, a major QTL affecting kernel oil and oleic acid content, encodes an acyl-CoA: diacylglycerol acyltransferase (DGAT1-2) and demonstrate that a phenylalanine (F469) in DGAT1-2 is responsible for the increase of oil and oleic acid contents. Ectopic expression of high oil DGAT1-2 increases kernel oil content by as much as 41%. Molecular cloning of qHO6 provides opportunities for direct selection of a major oil QTL using marker assisted selection and for transgenic high oil crop through genetic engineering of DGAT gene.

T23

Endogenous variation revealed by selection in su1 maize

(submitted by Michael Chandler <machandler@wisc.edu>)

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Divergent recurrent selection for starchiness (visual evaluation) in a population homozygous recessive for su1 has revealed significant levels of endogenous variation for kernel and endosperm traits. After six cycles of selection mature endosperm from the pseudo-starchy population contained 17% more starch, 50% less water soluble polysaccharide, 65% less sucrose, and 86% less glucose than the extreme sugary population. An evaluation of the genetic control of pseudo-starchiness revealed four unlinked QTL accounting for 50% of the phenotypic variation. The gene action of these QTL was dominant in the extreme sugary direction. Several QTL associated with endosperm carbohydrates, protein, and oil overlapped with visual starchiness. Correlations among these traits were as expected, with the exception of those involving protein. It appears that protein accumulation is not reduced at the expense of starch. In a separate experiment, RNA from cycle six pseudo-starchy and cycle six extreme sugary was interrogated on maize spotted micro-arrays at four day intervals from two to eighteen days after pollination (DAP). RNA collected at two and six DAP included whole kernels, while ten, fourteen, and eighteen was isolated from the endosperm. Unique time-course transcriptional profiles involving starch and protein synthesis were resolved. Differentially expressed genes included Brittle2, Sugary2, Amylose Extender, and many zein proteins. This research provides information on the genetic and molecular effects of modifying genes which are revealed in the presence of the qualitative mutation, sugary1.

T24

Genomic survey of gene expression diversity in *Zea mays* roots in response to water stress

(submitted by Lewis Lukens <llukens@uoguelph.ca>)

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Water stress is a major limitation to maize shoot and root growth, and we find that root growth rates significantly differ among maize genotypes grown under water stress. To investigate the transcriptional basis for this genetic variation, we surveyed the root-tip transcriptomes of seven diverse *Zea mays* inbred lines in control, water stress, and recovery conditions using 84 oligo chips. The transcriptomes of four biological replicates of each line were compared at three time points (before water stress, 24 hrs into the water stress, and 24 hrs into water-stress recovery) using a factorial, direct loop design. A linear model with empirical Bayes shrinkage of the standard errors estimated significant changes in gene expression across conditions within each of the genotypes. The transcriptional profiles within the recovery growth treatments show that water stress failed to induce lasting transcriptional changes: fewer than 30 genes differed between control and recovery within each genotype. In contrast, over 5,000 genes per genotype changed between control and stress treatments. A subset of these genes comprises a core transcriptional response to water stress. These genes fall into specific pathways and change across all genotypes. Nonetheless, many differentially expressed genes and a number of pathways were genotype-specific. Our results show that a significant fraction of genes that are part of the maize transcriptome response to water stress differs among inbred lines. We suggest that analyses of additional abiotic conditions may also show large, genotype-specific transcriptional responses.

T25

The nested association mapping (NAM) genetic map

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A goal of the Maize Diversity Project is the development of a genetic resource for conducting joint linkage-association analysis in maize. We have designated this resource as NAM for nested association mapping. NAM is constructed from 26 inbred lines chosen to maximize genetic diversity. NAM has a reference design, B73 was crossed to each of the 25 diverse lines (DL) and recombinant inbred line (RIL) populations of at least 200 individuals were generated from each cross. The 25 DL consists of 13 tropical/subtropical, three mixed temperate/tropical, six non-stiff stalk, two sweet corn and one popcorn. Essential to the use of NAM is a high resolution genetic linkage map. For genetic markers we used single nucleotide polymorphisms (SNP) derived from random and candidate gene diversity sequencing from the Maize Diversity Project, supplemented with markers contributed by Pioneer Hi-Bred. SNPs were chosen with B73 nucleotide as the rare allele to maximize marker informativeness across subpopulations. Genotypes were obtained for a panel of 1536 SNPs using Illumina GoldenGate assays. After quality control examination of the data a composite linkage map was constructed for 4699 individuals with 1106 markers, including 38 markers combining informative data from two SNPs from the same amplicon. The length of the genetic map is ~1400 cM for an average marker density of 1 marker/1.3 cM. By positioning the SNPs on sequenced BACs the map allows for an entirely independent anchoring of the physical map, placing many unanchored contigs and resolving anchoring discrepancies. To integrate NAM with previous genetic resources the same marker set was used to genotype 286 IBM individuals. With the reference design, the NAM map allows examination of inbred-specific segregation distortion and recombination. NAM represents the most powerful genetic resource for dissection of quantitative traits for any species.

T26

Maize phenomics: massively parallel phenotyping of the nested association mapping population

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How does the tremendous genetic variation within maize cause the phenotypic diversity displayed in this species? A goal of the Maize Diversity Project is the development of the genetic resources and methods to answer this question. We created a Nested Association Mapping (NAM) population consisting of 25 new recombinant inbred line (RIL) subpopulations, each derived from a cross between B73 (the reference parent) and one of 25 inbred lines selected to capture much of the diversity available globally among inbred lines. We created 200 RILs from each of the 25 crosses, and we are integrating the IBM population to form a 26th subpopulation. Each line has been genotyped at 1106 SNP markers. The combined NAM population of 5000 lines plus 200 IBM lines and an additional 281 diverse inbreds representing the maize association mapping platform has been phenotyped for up to 20 traits in up to 11 environments. Phenotyping nearly 5500 unique lines in presents many challenges, including the limitation that only one replication can be grown within each environment. To control error variation within environments, we employed a blocking design in which about 10% of the plots were planted to replicated check inbreds. Plots were labeled with bar-coded tags and trait data were collected with hand-held computers or scanning devices. Data analysis employs information on the repeated checks to adjust unreplicated RIL phenotypes for extraneous micro-environmental effects in the field. To obtain RIL phenotype values adjusted to remove extraneous error effects, we obtained Best Linear Unbiased Predictors (BLUPs) for each line. The BLUPs are the optimal phenotypic values to use for QTL mapping. The same analysis also permits estimation of important genetic architecture parameters such as trait heritabilities and the distribution of genetic variance among and within populations.

T27

***tie-dyed1* leaves contain increased cellulose: biofuel applicability and underlying mechanism**

(submitted by David Braun <dbraun@psu.edu>)

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My lab is interested in understanding the regulation of carbon partitioning in maize. To identify genes controlling this process, we are characterizing mutants that hyperaccumulate carbohydrates in their leaves. *tie-dyed1* (*tdy1*) is a recessive mutant that produces leaves containing approximately ten-fold higher levels of soluble sugars and starch. To determine if the elevated sugars result in synthesis of additional cell wall components, we examined cellulose and lignin deposition in wild type and *tdy1* mutants. We found that *tdy1* leaves contain increased cellulose, without changes in lignin, suggesting that *tdy1* stocks may be useful in the production of biofuels. We have combined *tdy1* with the *brown midrib* mutants which contain decreased lignin levels and are evaluating their potential for fermentation to ethanol.

To characterize the molecular function of *Tdy1* we cloned the gene by transposon tagging. We determined that *Tdy1* encodes a novel transmembrane protein. Translational fusion of a fluorescent protein to TDY1 demonstrated that TDY1 is localized in the endoplasmic reticulum. Expression analyses revealed that *Tdy1* is expressed in phloem cells of all organs. Therefore, we hypothesize that *Tdy1* may regulate the function of a sucrose transporter (SUT). SUTs are also expressed in phloem cells and function to load sucrose into the veins for distribution to growing tissues. Additionally, loss of SUT function resulted in carbohydrate hyperaccumulation in leaves. Evidence testing this hypothesis through genetic, cell biological and biochemical approaches will be presented.

T28

ABP9, a stress- and ABA-inducible bZIP transcription factor of maize, mediates ABA signaling and ROS scavenging and enhances tolerance to multiple environmental stresses in transgenic Arabidopsis

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Both ABA and ROS are produced/accumulated in plants under adverse environmental conditions such as drought, salinity and low temperature, and play important roles in signaling adaptive responses including stomata closure and antioxidant defense. However, ROS production/accumulation in excess causes oxidative stress leading to oxidative destruction of cellular components and thus must be controlled tightly. One mode of ABA action is involved in the regulation of ROS-producing and ROS-scavenging pathways. Previous study revealed that maize Cat1 is induced by ABA and osmotic stress and the ABRE2 element in its promoter is responsible for the response. To dissect the signaling pathway of Cat1 induction by ABA and osmotic stress, a cDNA library constructed with the mRNA extracted from 17-21dpp immature embryos was screened by using a yeast one-hybrid system with ABRE2 as the bait. One of the ABRE2 interacting clones, designated ABP9 (for ABRE Binding Protein 9), encodes a protein belonging to bZIP-type transcription factor family. In vitro and in vivo assays revealed that ABP9 specifically binds to ABRE2 motif of Cat1 promoter and activates the expression of downstream reporter genes. Expression analysis in maize showed that ABP9 is induced by ABA and stress conditions such as drought, high salt and H₂O₂. Constitutive expression of ABP9 in Arabidopsis resulted in enhanced tolerance to multiple environmental stresses including drought, salt, cold and oxidative stresses. Enhanced sensitivity to ABA in seed germination and stomatal closure, and reduced cellular level of ROS under both normal growth condition and stress/ABA treatment were further observed in transgenic plants expressing ABP9. Microarray and RT-PCR analysis revealed that a large body of stress/ABA responsive genes including those for cell protection and ROS-scavenging were elevated in ABP9 transgenic plants. These results demonstrate that ABP9 encodes a functional transcription factor with ABRE-binding specificity and trans-activation activity, and plays an important regulatory role in plant responses to abiotic stresses via mediating ABA signaling and ROS-scavenging.

T29

Coordinated control of the maize carbon/nitrogen balance

(submitted by Jeff Church <jbchurch@uiuc.edu>)

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The degree to which high nitrogen (N) uptake is translated into high yield varies widely among maize cultivars and is a likely consequence of metabolic changes in vegetative source tissues. In order to explore the molecular basis for altered vegetative N use, we utilized Illinois High Protein (IHP), a genotype with high N uptake and accumulation, but poor yield due to artificial selection for high grain protein concentration. We grew IHP and B73 plants under N-deficient and N-sufficient conditions in a hydroponics experiment, and analyses were conducted on plant growth, metabolite pools, enzyme activities and RNA expression. We confirmed lower growth and photosynthetic responses to N in IHP, coupled with greater N uptake and an enhanced ability to accumulate amino acids, even at lower internal concentrations of N. Microarray and qRT-PCR analyses revealed widespread regulation of the N assimilation genes, which responded to N more strongly in IHP and helped IHP funnel carbon preferentially toward asparagine and away from malate, a C₄ carrier and TCA intermediate. B73 showed a stronger preference for malate synthesis at the gene expression and metabolite levels, but both genotypes exhibited altered carbon balance in the form of lower malate-to-aspartate ratios with increasing N. Our experiments with IHP show that increased amino acid synthesis can occur independently of high internal N concentrations. As previous studies have attributed seed protein concentration to the size of leaf amino acid pools, we postulate that (1) leaf amino acid synthesis is controlled in a similarly coordinated manner, possibly by signals coming from the root and (2) deregulation of this signal or of N uptake may result in altered leaf carbon balance, higher N translocated to sink tissues, and reduced grain yield.

T30

Multiple non-redundant roles for plastidic 6-phosphogluconate dehydrogenase in maize

(submitted by Li Li <lili1982@ufl.edu>)

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The oxidative pentose phosphate pathway (OPPP) serves multiple roles in primary metabolism. Enzymes for the oxidative section of the OPPP are found both in the cytosol and plastid. Several mutant studies have suggested that cytosolic and plastidic OPPP enzymes are redundant with each other. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (6PGDH) catalyze the three non-reversible steps of the oxidative section of the OPPP. 6PGDH enzymes are more tractable for genetic analysis because they comprise a smaller gene family in plants. Maize mutations in the cytosolic 6PGDH enzymes, *pgd1* and *pgd2*, do not show obvious phenotypes beyond loss of enzyme activity. We identified two knockout alleles of the maize *Pgd3* locus. *pgd3* mutants disrupt plastid-localized 6PGDH activity and cause a rough endosperm (*rgl*) phenotype that affects both grain-fill and embryo development. Consistent with the reduced grain-fill phenotype, ¹³C-glucose labeling experiments during seed development suggest that *pgd3* mutants disrupt carbohydrate flux for starch synthesis. PGD1, PGD2, and PGD3 are all active in both the endosperm and embryo. These data suggest that plastid-localized 6PGDH has a non-redundant role for seed development. Moreover, homozygous *pgd3* mutants can be rescued through tissue culture. Mutant *pgd3* plants show normal morphology but are slow to green and late flowering. Interestingly, PGD3 activity is restricted to non-green tissues suggesting that the slow to green phenotype is due to disruptions in carbon metabolism during leaf expansion.

T31

Regulation of maize chloroplast gene expression by RNA editing

(submitted by Maureen Hanson <mrh5@cornell.edu>)

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Transcripts of maize chloroplasts undergo C-to-U RNA editing at 28 different C targets within 15 different genes. All but one of the known C targets is within the coding region and changes the amino acid predicted from the genomic sequence. RNA editing performs post-transcriptional correction of T-to-C genomic mutations so that functional proteins can be translated. In maize leaves, at least 80% of each C target is converted to U. However, in non-photosynthetic maize tissue, only 20% of the Cs in transcripts of certain genes is converted to U. The editing machinery is encoded by the nuclear genome; chloroplast ribosome-less *iojap* mutants undergo editing at all sites. We have exploited maize chloroplast extracts capable of editing exogenous RNAs for detailed characterization of sequence requirements for editing. We have found that conserved nucleotides 5' to two C targets of editing in maize are important for editing efficiency. Despite limited 5' sequence similarity, transcripts of these two genes can compete editing activity from each other in vitro. Excess transcripts of DNA sequences unrelated to the chloroplast editing sites do not cause competition in vitro. Mutational analysis has revealed that the signal causing competition overlaps the 5' cis-element required for editing efficiency. These results are consistent with a model in which a common protein is involved in editing of two different C targets. In order to identify trans-factors responsible for RNA editing, we have undertaken biochemical purification of the editing activity from maize seedling chloroplasts. We have found that the editing activity for maize *rpoB* transcripts separates as a 200-300 kD complex on size exclusion columns. Further purification steps have been performed to obtain candidate components of the complex. Supported by NSF MCB 0344007.

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T32

The revised carotenoid biosynthetic pathway in plants

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Carotenoids are a diverse group of pigments found in plants, fungi, and bacteria. They serve essential functions in plants and provide health benefits for humans and animals. In plants, it was thought that conversion of the C40 carotenoid backbone, 15-cis phytoene, to all-trans lycopene, the geometrical isomer required by downstream enzymes, required two desaturases (PDS and ZDS) plus a "carotene isomerase", CRTISO, in addition to light mediated photoisomerization of the 15-cis double bond; bacteria employ only a single enzyme, CRTI. Characterization of the maize y9 locus has brought to light a new isomerase required in plant carotenoid biosynthesis. We report that maize Y9 encodes a factor required for isomerase activity upstream of CRTISO, which we term Z-ISO, an activity that catalyzes the cis to trans conversion of the 15-cis bond in 9,15,9'-tri-cis-zeta-carotene, the product of PDS, to form 9,9'-di-cis-zeta-carotene, the substrate of ZDS. We show that recessive y9 alleles condition accumulation of 9,15,9'-tri-cis-zeta-carotene in dark tissues, such as roots and etiolated leaves, in contrast to accumulation of 9,9'-di-cis-zeta-carotene in a ZDS mutant, vp9. We also identify a locus in *Euglena* which is similarly required for Z-ISO activity. These data, taken together with the geometrical isomer substrate requirement of ZDS in evolutionarily distant plants, suggest that Z-ISO activity is not unique to maize but that it will be found in all higher plants. Further analysis of this new gene-controlled step is critical to understanding regulation of this essential biosynthetic pathway.

T33

Immediate paternal genome activation and enhanced trans-regulatory interactions in early maize F1 hybrid embryos

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Heterosis results from the combination and the expression of genetically-distant genomes after fertilisation. We observed high levels of heterosis in early hybrid maize embryos indicating a major contribution of both parental genomes to this early phase of kernel development. To explore the activation of the paternal genome after fertilization and the contribution of regulatory mechanism to early development we have analysed the allele-specific expression of 25 genes following fertilisation of the egg in maize. Sequence comparisons indicate these genes to be involved in a range of processes, and to be distributed throughout the genome. Our data confirm, in contrast to the situation in other plants and in animals, immediate equivalent parental genomic contribution to the maize zygote. Every gene expressed before the first cell division of the zygote showed paternal transcripts. The maternal effects in zygotes exhibit a mean value below 20% and disappear completely until 6 days after fertilization. Concerning the regulatory mechanisms leading to variation of gene expression in hybrid embryos compared to their parental lines we found large differences at 6 and 8 days after fertilization. Taken the mean of both reciprocal hybrids at 6 days trans-regulatory differences are attributable to the intraspecific variation of gene expression for 82% of the genes analysed whereas at 8 days only 42% of the genes were influenced by trans-regulatory interactions.

Our findings confirm that maize evolved a strategy to activate the paternal genome immediately following fertilization providing an explanation for the early appearance of hybrid vigour in maize embryos. Large-scale change of gene expression regulation may be related to the modulation of the diverse genomes after crossbreeding.

T34

A maize RNA Polymerase IV large subunit is required to maintain *trans*-generational epigenetic regulatory states

(submitted by Karl Erhard <karlerhard@berkeley.edu>)

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Meiotically-heritable, repressed epigenetic states at the *purple plant1* (*pl1*) locus are maintained by at least two components of a presumed RNA-based silencing pathway; Required to Maintain Repression1, a Rad54-like protein [1], and Mediator of Paramutation1, a likely RNA-dependent RNA polymerase [2,3]. At least 8 other factors of unknown function, defined by recessive mutations, are also required to maintain these repressed states. The *rmr6* locus, identified in EMS-mutagenesis screens for factors required to maintain repression at the *pl1* locus, was mapped to an ~800 kb region within bin 1.06 (*IL*) [4], that shows a high degree of synteny with the rice and sorghum genomes. According to maize genomic sequence resources, however, this synteny is interrupted by sequences having the potential to encode an ortholog of *NUCLEAR RNA POLYMERASE D1a*, the largest subunit of a plant-specific putative DNA-dependent RNA polymerase, Pol IVa [5,6]. Sequencing of genomic DNA from plants homozygous for the *rmr6-1* allele reveals an in-frame premature nonsense codon in the putative coding region of this NRPD1a gene model. Identity of RMR6 as a maize NRPD1a ortholog is further supported by molecular genomic phenotypes of mutant *rmr6* plants that are similar to those seen in *Arabidopsis nrpd1a* mutants [6,7,9]. However, whereas *nrpd1a* mutants appear developmentally normal [7,8], RMR6 acts to canalize both leaf and inflorescence development by prohibiting ectopic action of specific developmental regulators [9]. These features implicate the potential cooption of Pol IV function in the morphological evolution of *Zea mays*.

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T35

Gene silencing by artificial microRNAs in monocots

(submitted by Philippe Herve <p.herve@cgiar.org>)

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Two major challenges in biology still persist. A large proportion of gene functions remain unknown. And the complexity of regulatory networks is still to be discovered. It is also striking that several examples illustrate that loss of function mutations and gene silencing through RNA interference (RNAi) show great potential for crop improvement. Main concerns with gene silencing have however been simplicity of the procedure, reproducibility and specificity. Artificial microRNAs (miRNAs) present a new and highly specific technology for gene silencing. Based on endogenous rice sequences, we achieved artificial miRNA-directed suppression of the intended target genes in both Nipponbare (japonica) and IR64 (indica). We developed new vectors derived from rice microRNA sequences, and discovered previously unrecognized features important for the design of more effective artificial microRNAs. In addition, we provide a webtool that allows design of artificial microRNAs for a wide variety of monocot crops including maize. This tool incorporates design advances over the original algorithms (<http://wmd2.weigelworld.org>). This is the first report that artificial miRNAs efficiently trigger gene silencing and mimic mutant phenotypes in monocots. We will discuss how this innovative technology may rapidly offer unexplored ways and great potential for both functional genomics and molecular breeding for inbred and hybrid crops.

T36

Short and long-distance placement of gene copies in the maize genome

(submitted by Mihai Miclaus <mihai@waksman.rutgers.edu>)

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To better understand neofunctionalization and subfunctionalization we have investigated an entire gene family in two different inbred lines of maize. Based on genomic libraries of B73 and BSSS53, we have isolated overlapping BAC clones comprising all copies of alpha zein genes, the major nitrogen storage of the seed and a moderately sized family of 42, dependent on haplotypes. We have discovered eight major features that could provide a paradigm for gene organization in plants because all plant genomes sequenced so far have extensive gene families. First, although seed storage proteins can be found in most species of the plant kingdom, their structure has been formed in independent lineages; alpha zein genes are single exon genes that arose after the progenitor of maize and rice separated but before the progenitors of maize and sorghum split. Based on orthology, the first alpha zein gene copy was an insertion of a new gene in maize chromosome 1L. Second, a copy of this gene was inserted into another chromosome, 4S. Third, additional movement to other chromosomes occurred subsequently, first to chromosome 7S, back to 4S and then closer to the centromeric region of 4S; the latter occurred after tetraploidization and constituted paralogous loci. Fourth, single gene insertions on homoeologous chromosomes were lost after allotetraploidization. Fifth, these movements were followed by tandem duplications, except for one copy on chromosome 4S, all after tetraploidization. Sixth, intergenic space diverged mostly by retrotransposition in sequence and length. Seventh, tandem expansions and contractions occurred in a haplotype specific manner. Eighth, expression studies show differences in how much each gene copy contributes to the total gene product. Furthermore, allelic and non-allelic gene expression diverged as well, providing a model for rapid adaptation of gene function by copying genes and placing copies in different positions.

T37

Zinc finger nuclease-mediated gene targeting in maize

(submitted by Vipula Shukla <vkshukla@dow.com>)

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Gene targeting via homology-driven repair occurs at a very low frequency in plant cells compared to random integration, rendering targeted gene modifications impractical. Recently, substantial increases in the frequency of targeted integration has been observed following the induction of double stranded breaks in host cell DNA followed by the apparent stimulation of cellular repair mechanisms. Strategies to achieve targeted DNA double strand breaks have been developed by fusing engineered zinc finger DNA binding proteins with sequence-independent nuclease domains derived from Type IIS restriction endonucleases. Using this strategy, we have demonstrated relatively high frequency site-specific transgene integration and concomitant targeted disruption of a native gene in maize. Implications for novel trait development and crop improvement will be discussed.

T38

The codifying genome of the Palomero Toluqueno Mexican landrace

(submitted by Jean-Philippe Vielle-Calzada <vielle@ira.cinvestav.mx>)

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Large-scale sequencing efforts concentrated in B73 will not be sufficient to fully understand maize genome organization and identify all functional units available in the domesticated gene pool. To complement the large-scale B73 sequencing initiative and explore landrace genomic diversity, we undertook the structural and functional characterization of the Palomero Toluqueno genome after estimating its genome to be 22% smaller than B73. Palomero Toluqueno is an ancient popcorn landrace and a member of the Central and Northern Highlands Group that produce short individuals (140-180 cm) and grow at elevations above 2000 meters. A total of 1.2 million Sanger reads (10% HCot; 90% enzyme-based methyl-filtration) and 213 pyrosequencing runs (50% methyl-filtered, 50% whole genome sequencing) were sequenced for a 3.2X coverage. A total of 199.2 Mb have been assembled in close to 220,000 gene-enriched contigs averaging 0.91 kb in size. This effort has been complemented by an in-depth pyrosequence-based global transcriptional analysis of the same genotype. As expected, a significant percentage of codifying transcripts are not reported in public databases, suggesting that a large portion of the molecular and functional diversity contained in landraces remains unexplored. Our results represent an initial landmark to explore the functional diversity of Mexican landraces in the context of maize domestication and enhancement.

T39

Detailed sequence analysis of a 22 MB region of the maize genome

(submitted by Richard McCombie <mccombie@cshl.edu>)

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As part of the maize genome sequencing project, in addition to the overall sequencing of the genome, a region was selected for accelerated sequencing. This region on chromosome 4 is comprised of the adjacent fpc contigs 181 (partial) and all of 182. This is a total of about 22 million bases of sequence. This region is comprised of 206 BACs, which have been taken through the automated sequence and manual sequence improvement process. The reason for doing this accelerated region was to provide a large contiguous region early in the process that would be available for analysis and comparison with other genomes. Moreover, such a region would allow the comparison of a complete sequence with the results of gene enrichment methods such as methyl filtration and Cot filtering. This region has now been analyzed and is found to contain 765 non-transposable element gene predictions that show homology to GenBank proteins, having an average gene size of 2763 bp. The extent of synteny, gene loss, and movement compared to corresponding regions of the rice and sorghum genomes will be presented.

T40

In-depth analysis of the maize genome: case studies in a 22-Mb region and two orthologous regions totaling 14 Mb

(submitted by Fusheng Wei <fushengw@ag.arizona.edu>)

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Maize (*Zea mays* L.) is one of the most important cereal crops and a model for genetics, evolution, and domestication studies. To better understand maize genome composition and organization, we sequenced a 22-MB region and two orthologous regions totaling 14-Mb using a BAC-by-BAC approach as part of the NSF funded maize genome sequencing project. The 22-MB region from chromosome 4 is composed of 178 BACs that were draft sequenced, ordered and orientated. On average, there are about 5 gaps in each BAC. The low copy regions, including genes and intergenic regions were fully sequenced while high copy regions, mostly transposable elements, were draft sequenced at 5x redundancy. Syntenic to the tip of the short arm of rice chromosome 3, the two orthologous regions, 7.2 Mb in chromosome 1 and 6.9 Mb in chromosome 9, were fully sequenced with less than two gaps in each BAC. Annotation of all 3 regions is in progress and will be presented at the meeting.

T41

Evidence-based gene builds in plant genomes

(submitted by Chengzhi Liang <liang@cshl.edu>)

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Evidence-based gene building is becoming the preferred method for constructing gene annotations in organisms where large numbers of gene products exist. Gramene has developed a modified Ensembl pipeline for building reliable gene sets in sequenced plant genomes. The Ensembl pipeline utilizes proteins as the primary source of evidence. Gramene extends the Ensembl pipeline with the inclusion of cDNAs and ESTs of the same species or cross-species as additional forms of evidence. To evaluate the quality, the rice genes generated from the Gramene pipeline were compared with the predictions from several other programs. The results showed that the pure evidence-based genes using only ESTs and proteins are more reliable than those from the other prediction methods at both gene level and exon level. Furthermore, cross-species mRNAs (e.g., between maize and rice) were shown to be a highly reliable source of evidence. This becomes critical since it is anticipated that many of the newly sequenced genomes will be lacking in species-specific full-length cDNAs and ESTs. Analyses using TBLASTX showed that the predicted rice genes from Fgenesh and Twinscan not supported by cDNA/EST evidence usually share low sequence identity to both the maize and sorghum genomes. These results suggest that for gene structure annotation purposes, genome sequence similarity between rice and maize might provide only very limited information when utilizing the existing expression data. The pipeline was run on part of the maize genome and comparable results to those in rice were obtained. Comparing evidence-based genes and Fgenesh predicted genes in maize showed that more than 50% of the overlapping genes contain at least one different intron, where most of the evidence gene models are correct.

T42

Maize full-length gene identification, annotation, and comparison with model species

(submitted by Jon Duvick <jduvick@iastate.edu>)

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The maize genome is currently represented by approx. 15,500 BACs from inbred B73, ordered as a minimum tiling path across all 10 chromosomes and shotgun sequenced, as well as by 2.68 million genome survey sequences (GSS) assembled into contigs. To gain insight into the gene content of maize, we used a similarity-based strategy to identify maize genes from the BACs and GSS assemblies. Our method is based on the accurate spliced alignment of maize genomic DNA to model plant species predicted protein sequences, as well as to maize EST/cDNA assemblies, using the GenomeThreader program.

GenomeThreader uses plant specific-trained Bayesian splice site models to compute consensus spliced alignments and uncover alternative splicing. The EST/cDNA-only alignments may indicate genes not shared by other sequenced plant genomes, while the full length or near-full length protein alignments permit a more detailed analysis of gene structure and functional annotation for maize. From the protein alignments, maize genes were grouped into functional classifications based on GO ontologies, and the molecular evolution dynamics for each maize gene and GO category was assessed by measuring Ka/Ks ratios of each maize gene with its top model species pair. In addition, microsynteny between maize and model species was assessed by identifying instances of multiple loci per BAC with matches localized to a single annotated region of a model species. The results of this analysis will be discussed in the context of maize's evolutionary dynamics.

T43

Maize syntenic analyses using all grass genomes as outgroups

(submitted by Eric Lyons <elyons@nature.berkeley.edu>)

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Given the rice genome sequence, the pending release of sorghum and Brachypodium genomic sequence, the growing maize assembly, and early efforts in foxtail millet, the diverse grass family will soon be uniquely rich in genomic sequence. While all grasses share a common tetraploidy (~60mya), maize had an additional tetraploidy about 12 million years ago. As in many other plant families, many grasses had very recent tetraploidies, including wheat, sugarcane and most biofuel perennials. Following each tetraploidy, genomes undergo large-scale reorganization through massive gene loss (fractionation), inversions, translocations, local duplications, segmental duplications and perhaps transpositions. It is a formidable problem to simply identify all orthologous and homeologous grass chromosomal regions, present them to a sequence alignment algorithm, and visualize the results. However, such multi-region alignments generate invaluable data. Annotation errors are exposed, putative gene regulatory regions are discovered as conserved noncoding sequences, and cases of sequence subfunctionalization following duplication can often be evidenced. We have built a software system (CoGe) that stores multiple genomes in a useful way, facilitates such multiple genomic region comparisons, displays the result graphically using an interactive viewer, and can compare up to 10 chromosomal regions in a single user-configured analysis. This research tool that is available to maize geneticists at <http://synteny.cnr.berkeley.edu/CoGe/>. Read: Lyons and Freeling, 2007-8, *The Plant Journal*, in press. Supported by NSF.

T44

In vitro assembled autonomous maize mini-chromosomes

(submitted by Shawn Carlson <scarlson@chromatininc.com>)

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Autonomous maize mini-chromosomes (MMCs), constructed using a bottom-up approach, allow simultaneous delivery of multiple genes to maize. We constructed plasmids carrying maize centromere sequences, delivered purified constructs to embryogenic maize tissue, and identified centromere fragments that formed autonomous MMCs. These vectors have been propagated in several maize germplasms with reporter genes expressed through six generations. Segregation analysis demonstrated that MMCs can be mitotically and meiotically transmitted; overall frequencies of inheritance approach Mendelian expectations, while notable cases of marker mis-segregation (and PCR confirmation of marker loss) support the interpretation of chromosome autonomy. Moreover, fluorescent in situ hybridization (FISH) showed co-localization of marker genes, centromere sequences and autonomous DAPI-staining bodies that are much smaller than natural maize chromosomes. This novel approach for plant transformation could significantly improve commercial corn production by i) combining a stack of trait genes on a single DNA fragment, ii) arranging genes in a defined sequence context, and iii) providing an independent linkage group that can be rapidly introgressed into elite germplasms.

T45

Artificial chromosome construction in *Zea mays* L.

(submitted by Sergei Svtashev <sergei.svtashev@pioneer.com>)

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We report on the de novo assembly of plant artificial chromosomes from selected BAC clones containing maize centromeric segments, and retrofitted with selectable and visible markers, origins of replication, and telomeric sequences. Linear minichromosome constructs were delivered to scutellar cells of maize immature embryos by microprojectile bombardment. Several independent events were found containing artificial chromosomes in addition to 20 normal maize chromosomes. Artificial chromosomes were positive for centromeric, telomeric, and exogenous marker sequences as shown by FISH. Immunostaining revealed that the artificial chromosomes recruited, in vivo, centromeric protein C (CENPC), which is a specific component of the centromere/kinetochore complex of normal chromosomes. FISH and cytological observations indicated that the artificial chromosome formation apparently involved rearrangements and concatenation of the original constructs delivered to the cells. Artificial chromosomes were mitotically stable for more than a year in actively dividing callus culture. Plants were regenerated and artificial chromosomes were detected in root tips, providing evidence of their normal replication and transmission during mitotic cell division. Creation of a plant artificial chromosome is the first step toward developing new plant vectors capable of carrying a large volume of genetic information.

T46

The iPlant collaborative: Empowering a new plant biology

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Notes on the iPlant collaborative and how it will affect plant biology research.

Poster Abstracts

P1

***Tie-dyed1* and sucrose transporters are expressed in the same tissues**

(submitted by Yi Ma <yum105@psu.edu>)

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A maize mutant, *tie-dyed1* (*tdy1*), with variegated yellow and green leaf sectors was discovered to have defects in carbohydrate partitioning. To learn how *Tdy1* regulates carbohydrate transport, the gene was cloned using transposon tagging. TDY1 is predicted to be a novel transmembrane protein. Sequence alignment analyses showed that TDY1 is highly conserved in grasses, such as rice, sorghum and sugarcane, while no conserved full length protein sequences were found in dicots. However, two stretches of amino acid sequences were found to be conserved among both dicot and monocot plants indicating that these two domains may be important for TDY1 function. None of the related genes found in other plants are of known function. To characterize the expression of *Tdy1*, semi-quantitative and quantitative RT-PCR were performed on RNA isolated from different wild type tissues. Interestingly, even though we only observe a mutant phenotype in mature leaf tissues, *Tdy1* transcripts were detected in immature leaves, ears, tassels and roots. This result suggests that *Tdy1* may function throughout the plant. Because of the carbohydrate retention in *tdy1* mutant leaves, we propose that carbohydrate export is altered. Therefore, we hypothesize that TDY1 may regulate sucrose transporters (SUTs), which function to transport sucrose into or out of veins. To characterize which *SUTs* are co-expressed with *Tdy1*, the expression of three different *SUTs* were examined in the same wild type tissues. The results showed that *SUT2* and *SUT4* are expressed in all of the tissues tested while *SUT1* is not expressed in ears and tassels. We are currently determining whether the expression of *SUTs* is changed in *tdy1* mutants to understand the relationship between *Tdy1* and *SUTs*.

P2

A Jacalin-related Chimeric Lectin is Responsible for beta Glucosidase Null Phenotype in Maize

(submitted by Christopher Diehl <cdiehl@vt.edu>)

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Beta-Glucosidases hydrolyze Beta-glycosidic linkage(s) in alkyl and aryl Beta-D-glucosides, glycoproteins, and glycolipids and that between two glucose residues in Beta-linked oligosaccharides. In maize, two isozymes of Beta-glucosidase have been identified and are believed to be involved in the defense against pathogens and herbivores by releasing toxic aglycones, such as hydroxamic acids, from their glucosides (e.g., DIMBOAGlc). Certain maize genotypes, however, were reported to lack Beta-glucosidase activity and classified as "null" for Beta-glucosidase. Our studies have shown that these null-lines have Beta-glucosidase but it is present in the form of large insoluble complexes. These complexes become soluble in the presence of galactose or lactose. Furthermore, we have shown that a lectin also known as Beta-glucosidase aggregating factor (BGAF) is responsible for aggregation of Beta-glucosidase in a maize null-line, H95. There are at least 14 such null-lines and it is not known what causes null-phenotype in these inbred lines. It is possible that the same lectin is responsible for Beta-glucosidase null phenotype in 14 other maize inbred lines besides H95. To test this hypothesis, tissue homogenates from maize null-lines were extracted with PBS buffer (with and without lactose) and the extracts were assayed for Beta-glucosidase activity and protein. SDS-PAGE, western blotting and Beta-glucosidase activity measurements indicated that BGAF is responsible for Beta-glucosidase aggregation in these null-lines too. BGAF is also expressed in wild-type maize (B73 and K55) but its expression level is significantly lower than in null lines. Over-expression of BGAF in null lines leads to formation of large insoluble Beta-glucosidase aggregates, which is reflected as Beta-glucosidase null-phenotype in maize.

P3

A knockout mutation of a *Zea mays* U2AF35 related protein alters splicing in a subset of maize genes

(submitted by Federico Martin <fmartin@ufl.edu>)

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Alternative RNA splicing produces multiple mRNA species and allows genes to increase their range of functions. EST sequencing projects have shown that plants produce a significant fraction of alternatively spliced messages, but little is known about the control of alternative splicing. We have identified a Mutator insertion in a U2AF35 related gene from the UniformMu transposon tagging population. We are calling this gene ZmUrp for *Zea mays* U2AF35 related protein. U2AF35 proteins identify splice acceptor sites during RNA processing. The zmurp mutation is tightly-linked to the rough endosperm3 (rgh3) mutation which causes both defective seed and seedling development. RT-PCR analysis from rgh3 mutant RNA indicates that the zmurp insertion allele accumulates a defective transcript with multiple stop codons. RT-PCR analysis of normal RNA identified a series of alternatively spliced ZmURP mRNAs with only one variant predicted to encode a protein. We tested a series of alternatively spliced candidate genes to determine if mRNA splicing is disrupted in zmurp/rgh3 mutants. Candidates were selected from maize orthologs of rice genes that were characterized in the ASIP database at PlantGDB. We selected alternative splice variants that either had alternative donor or alternative acceptor sites. No differences in alternative donor splicing patterns were detected in zmurp/rgh3 RNA. Consistent with U2AF35's role in defining splice acceptor sites, a subset of alternative acceptor candidates showed splicing differences in zmurp/rgh3 mutants. These data suggest that the ZmUrp/Rgh3 locus influences splice acceptor site selection for a specific subset of maize genes.

P4

A sorbitol dehydrogenase deficiency increases sugar levels during maize development

(submitted by Sylvia Morais de Sousa <smsousa@ufl.edu>)

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Sorbitol dehydrogenase (SDH) is highly active in maize endosperm, but since kernels do not import or store sorbitol, an intermediary role is likely for this sugar alcohol and enzyme. The reaction reversibly interconverts fructose + NADPH to sorbitol + NAD, and could provide an alternate path for use of fructose derived from imported sucrose. To help test hypotheses for the significance of sorbitol dehydrogenase in maize, we identified an sdh1 mutant by screening the UniformMu maize population. Since this population is highly inbred, the wild type material provides a uniform set of controls for functional analysis of the mutants. The sdh1 mutation reduced maximal SDH activity in developing kernels to less than 6% of wild-type levels. The resulting phenotype was a 20% smaller kernel under field conditions (dried-seed weight at maturity, significant to $p < .001$). The sdh1 mutation also increased sugar levels during development; especially at 25 DAP (near harvest date for many sweet corns). At this stage, hexose levels were more than doubled and sucrose levels were elevated by 16%. The sdh1 mutation may thus have potential value for sweet corn improvement. The 2-fold hexose increases also indicated a central role for sorbitol metabolism in the sugar balance of developing maize kernels. These changes were accompanied by 33% reduction in sorbitol levels, but other paths of sorbitol biosynthesis appear likely in kernels, given the presence of significant residual sorbitol in the sdh1 mutant. Elucidation of the sorbitol pathway will allow an appraisal of its importance to the metabolic and physical flux of carbon in the developing maize kernel.

P5

Altered ethylene levels and ethylene-related transcripts are seen in developing seeds of two sugar mutants in maize

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In this study we explore maize kernel development with focus on sugar metabolism and starch production. Specifically, we use sugar metabolism mutants *miniature1-1* (*mn1-1*, cell-wall-invertase deficient) and *sugary1* (*su1*, starch-synthase deficient) to understand the possible significance of sugar signals related to levels of the phytohormone ethylene and expression profiles of ethylene-related genes. Our data combine transcriptional profiling of *Hexokinase* (*HxK*) and key ethylene biosynthesis and perception genes with direct hormone analysis to provide deeper understanding of the way ethylene action is influenced by the sugar status of the cell, and ultimately how this crosstalk modulates development. Wild-type *Mn1* and *Su1* maize kernels exhibit two peaks of ethylene, one ~14 days-after-pollination (DAP) and the second ~30 DAP. Preliminary data show that *mn1-1* kernels (vs. *Mn1*) have slightly higher ethylene production during early development and peaks significantly earlier during final maturation. Despite ~70% reduced seed mass, *mn1-1* produced comparable ethylene levels per kernel as *Mn1*. During early stages, *HxK* and the ethylene receptor *Ethylene response 2* (*ETR2-40*) transcripts show pronounced differences from wild-type, while receptor *Ethylene response sensor 1* (*ERS1-14*) begins to diverge at 16 DAP onward. *ACC-synthase* (*ACS47*) is variable throughout development, showing lower expression early in the *mn1-1* line and peaks five days earlier (29 DAP), coinciding with rising ethylene production. For the starch-deficient *sugary1* mutant, ethylene production peaks are phase-shifted four days earlier while retaining similar trends in amplitude. *HxK* and *ERS1-14* show most difference during early development. *ETR2-40* exhibits a similar trend in *Su1* and *su1* genotypes; however, the transcript is 2-3 fold more abundant in the mutant. *ACS47* shows highest differences during early (8 DAP) and late (28 DAP) development. Overall, we observed altered levels of both ethylene hormone and related transcripts in developing seeds of two maize sugar mutants relative to the corresponding wild-types.

P6

Biomass composition diversity in maize

(submitted by Andrew Hauck <ahauck@uiuc.edu>)

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The emerging importance of energy produced from cellulosic sources has been made well known given reports such as USDA's "Biomass as Feedstock for a Bioenergy and Bioproducts Industry" and the DOE's "Breaking the Biological Barriers to Cellulosic Ethanol". Since corn stover represents the largest source of readily available biomass, use of this supply will inevitably be sought as soon as cellulose conversion technologies are brought online. Extraction of usable sugars from a complex biomass such as corn stover is complicated by a large fraction of the cellulose being embedded in a recalcitrant cell wall matrix and the production of fermentative inhibitors from the processes used to facilitate extraction of cellulosic sugars. In order to ascertain the maize stover phenotypes more amenable for bioenergy purposes, we estimated the stover composition using the National Renewable Energy Laboratory's NIR methods across a set of lines that represent approximately 80% of the SNP diversity in maize. In addition to the core diversity lines, several other lines with known variation for cell wall composition were tested as well. Additionally, the lines resulting from test crosses with B73 were grown for comparison. Material for the experiment was grown in two replications in Illinois, Missouri, and North Carolina in the summer of 2005, then shipped to NREL at Golden Colorado for processing and analysis. Results indicate that inbred trait values are not predictive of hybrid performance. There is variation across the lines for meaningful traits such as sugar content, as well as other constituents.

P7

Biosynthesis of DIMBOA in maize is solved

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Benzoxazinoids are natural pesticides, insecticides and allelopathic substances characteristically found in the grasses. In maize, DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one) is the predominant substance. The inactive glucosylated substance is stored in the vacuole. When the integrity of the cell is destroyed, the toxic aglucon is released by a specific glucosidase. We have isolated the last two missing genes of the biosynthetic pathway, Bx6 and Bx7, encoding a 2-oxoglutarate dependent dioxygenase and a methyltransferase. All genes of the pathway are localized on the short arm of chromosome 4.

Benzoxazinoids are found sporadically in some species of the dicots (e.g. *Consolida orientalis* (Ranunculaceae), *Lamium galeobdolon* (Lamiaceae)). Like in maize, the branch point reaction of the biosynthesis is the generation of indole. The enzyme of maize (Bx1) and the enzymes of the dicots are homologs of the tryptophan synthase alpha, but have an independent phylogenetic evolution. To elucidate the evolution of the glucosyltransferase of the pathway, the enzyme is purified from *Consolida orientalis* and *Lamium galeobdolon*.

P8

Centromere Protein C (CENP-C) targets centromeres by a novel DNA/RNA interaction

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Centromeric DNAs are highly repetitive and non-conserved, making it difficult to understand how centromeres maintain their interactions with kinetochore proteins. Currently the best candidate for the protein that mediates this interaction is Centromere Protein C (CENP-C). Our data from gel mobility shift assays show that maize CENP-C is a DNA binding protein, and further, that DNA binding activity is localized to a small exon duplication. The same exon duplication region binds to RNA. Transient expression of CENP-C variant without the exon duplication region resulted in reduction of centromere localization to 58% of the control, as indicated by a reduced signal at kinetochore. This decrease was also observed after the replacement with HIV integrase DNA binding domain (64%). Together, our results demonstrate that nucleic acid binding properties, especially RNA binding, as an important mechanism for centromere maintenance of CENP-C.

P9

Characterization of dull1 plants transformed with a mutagenized Du1 coding sequence containing an altered 14-3-3 binding site

(submitted by Qiaohui Lin <qhlin@iastate.edu>)

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Amylopectin (Ap) is the most abundant α -D-glucose polymer in starch. Several starch synthases catalyze the reactions that utilize ADP-glucose to build linear Ap chains. One of these, starch synthase III (SSIII), is the product of the maize *dull1* gene. SSIII is responsible for producing the longest Ap chains, and also may regulate the activities of other starch biosynthetic enzymes via protein-protein interactions. SSIII itself may be regulated by interaction with 14-3-3 proteins, regulatory factors that bind specific phosphorylated peptide motifs. Our immunoprecipitation experiments confirm that maize SSIII binds 14-3-3s and that this interaction is phosphorylation dependent. A previous study of SSIII/14-3-3 interaction in Arabidopsis hypothesized that 14-3-3 binding negatively regulates SSIII and has an inhibitory effect on starch synthesis. We used a transgenic approach to test this hypothesis in planta, altering the phosphorylation target (Thr1583) within the 14-3-3 consensus binding sequence of maize SSIII to prevent binding. Plasmid pVR5-T1583A was constructed to express full-length Du1 cDNA, with nucleotides coding for Thr1583 mutagenized to encode alanine. Mutant *du1-M3* plants in the Oh43 background were transformed with pVR5-T1583A by microprojectile bombardment. One class of F2 plants containing the transgene has kernels that appear wild type and starch chain profiles that differ from either wild type or *du1* starches. This suggests the transgene complements the *dull* mutation, restoring SSIII activity. The novel starch in these kernels has a greater abundance of linear chains of DP 40-60, suggesting possible effects of removal of 14-3-3 regulation are increased SSIII activity and more long Ap chains. Other possible effects of loss of SSIII/14-3-3 interaction, including changes in the total amount of starch produced or altered activities of other starch biosynthetic enzymes, are being tested. MS analysis of immunoprecipitated 14-3-3 proteins also will be applied to identify specific 14-3-3s that interact with SSIII.

P10

Characterization of two high-molecular weight complexes from developing maize endosperm containing multiple starch biosynthetic enzymes

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In order to explain synthetic phenotypes and pleiotropic effects observed in maize mutants, starch biosynthetic enzyme functions are proposed to be coordinated through physical association in multi-subunit complexes. Previous work demonstrated pair-wise interactions between specific starch synthases (SSs) and starch branching enzymes (BEs) using yeast two-hybrid tests, immunoprecipitation, and affinity purification using fusion-tagged recombinant proteins as solid-phase ligands. In this study gel permeation chromatography was used to separate components present in wild type maize amyloplast- or total endosperm soluble extracts based in large part on native molecular mass. Fractions were then examined for the presence of SSIIa, SSIII, BEI, BEIIa, and BEIIb by immunoblot analyses. Three elution peaks containing starch biosynthetic enzymes were observed, a presumed monomer fraction at approximately 80 kDa, and two different high molecular weight forms at approximately 300 kDa (C300) and 600 kDa (C600). SSIIa, BEIIa, and BEIIb were present in C300, and these three proteins, along with SSIII, were present in C600. Both high molecular weight peaks were present in 1 M, 150 mM, and no NaCl conditions. At 1 M NaCl an increase of signal intensity for C600 components was observed, suggesting that the hydrophobic effect is significantly involved in complex stability. C300 and C600 formation is unlikely due to random aggregation because in contrast to BEIIa and BEIIb, the BEI isoform was observed exclusively as a monomer. Analysis of mutant extracts revealed SSIIa is required for BEIIa and BEIIb inclusion in C300 and SSIII is necessary for SSIIa, BEIIa, and BEIIb assembly into C600. Ongoing investigations will characterize these complexes *in vivo*, purify and identify their components, and compare SS and BE enzymatic activities among the three assembly states. These analyses will further define the role of specific enzymes in determining amylopectin structure required to form crystalline starch.

P11

Complementary sources of a high-lutein phenotype in yellow dent inbred lines

(submitted by Andrew Burt <aburt@uoguelph.ca>)

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Carotenoid profiles in maize grain may be most simply characterized, according to the major carotenoid constituent, as either high-lutein or high-zeaxanthin. Lutein and zeaxanthin are hydroxylated carotenoids that are important for eye health. Biosynthetically these compounds are created by the cyclization of lycopene where the pathway branches into 3,3'-beta-ring carotenoids (zeaxanthin) and 3-beta, 3'-epsilon-ring carotenoids (lutein). As both sides of the pathway contain beta-rings, it is generally thought that only lycopene-cyclase determines the fate of the biosynthetic flux. We have discovered two complementary sources of the high-lutein phenotype in yellow dent inbred lines. Reciprocal crosses between high-zeaxanthin inbreds and these high-lutein lines show different modes of inheritance. Reciprocal crosses between the two inbred lines show over-dominance and maternal effects on lutein:zeaxanthin ratio. A mapping population has been created and is being analyzed to clarify the mechanism(s) underlying these high-lutein phenotypes. The hypothesized mechanism is that lycopene-cyclase, as well as lycopene-cyclase, is involved in determining this phenotype.

P12

Consequences of C4 differentiation for chloroplast membrane proteomes in maize mesophyll and bundle sheath cells

(submitted by Wojtek Majeran <wm48@cornell.edu>)

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Chloroplasts of maize leaves differentiate into specific bundle sheath (BS) and mesophyll (M) types to accommodate C4 photosynthesis. Chloroplasts contain thylakoid and envelope membranes which contain the photosynthetic machineries and (metabolite) transporters, but also proteins involved in e.g. protein homeostasis, anti-oxidative stress response. Based on previous information, it can be hypothesized that these chloroplast membranes must be specialized within each cell type to accommodate C4 photosynthesis and regulate metabolic fluxes and activities. This quantitative study determines the differentiated state of BS and M chloroplast thylakoid and envelope membrane proteomes and their oligomeric states, using innovative gel-based and mass spectrometry based protein quantifications. This includes native gels, iTRAQ and label-free quantification using a LTQ-Orbitrap. Subunits of Photosystem I, II, the cytochrome b6f and ATP-synthase complexes showed average BS/M accumulation ratios of respectively 1.6, 0.45, 1.0, 1.33, while ratios for the LCHI and the LHCII families were respectively 1.72 and 0.68. BS specific NDH complexes with associated proteins of unknown function containing more than 15 proteins were observed; we speculate that this novel complex possibly functions in inorganic carbon concentration when carboxylation rates by Rubisco are lower than decarboxylation rates by malic enzyme. Differential accumulation of thylakoid proteases (Egy, DegP), state transition kinases (STN7,8) and PSII and PSI assembly factors was observed, suggesting that cell-specific photosynthetic electron transport depends on post-translational regulatory mechanisms. BS/M ratios for inner envelope transporters PPT, Dit1, Dit2 and Mex1 were determined and are consistent with expected metabolic fluxes. A wide variety of other proteins showed differential BS/M accumulation. Mass spectral information and other relevant information are available through the Plant Proteome DataBase. These data are integrated with previous data, resulting in a model for C4 photosynthesis thereby providing new rationales for metabolic engineering of C4 pathways and targeted analysis of genetic networks that coordinate C4 differentiation.

P13

Effect of mutagens on imprinting expression in apomictic maize-Tripsacum hybrids

(submitted by Victor Sokolov <sokolov@bionet.nsc.ru>)

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It has been long believed that apomixis is determined by two components - apomeiosis and partenogenesis. The current work shows the considerable role of imprinting in viable maize-Tripsacum seed formation of pseudogamic asexual seeds. In apomicts, the embryo sacs develop out of diploid cells, and the central cell is tetraploid until fertilization. In the initial endosperm cell development, the ratio of female to male genomes will be 4F:1M in its nucleus. Such a combination of male and female genomes in the cell nuclei of grain storage tissue is abnormally different from that of the regular 2F:1M necessary for viable seed formation. Thus, only 20% of asexual Tripsacum florets actually give rise to viable seeds. Despite a considerable deviation of maize genomes in endosperm cells from that of regular 2F:1M, we obtain up to 50% of viable seeds in the apomictic maize x Tripsacum hybrids produced. The mechanism of the 5-azacytidine effects has been connected with methyl-cytosine demethylation, one way of imprint marking. However, due to complicated interactions, its expression is most likely to be unstable. Thus, it is possible to conclude that, imprinting expression and its strict dosal dependence is different from that observed in diploid maize of apomictic maize x Tripsacum hybrids in the presence of some of the wild parent (Tripsacum) chromosomes. The presence of Tripsacum chromosomes is likely, in some way, to affect the imprinting signal-setting and modify its expression. In this connection, we tried to influence its expression experimentally with 5-azacytidine treatment of germinating seeds. We have observed grain weight increases traced through the M1 and in the subsequent generations. At present, we are left to dwell on the results of our investigation and consider those of the future in using 5-azacytidine for modification of imprinting expression in apomictic maize x Tripsacum hybrids.

P14

Evolution and functional divergence of ADP-glucose pyrophosphorylase subunits in angiosperms

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ADP-glucose pyrophosphorylase (AGPase) catalyses a rate-limiting step in starch synthesis. In plants, AGPase consists of two identical large and two identical small subunits. Earlier, we showed that the overall ratio of nonsynonymous substitutions per nonsynonymous site to synonymous substitutions per synonymous site (Ω) is 2.7-fold greater for the large subunit than for the small subunit in angiosperms. Herein, we verify and complement the Ω analysis by estimating the absolute rate of amino acid evolution for the large and small subunit in angiosperms. Collectively, these results show that large subunits evolve faster than do small subunits in angiosperms. Our results also illustrate that the absolute rate of evolution is a valid means of estimating evolutionary constraints. Hence it was used to validate Ω analysis. It can also be an alternative to Ω analysis in gene families where the use of Ω is not appropriate. Positive selection was detected in specific lineages and in sites of both subunits in angiosperms. However, the limited extent of positive selection cannot explain the faster rate of evolution of the large subunit compared to the small subunit. Collectively, our results strongly suggest that the large subunit evolves faster than does the small subunit because of relaxed evolutionary constraints. Further study of the evolutionary history of the two AGPase subunits in angiosperms indicates that the large subunit had more successful duplications than did the small subunit. The large subunit had its first successful duplication ~400 million years ago, well before the divergence between monocots and eudicots. In contrast, the small subunit had its first successful duplication after the divergence of monocots and eudicots. Gene duplications have led to tissue-specific large subunit groups. We provide evidence that the large subunit groups have functionally diverged at the protein level. We also show that the small subunits from monocots have functionally diverged from the small subunits from eudicots. We identified candidate sites that may account for the functional divergences.

P15

Functional genomic analysis of maize chromatin genes

(submitted by Karen Cone <ConeK@missouri.edu>)

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Chromatin-level regulation has the potential to modulate expression of many genes throughout the genome. To discover and analyze such epigenetic regulation, we have used RNA interference (RNAi) to produce lines in which chromatin gene expression is reduced. The genes targeted encode enzymes that modify DNA and protein components of chromatin and proteins that are involved in nucleosome assembly, chromatin remodeling, gene silencing and modulation of chromatin condensation. We have also used TILLING to isolate recessive mutations in other types of genes, including those involved in the RNAi pathway. Chromatin mutant lines are analyzed using genetic, biochemical and cytological assays in order to define the function of individual chromatin genes on paramutation, phenotypic variegation, imprinting and DNA methylation. The global effects of chromatin gene mutants are studied using transposon display and microarrays to define genome-wide changes in chromatin modification and gene expression. Chromatin mutant lines and a series of Gateway compatible plasmid vectors--suitable for epitope tagging, fusion to fluorescent proteins and expression in plant and bacterial cells--are available for use by other researchers.

P16

Genetic and molecular regulation of cyclic carotenoid biosynthesis in maize seeds

(submitted by Ling Bai <lb226@cornell.edu>)

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Carotenoids are essential pigments for all photosynthetic tissues with roles in photoprotection, the reaction center core and as accessory light harvesting pigments. In maize, human selection for yellow seeds has led to increased carotenoid accumulation in endosperm tissues and the development of high carotenoid grain. Further manipulation of grain carotenoids to improve grain nutritional quality requires a detailed understanding of carotenoid accumulation profiles and carotenoid pathway regulation in developing seed. Here, we show that carotenoid accumulation patterns differ in maize embryo and endosperm tissues and that this tissue-specificity of the pathway is largely mediated through the differential expression of genes encoding lycopene b-cyclase (LCYB) and lycopene e-cyclase (LCYE). Using a null mutation in the Lcy-b gene (*lcyb-m2.1*), we demonstrated that the LCYB is necessary for the synthesis of nearly all of the b-carotene-derived carotenoids in maize. In the absence of LcyB, a number of unusual carotenoids accumulate in endosperm tissue including d-carotene, e-carotene and lactucaxanthin (e,e-carotene-3,3-diol), suggesting that a block in the biosynthesis of carotenoids may lead to novel accumulation profiles. Functional assays confirm that maize LCYE is able to produce such an unusual carotenoid accumulation profile in *E. coli* systems. To address potential negative feedback regulation of the pathway, we examined the transcription profiles of the *Psy1* gene encoding phytoene synthase in two different seed tissues. Interestingly, *Psy1* transcript abundance increased in both *lcyb-m2.1* mutant embryo and endosperm tissues indicating the presence of negative feedback regulation by a carotenoid intermediate.

P17

Genetic control of provitamin A synthesis, modification and degradation in maize grain

(submitted by Catherine Kandianis <cbermude@uiuc.edu>)

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The production and stability of provitamin A (ProVA) pools in plants is dynamically controlled by the synthesis, modification and degradation of carotenoid precursors and metabolites. A significant proportion of the variation in maize grain ProVA levels can be explained by several major QTL, including those that localize to carotenoid synthesis genes, including PSY1, DXS, LCYb, LCYe and HYDb. It is essential to determine how these genes function in combination, as well as how the balance between synthesis and degradation is achieved. Reconciliation of previously unknown QTL with recent association mapping results for carotenoid composition will be shown using genetically mapped functional markers for LCYe and HYDb1 on two F2:3 maize mapping populations developed to explore the genetic basis for alterations in ProVA levels. Results also suggest that maize CCD1, the locus underlying the white cap1 (wc1) phenotype, plays a significant role in reducing certain carotenoid pools. Genome-wide epistatic interactions among genetic markers and functional markers for candidate genes are being evaluated in an effort to establish an epistasis-inclusive pathway model describing the regulation of the carotenoid biosynthesis pathway. Comparisons of QTL results across multiple studies demonstrate that several loci consistently contribute large effects on carotenoid composition, including known major QTL, and now CCD1. Our results point to new genetic targets that we have started using in marker assisted selection to increase ProVA concentration and thus improve the nutritional value of maize grain.

P18

Maize has two Mre11 genes: one is novel and both appear to be developmentally regulated

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Mre11-Rad50-NBS1 (MRN) is a highly conserved protein complex in eukaryotes, involved in DNA double strand break (DSB) repair, homologous recombination, DNA damage signaling/ cell cycle checkpoint regulation, and maintaining telomere length. The core of the complex, Mre11 has been widely studied in yeast and humans. We have previously reported that maize has two Mre11 genes, Mre11A and Mre11B, a duplication event that, so far, has only been reported in the grasses. In maize and in sorghum (but not in rice or wheat), rather than a simple duplication, the proteins encoded by the duplicate genes are similar over most of their length, but Mre11B has a novel C-terminal domain found nowhere else among the databases.

Mre11A is alternatively spliced and this alternative splicing may be developmentally regulated. One of these messages makes a full-length protein while the other makes a C-terminally truncated protein of as yet unknown function. The truncated protein appears to be relatively underrepresented in dividing cells while the full-length Mre11A is present in all tissues examined. Here we report that Mre11B expression is also developmentally regulated. Our results suggest that Mre11B protein is localized to the nucleus and is detectable only in dividing cells, suggesting it may be regulated in a cell cycle-dependent manner and may be an excellent marker for dividing cells and the signals that control division.

Mre11B is able to interact with full-length Mre11A in yeast two-hybrid assays to form heterodimers. Interestingly, however, Mre11B does not interact with Nbs1, the signaling component of MRN, while Mre11A does. The truncated protein made by the splicing variant of Mre11A does not interact with either Mre11B or with Nbs1. We are currently testing whether Mre11B interacts with maize Rad50. We are also analyzing other interaction partners of Mre11B from maize nuclear extracts.

P19

Maize syntenic analyses using all grass genomes as outgroups

(submitted by Eric Lyons <elyons@nature.berkeley.edu>)

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Given the rice genome sequence, the pending release of sorghum and Brachypodium genomic sequence, the growing maize assembly, and early efforts in foxtail millet, the diverse grass family will soon be uniquely rich in genomic sequence. While all grasses share a common tetraploidy (~60mya), maize had an additional tetraploidy about 12 million years ago. As in many other plant families, many grasses had very recent tetraploidies, including wheat, sugarcane and most biofuel perennials. Following each tetraploidy, genomes undergo large-scale reorganization through massive gene loss (fractionation), inversions, translocations, local duplications, segmental duplications and perhaps transpositions. It is a formidable problem to simply identify all orthologous and homeologous grass chromosomal regions, present them to a sequence alignment algorithm, and visualize the results. However, such multi-region alignments generate invaluable data. Annotation errors are exposed, putative gene regulatory regions are discovered as conserved noncoding sequences, and cases of sequence subfunctionalization following duplication can often be evidenced. We have built a software system (CoGe) that stores multiple genomes in a useful way, facilitates such multiple genomic region comparisons, displays the result graphically using an interactive viewer, and can compare up to 10 chromosomal regions in a single user-configured analysis. This research tool that is available to maize geneticists at <http://synteny.cnr.berkeley.edu/CoGe/>. Read: Lyons and Freeling, 2007-8, The Plant Journal, in press. Supported by NSF.

P20

Molecular analysis of autophagic pathway in maize

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Autophagy is a self-eating, bulk degradation process in eukaryotes that plays a key role in nutrient remobilization during starvation. Genetic studies in yeast have defined a set of *ATG* (autophagy) genes required for proper autophagic engulfment. Two ubiquitin-like conjugation pathways involving ATG8 and ATG12 tags are critical for autophagic vesicle expansion and deposition, and many of them are conserved in animals and plants. We previously showed that mutations in several *Arabidopsis* *ATG* genes result in a hypersensitivity to nitrogen and carbon limitations, demonstrating the importance of these genes in the adaptation of plants under nutrient starvation. To elucidate roles of autophagy in crop species, we identified several *Atg* genes in the maize B73 genome and examined their expression patterns. Most maize *Atg* genes in the ATG8 and ATG12 pathways are highly conserved, except for the *Atg7*, the gene encoding the E1 enzyme required for both conjugation pathways. Unlike animal and dicot ATG7 homologues, the maize and rice ATG7 proteins have duplicated C-terminal domains, only one of which has a conserved cysteine active site. Although transcripts of maize *Atg* genes are ubiquitously distributed, as in *Arabidopsis*, starvation significantly increases the levels of several *Atg* mRNAs including those encoding ATG8. Furthermore, ATG8 conjugation to phosphatidylethanolamine (PE), a marker of autophagy, depends on age and nutritional supply, suggesting transcriptional and post-translational control of autophagy in maize by developmental and environmental factors. We have also identified mutations in several maize *Atg* genes that will be useful tools to study autophagy genetically in a crop species.

P21

Molecular studies of maize photosynthetic mutants with transposon insertions in nuclear genes encoding chloroplast targeted pentatricopeptide repeat (PPR) proteins

(submitted by Maureen Hanson <mrh5@cornell.edu>)

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Pentatricopeptide repeat (PPR) proteins of higher plants comprise a large family (~450 members) of nuclear encoded proteins. Various family members have been characterized as having roles in numerous aspects of organellar RNA metabolism. To determine specific roles of other PPR proteins in chloroplast RNA metabolism of maize, we have screened The Photosynthetic Mutant Library (PML) (<http://pml.uoregon.edu>) for photosynthesis impaired mutants of chloroplast targeted PPR proteins that are representative of distinct PPR protein sub-families. Mutants with Mutator insertions associated with a different pigmentation phenotype were used to generate heteroallelic mutants, which were then evaluated, at both protein and RNA levels, for molecular aberrations of the chloroplast photosynthetic machinery. Components of the chloroplast membrane complexes in mutant plants were measured using immunoblot analysis. Chloroplast encoded RNA populations have also been evaluated for processing, editing and splicing defects. Notably, allelic mutants showing a pale yellow green (pyg) phenotype presented severely diminished levels of both Cytochrome B6/f and Photosystem I complex proteins, as well as moderately reduced levels of both Photosystem II and ATP Synthase complex components. Analysis of chloroplast RNAs in these pyg mutants also revealed that *ycf3* transcript splicing is highly impaired.

P22

Natural genetic diversity as a tool towards developing metabolic engineering strategies to improve or modulate endosperm carotenogenesis

(submitted by Ratnakar Vallabhaneni <ratnakarvallabhaneni@yahoo.com>)

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Global vitamin A deficiency is linked to diets deficient in pro-vitamin A carotenoids. One approach to alleviating this deficiency is to enhance pro-vitamin A carotenoid content in endosperm of crops such as maize, sorghum, and wheat, related grasses in the Poaceae and important food staples worldwide. Carotenoids are synthesized in plastids from isoprenoid precursors; accumulation is a balance of synthesis and degradation. One approach to investigate regulation of endosperm carotenoid content/composition is to utilize germplasm resources that exhibit natural genetic variation. A collection of 300 diverse inbred lines in maize exhibits 80% of the known allelic variation and shows quantitative variation in endosperm carotenoids. To utilize this resource for investigating the underlying gene expression, we identified gene family members encoding enzymes of the carotenoid pathway and for pathways that impact carotenoid accumulation. Using a subset of this maize diversity collection, we dissected endosperms at various developmental time points, for which we profiled transcript levels and carotenoid composition. Such correlation analysis revealed specific gene family members whose expression at certain endosperm developmental time points correlated with carotenoid content and/or composition. This approach will lead to better selection of breeding alleles and/or temporal optimization of transgene expression during endosperm development. Further application of comparative genomics also allowed us to identify paralogous genes that may serve as targets for enhancing carotenoid content of related crops, some of which may lack resources of germplasm diversity collections.

P23

Oh *tie-dyed*, where art thou?

(submitted by Robert Baker <rfb11@psu.edu>)

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Carbon partitioning is essential for plant growth and development, but the genetic regulation of this process remains poorly understood. In maize, recessive *tie-dyed1* (*tdy1*) mutations condition a yellow and green leaf variegation pattern that results from a defect in carbon partitioning. Yellow tissues accumulate excessive carbohydrate levels while green tissues are essentially like wild type. To localize the site of *Tdy1* function, we performed a clonal mosaic analysis and determined that *Tdy1* functions in the innermost leaf layer, which contains the interveinal mesophyll cells, bundle sheath cells and veins. Additional work examining the *tdy1* sector phenotype in relation to lateral veins further implicated a role for *Tdy1* in the veins. Cloning *Tdy1* revealed that it encodes a novel membrane localized protein, and RT-PCR analyses showed it is expressed in all organs. To determine the cell-type specific expression of *Tdy1*, RNA in situ hybridizations were performed on developing leaves, roots and inflorescences. In all cases, *Tdy1* was found to be exclusively expressed in phloem cells. The *Tdy1* expression pattern and the carbon accumulation defect in *tdy1* mutants suggest a role for *Tdy1* in regulating sucrose transporters (SUTs), which are involved in loading and unloading sucrose from the veins. RNA in situ hybridization of *ZmSUT1* in developing roots and leaves showed that it is co-expressed with *Tdy1* in phloem cells, consistent with the hypothesis that *Tdy1* may regulate SUT function.

P24

Regulation of hormone pathways by the ramosa genes in maize inflorescence development

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RAMOSA 1 (RA1) is a plant specific EPF protein with a Cys2-His2 zinc finger DNA binding domain and an EAR repression motif that regulates branch architecture of the maize inflorescence. RA1 is expressed at the junction between each second-order meristem and the main inflorescence axis, and regulates the fate of those second-order meristems. Besides ra1, two other ramosa genes (ra2 and ra3) have been identified to perform similar developmental functions. ra2 encodes a LOB domain protein, expressed in the edge of the bract and meristem early in inflorescence development. ra3 encodes a trehalose-6-phosphate phosphatase, expressed in discrete domains subtending axillary inflorescence meristems. Further research suggests that the three ramosa genes function in the same ramosa pathway with ra2 and ra3 acting upstream of ra1 to regulate second-order meristem determinacy and therefore inflorescence architecture. We have used GFP-RA1 fusions to demonstrate the proteins localization to the nucleus. To investigate the mechanism of ra1 function, we used yeast two hybrid analysis and identified RA1 interacting proteins, including several putative transcriptional regulatory proteins. As suggested by interaction with the KNOTTED1 protein, gibberellin and cytokinin may be involved in the ramosa pathway. We address this hypothesis by analyzing the transcription level of hormone biosynthesis genes in developing normal and mutant inflorescences.

P25

Role of HC-toxin in disease susceptibility: redefining the paradigm

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HC-toxin enables *Cochliobolus carbonum* race 1 to cause a lethal leaf blight/ear mold disease in maize. The mode of action by which HC-toxin facilitates host colonization remains unclear. One mechanism, based on the observation that HC-toxin inhibits histone deacetylases (HDACs) across diverse species, presumes that HC-toxin inhibits the induction of defense response genes, thereby leaving the host vulnerable to pathogen attack. According to this scenario, HC-toxin should be ineffective in inducing susceptibility if defense responses are already up and operating at the time of pathogen infection. Our results, described below, do not support this hypothesis and suggest that HC-toxin induces susceptibility by a mechanism(s) other than interfering with the induction of defense responses. Moreover, it may not be the HDAC inhibitory activity of HC-toxin that induces susceptibility in maize to *C. carbonum* race 1.

P26

Starch branching enzyme (SBE) IIa is required for diurnal cycling of starch within the maize leaf

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Maize leaf chloroplasts undergo starch synthesis and degradation in accordance with the diurnal cycle: starch accumulates during the day and is degraded at night to maintain energy homeostasis. Starch is composed of two glucose polymers, amylose and amylopectin. Amylopectin contains many branches, which are catalyzed by starch branching enzymes (SBEs). Of the three maize SBE isoforms, SBEIIa plays a pivotal role in starch biosynthesis within the leaf and starch produced in the *sbe2a* mutant contains very few branches. To study the effect of the *sbe2a* mutation on the diurnal cycling of starch, starch content was measured in wildtype and *sbe2a* mutant leaves. In wildtype leaves, the amount of starch present at the end of the dark phase was significantly reduced (~five-fold) as compared to the end of the light phase. However, in *sbe2a* leaves starch content was reduced less than two-fold at the end of the dark phase, which suggests that starch fails to fully degrade in plants lacking SBEIIa. Additionally, *sbe2a* starch granules are ~three-fold larger and are more resistant to enzymatic digestion than wildtype. This accumulation of abnormal starch within chloroplasts may trigger the premature leaf senescence observed in *sbe2a* mutants (Blauth et al, 2001).

Since starch biosynthesis and photosynthesis are intimately associated and photosynthesis is a well-known driver of cellular redox changes during the diurnal cycle, it seems likely that SBEIIa might be post-translationally regulated via the redox cycle. To begin to test this hypothesis, crude protein extracts and leaf discs from wildtype W64A leaves were treated with DTT (reducing conditions) or oxidized DTT (oxidizing conditions). SBE activity was two to three-fold higher in samples treated with reduced DTT. This result is consistent with the fact that starch synthesis occurs during the light phase when photosynthesis maintains the chloroplast in reducing conditions.

P27

Sucrose hyperaccumulation and ionic changes in maize leaves over development

(submitted by Clifford Weil <cweil@purdue.edu>)

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Maize varieties that accumulate large amounts of sucrose in their vegetative tissues instead of producing large ears can be an outstanding alternative feedstock for biofuel production. Tropical varieties grown in temperate U.S. conditions often flower very late or make no ears at all and, instead, accumulate levels of sugar in their vegetative tissues that exceed those of sweet sorghum and can rival sugarcane. In contrast, temperate-adapted varieties like B73, if the ear is removed as a carbon sink, also show hyperaccumulation of sucrose, but also show premature senescence. To better understand the basis of this difference, we have used the *tie-dyed1* (*tdy1*) mutant of B73, which produces nonclonal sectors of yellow leaf tissue associated with hyperaccumulation of sucrose. In addition these sectors senesce earlier than green tissue in the same leaf, providing a useful test system for responses to increased sugar levels. We have examined mineral levels and patterns of accumulation over development in hyperaccumulating as compared with nonaccumulating and nonmutant leaf tissue using Inductive Plasma-Coupled Mass Spectrometry (IPC-MS). Sucrose accumulation is associated with specific changes in the ability to accumulate Ca, Cu, Mg, S, Mo, Fe and Mn, particularly in older leaves. Sucrose-nonaccumulating sectors of *tdy1* leaves show increased levels of K and P compared to sucrose-accumulating and nonmutant tissue. These metabolic profiles will provide a better understanding of what changes are associated with sugar accumulation and other physiological adaptations to excess carbohydrate levels.

P28

The *camouflage1* mutant is defective in tetrapyrrole synthesis and displays nonclonal sectors in its leaves

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camouflage1 (*cf1*) is a recessive maize mutant which exhibits a zebra banding pattern with alternating yellow-green and green leaf sectors. Fluorescent light microscopy of *cf1* leaf tissues reveals that bundle sheath cells in the yellow-green sector are specifically affected and undergo cell death, while in the green sectors the bundle sheath cells remain healthy. The selective effect on bundle sheath cells was confirmed by examining the expression of Rubisco, a bundle sheath cell-specific protein. The large subunit of Rubisco is absent in the *cf1* yellow-green sectors by SDS-PAGE analysis, and the mRNA of the small subunit is undetectable by reverse transcription-PCR (RT-PCR) analysis. The *cf1* gene was cloned via *Mutator* (*Mu*) transposon tagging, and it encodes porphobilinogen deaminase (PBGD), an enzyme in the chlorophyll and heme biosynthesis pathway. Both yellow-green and green sectors have much reduced PBGD activity and an elevated level of the precursor PBG. Interestingly, development of *cf1* sectors is dependent on light/dark cycling; plants grown in continuous light do not show the *cf1* phenotype. Moreover, in double mutant studies between *cf1* and mutants with defective chloroplasts in leaf tissues such as *striate2*, bundle sheath cells in albino tissues do not die even if they are located in the *cf1* yellow-green sectors. This result illustrates that functional chloroplasts are required for the bundle sheath cell-specific death in *cf1* mutants. Further investigations of the nonclonal sector phenotype and the function of the gene are underway.

P29

Tie-dyed1 localizes to the endoplasmic reticulum and co-localizes with sucrose transporter1

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The Tie-dyed1 (*Tdy1*) gene encodes a novel transmembrane protein that we hypothesize plays an important role in carbon partitioning in maize leaves. As one approach to characterize the genes function, we are determining its expression pattern at the tissue, cellular and subcellular levels. Expression analyses determined that *Tdy1* mRNA is exclusively present in the phloem of all tissue types. To determine where in the cell the TDY1 protein resides, we produced translational fusions of TDY1 to the fluorescent reporter proteins YFP or mCherry. Co-expression with known subcellular markers indicates that TDY1 localizes to the endoplasmic reticulum. When TDY1-mCherry is co-expressed with a maize sucrose transporter translationally fused to YFP (*ZMSUT1*-YFP), we observe that both proteins co-localize to the ER membrane while *ZMSUT1* also localizes to the plasma membrane and small mobile bodies. RNA in situ hybridizations also localize *ZmSut1* transcripts to developing phloem cells revealing an overlapping expression pattern with *Tdy1*. We hypothesize that TDY1 may control carbon partitioning by interacting with or regulating *ZMSUT1* within phloem tissue.

P30

Using the Corngrass1 gene as a tool for biofuel improvement

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The dominant Corngrass1 (Cg1) mutant displays several phenotypes that make it ideal for biofuel studies. Cg1 mutant plants increase biomass of vegetative shoots by continuously initiating extra axillary meristems and leaves. These leaves have reduced adult characteristics and exhibit juvenile leaf cell morphology throughout development. Furthermore, previous studies have shown that Cg1 mutant leaves contain decreased amounts of lignin and increased glucose, much like wild type juvenile leaves¹. Thus, the Cg1 gene keeps the maize plant in a prolonged juvenile state, causing increased biomass and providing an improved substrate for saccharification. Understanding the pathways that the Cg1 gene regulates to cause these changes can allow us to design better biofuel crops.

We recently cloned the Cg1 gene and showed that it is a tandem microRNA gene that is overexpressed in the mutant. This microRNA belongs to the MIR156 class that is known to target the SQUAMOSA PROMOTER BINDING LIKE (SPL) family of transcription factors. The overexpression of MIR156 causes inappropriate cleavage of its targets, demonstrating that the Cg1 mutant phenotype is caused by loss of function of several SPL target genes. To identify the targets of these transcription factors, a microarray analysis was done on Cg1 shoots compared to wildtype. Over 1,000 differentially expressed genes were identified, many of which are involved in auxin and gibberellin biosynthesis, as well as lignin biosynthesis and sugar metabolism. This analysis also helped identify all the cell wall components that distinguish juvenile versus adult tissues. A similar analysis done on Cg1 inflorescences revealed that several known branching regulators, as well as MADS box transcription factors, are downregulated in Cg1. Taken together, these results indicate that SPL genes effect phase change by altering the internal hormone balance necessary for the activation of floral specific transcription factors during flowering. Thus, it should be possible to enhance specific biofuel traits through transgenic experiments with select SPL genes.

1 - Abedon, B.G., Hatfield, R.D., and Tracy, W.F. (2006). Cell wall composition in juvenile and adult leaves of maize (*Zea mays* L.). *J. Agric. Food Chem.*

P31

A RepMiner analysis of maize LTR Retrotransposons reveals a previously unrecognized split in the Huck family

(submitted by James Estill <jestill@plantbio.uga.edu>)

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The taxonomic assignment of maize transposable elements (TEs) to families is a fundamental step of TE annotation that provides a foundation for the study of TE evolution. Our RepMiner package takes a graph theory based approach to taxonomic assignment that allows for the clustering of TEs into families based on networks of shared homology. The visualization of these networks allows us to map biologically relevant sequence features onto these families, and to compare our cluster based taxonomies to existing canonical databases. We have applied the RepMiner approach to our database of full length maize LTR Retrotransposons to illustrate the utility of this approach. RepMiner succinctly illustrates previously identified relationships among the Ji/Opie and Ciful/Zeon families as well as identifies a previously unrecognized split in the Huck family that would indicate that Huck should be treated as two separate families.

P32

An efficient algorithm to strengthen the power of nested association mapping experimental design

(submitted by Zhiwu Zhang <zz9@cornell.edu>)

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Recombinant inbred lines (RILs) are powerful tools for QTL association mapping. As demonstrated in nested association mapping (NAM) populations, the power of RILs is strengthened when a mapping population from a single cross between two inbred parents is extended to populations from multiple (n) crosses with a common parent. However, the strength can be reduced due to over fitting of statistical parameters with increasing number of populations. This study proposed an efficient algorithm to avoid this problem. For each detected QTL, the algorithm collapsed populations into k (less than n) groups. The collapsing was based on the estimated effects of a genetic marker for each population. The NAM populations in maize were used to illustrate the algorithm. The NAM population contained 25 inbred lines crossed to a common parent (B73). Each cross generated 200 recombinant inbred lines (RILs), which were collectively defined as a population. In total there were 5000 RILs across 25 populations. The mapping trait was flowering time recorded as days to silk over two years in eight locations. The best linear unbiased prediction (BLUP) of genetic effect for each RIL was derived using ASREML. The BLUP was used to map QTLs over eleven hundred genetic markers using SAS Procedure GLMSELECT. The genetic markers were chosen from candidate genes across the maize genome with B73 specific alleles. With the non-B73 marker alleles representing a specific genetic background for each population, a marker effect nested within population was used for detecting the association. Model fitting suggests that collapsing works as well or better for adjusted R square, while requiring the use of 1/3 as many statistical parameters.

P33

Ask for a MaizeGDB outreach visit to your institution!

(submitted by Lisa Harper <ligule@nature.berkeley.edu>)

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The MaizeGDB team is committed to providing easy access to maize data. We now offer outreach visits where an expert curator will come to your institution, present a general tutorial, then answer specific questions and create specific work flows one to one or in small groups. We are budgeted for three outreach visits in 2008. Presentations will be customized to suit your needs. In this poster, we present example usage cases for using MaizeGDB to help you find mutants that look like yours, estimate the cytological position of your gene or the genetic position of your cytological marker, and walking to genes. These examples are similar to what can be presented at your institution. To request an outreach visit, email us at: ligule@nature.berkeley.edu.

P34

GRASSIUS: A blueprint for comparative regulatory genomics across the grasses

(submitted by Erich Grotewold <grotewold.1@osu.edu>)

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An emerging premise in biology is the identification of the regulatory networks in which transcription factors (TFs) participate to specify the temporal and spatial expression of all genes in an organism. This fact underscores that it is vital to establish the architecture of plant promoters, cis-elements, TFs and their direct targets to understand the functionalities of the motifs that compose the network. The increasing amount and availability of genomic data from maize and other grasses has provided the necessity for integrated, comparative regulatory genomics-based resources and tools that contribute to defining the building block of the regulatory network. We provide here an update on the development of GRASSIUS, Grass Regulatory Information Server (<http://grassius.org/>), which integrates data from experimental results, literature and other publicly available repositories and resources with capabilities to visualize and annotate gene expression information. GRASSIUS houses three databases, GRASSTFDB (Grass Transcription Factor Database), GRASSPROMDB (Grass Promoter Database) and GRASSREGNET (Grass Regulatory Network Database). GRASSTFDB provides a collection of TFs from maize, sugarcane, and rice. Other grasses will be included as sequence information becomes available. GRASSPROMDB is designed to provide a collection of sequences for promoters from maize, sugarcane, sorghum and rice genes. In these promoters, predicted and experimentally verified cis-regulatory elements, presumably recognized by TFs, are indicated. GRASSREGNET will provide a dynamic relationship between the contents of GRASSTFDB and GRASSPROMDB in the light of experimentally verified interactions, helping visualize spatio-temporal gene regulation. GRASSIUS is expected to significantly benefit from community input, for example through voluntary curation contributions (Supported by NSF DBI-0701405).

P35

Identifying active transposable elements candidates: faster and simpler

(submitted by Yujun Han <yhan@plantbio.uga.edu>)

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The vast majority of transposable elements (TEs) in the genomes of multicellular organisms are inactive. Because active TEs are a valuable resource for functional genomics and evolutionary studies, a major goal of our lab has been to use computer-assisted methods to distinguish the rare active TEs from the inactive background. To this end we have developed three different bioinformatic approaches to identify active candidates: Identical DNA finder (IDF), Identical Transposase Finder (ITF) and Empty-Site Finder (ESF). IDF is similar to the program RECON that identifies repeats by self-comparison of whole genomes. IDF is simpler and faster than RECON because it only identifies repeats that have highly similar copies. More importantly, IDF is completely automated - from searching for identical repeats to determining complete elements. As such, IDF is a powerful tool to identify young de novo elements. ITF searches nearly identical amino acid sequences of transposases. First, it performs tblastn searches with known transposase sequences as queries, then extracts the copies from the blast output, joins the disrupted hits, eliminates introns, performs multiple-alignment and generates a phylogenetic tree. Young autonomous TEs are easily visualized by their short branches. Unlike IDF and ITF, ESF identifies polymorphisms generated by transposition events in otherwise identical genomic sequence. ESF then mines the unaligned regions (empty site) for TEs. Comparisons can be carried out in one of two modes, inter-subspecies comparison (ESC) or intra-subspecies comparison (ASC). Obviously, candidates from ASC will be more likely to be (recently) active. Using these approaches, we detected active TE candidates in rice and maize genomic sequence, together with several previously identified active TEs including mPing, Tos17 and Osmar5. Experimental testing of these candidates is underway.

P36

Maize Microarray Platform Translator, a web-based tool at PLEXdb to enhance capabilities for meta-analysis of gene expression profiling data

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

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PLEXdb (Plant Expression Database, <http://www.plexdb.org>) is a public resource that enables access to and analysis of gene expression data for plants and plant pathogens. Maize currently has several publicly available gene expression profiling platforms. In order to facilitate meta-analyses among these platforms and across taxa, we have developed a Microarray Platform Translator, a web-based tool that allows a user to input a genelist from one platform and receive the corresponding gene list for any of the other platforms. For genes on such a list that exist in the query platform but not in the subject platform, a message of "no match found" is returned. The connections between platforms are made via blastn of the platforms' design sequences to the current TIGR Transcript Assembly (TA). We will update the blast results as the TA is updated and the genespace is predicted, which will allow more matches to be found. The e-value criterion and selection of top hits to be included can be controlled by the user, allowing stringent or relaxed translations to be made. This feature is more important for translation across species, which has been implemented for the grass family in our most recent version. These tools will promote maximal use of future and existing data within our community by expanding our capabilities to develop and test hypotheses in silico.

P37

Maize allelic diversity project

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Of the estimated 250-300 races of maize, only 24 races are represented in materials utilized by the Germplasm Enhancement of Maize (GEM) project, a collaborative effort between USDA-ARS and public and private sector research scientists. This is largely a result of poor performance of many races in temperate environments and the absence of some germplasm from the LAMP project since LAMP accessions were selected based on agronomic performance. This was done of out necessity to reduce the number of accessions to manageable numbers and to eliminate poor-performing germplasm early in the process. However, to fully exploit the available genetic diversity in maize the acquisition and sampling of other exotic sources is required. The development of a set of inbred lines with a common recurrent parent and incorporating as many of the races of maize as possible would be a powerful tool for maize researchers to study maize allelic diversity. Such a set of lines would allow for greater use of the germplasm resources held in trust by public institutions. It would allow researchers to evaluate germplasm for traits of interest from different mega-environments (e.g., highland, tropical) in a single environment and genetic background and perform association mapping, which has been promoted as a powerful tool for mapping genes underlying traits of interest. The use of backcrosses to a publicly available inbred line is necessary to produce germplasm with adaptation to the target environment; while some exotic accessions can be evaluated per se in the Midwest, most need to undergo backcrossing and mild selection beforehand. While the set of lines to be produced is potentially large (5-7 per race, ~1500 lines total), researchers can use evaluations conducted on accessions per se in the mega-environments of origin to select lines derived from accessions that are more likely to contain traits of interest.

P38

Maize trained TWINSCAN and ab initio gene finding in maize

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Maize genome sequence is the knowledge infrastructure for the next generation of plant molecular genetics and crop improvement, and will provide the foundation for improving maize and other cereal crops. A large scale effort to sequence the maize genome is underway, and high throughput computational tools that can accurately identify genes within maize genomic sequence are absolutely necessary to annotate and understand the maize genome. A significant improvement in gene prediction accuracy has come from dual-genome prediction programs, such as TWINSCAN, which integrate traditional probability models like those underlying GENSCAN and FGENESH with information from the alignments between two genomes. The essential idea is that functional sequences, such as protein coding regions and splice sites, show different patterns of evolutionary conservation than sequences under little selective pressure, such as the central regions of introns.

Here we describe an NSF funded project to improve gene prediction in maize by identifying a comprehensive "training set" of complete and annotated maize gene models; and, using these to optimize TWINSCAN to accurately identify maize genes in un-annotated maize genome sequence. The training set will consist of EST validated maize gene models collected from both public and proprietary sources. During the course of this project maize trained TWINSCAN will be thoroughly benchmarked and used to re-annotate available public maize genomic sequence. Maize trained TWINSCAN will be made publicly available through the open-source software agreement.

P39

MaizeGDB as chromosome walking companion

(submitted by Mary Schaeffer (Polacco) <Mary.Schaeffer@ars.usda.gov>)

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MaizeGDB, www.maizegdb.org is the primary repository for genetic and cytogenetic maps, and contains many details about chromosome markers, genes, QTL, phenotypic variations and sequences. It links these data to various external resources: GenBank; the EST and GSS contigs at PlantGDB, www.plantgdb.org and Dana Farber (previously at TIGR), compbio.dfci.harvard.edu; the FPC BAC contig assemblies at Arizona www.genome.arizona.edu and their sequenced counterpart at the Maize Sequencing Project, www.maizesequence.org; and the SNP allele alignments at Panzea, www.panzea.org. The focus of this poster is usage examples, targeting groups interested in chromosome walking to sequences encoding a gene defined by a phenotype of interest and groups that use reverse genetics to discover function. The examples use the current tools, although we are working to add a sequence-based genome browser shortly after the B73 sequencing project is completed this year (2008; see Taner et. al, this meeting) In addition we summarize new genetic and cytogenetic map data added in 2007 which includes the first new generation SNP maps from the Maize Diversity Project; additional INDEL mappings from Pat Schnable; data from the TILLING Project; and RNAi resources and data from the Maize Chromatin project. Assistance in using MaizeGDB is available by on-line tutorials, or direct contact. Email queries are typically answered within 24 hours. In addition, researchers may request a MaizeGDB site visit for more extensive assistance; we currently schedule 3 visits/year from an expert curator for this purpose (see Harper et. al, this meeting). Funded by the USDA ARS.

P40

MaizeGDB's new genome browser project

(submitted by Taner Sen <Taner.Sen@ars.usda.gov>)

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MaizeGDB (<http://www.maizegdb.org>) is the community database for maize genetics and genomics. Based upon the 2006 MaizeGDB Working Group Report (available at http://www.maizegdb.org/working_group.php) and the Allerton Report (<http://www.maizegdb.org/AllertonReport.doc>), it has become evident that the focus of MaizeGDB must be shifted to better accommodate a sequence-centric paradigm. In order to (1) show how the data at MaizeGDB relate to the maize genome, (2) relate MaizeGDB's current sequence data with other sequence information as it becomes available, (3) become the keeper of maize's "official" set of gene models (which will enforce proper nomenclature), and (4) create a way to compare the various assemblies and annotations simultaneously, the feasibility of implementing of a genome browser at MaizeGDB was investigated and various available genome browser software were evaluated. Because the maize community communicates well, has a clear vision of their research problems, and has good ideas on how best visualize a sequenced maize genome, we prepared a survey to gauge cooperators' impressions of existing software and to find out what sorts of functionalities they would like to have in the MaizeGDB Genome Browser. Here we present the survey results of that survey as well as the rationale for why we chose GBrowse as MaizeGDB's Genome Browser software, and the roadmap for implementation.

P41

Structural annotation of maize genes: training and tuning of the Eugene combiner

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Maize (*Zea mays*) genome sequencing projects provide the community with large amount of genomic sequences. One of the next steps is to predict the gene content of these sequences as reliably as possible. To this end, several softwares are available and among them the Eugene package. Like most of other similar software, Eugene needs to be trained with gene sets which are structurally well characterized. It is advised that a set should include at least a few hundred genes to roughly represent a genome.

The first goal of this project was to build such a gene set for maize. Genomic maize sequences and full length cDNA sequences from maize and rice were retrieved from public databases and aligned with SIM4, GenomeThreader or Blat. Structural and functional annotations of each gene are manually curated. The annotated genomic DNA/cDNA cognates are used to train the Eugene software.

A major limitation of this approach is the availability of cognate genomic, cDNA and protein sequences for maize. The maize high quality training set contains 251 DNA/cDNA cognate sequences.

The preliminary results (Exons : 71.1% sensitivity, 64.2% specificity) are encouraging. Comparative genomic approach using rice genome should improve these results.

The maize-tuned version of Eugene will be available to the community shortly. This work is funded by Genoplante.

P42

Toward a better understanding of cereal genome evolution through Ensembl Compara

(submitted by Apurva Narechania <apurva@cshl.edu>)

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The maize genome has been largely shaped by its history of tetraploidization, subsequent rearrangement and duplicate gene loss. Disruption of synteny has also resulted from apparent gene movement in both maize and sorghum relative to rice. Many questions remain concerning the evolution of cereals, including the extent of lineage-specific rearrangements, selective forces that dictated the retention of duplicate genes, and the extent of conserved non-coding regions. The availability of three nearly complete cereal genomes (maize, rice and sorghum) provides an unprecedented opportunity to use comparative genomics to answer these and other questions in the evolution of plant genomes. As part of the Maize Genome Sequencing Project, we describe the use of the Ensembl Compara whole genome alignment pipeline to construct sequence-based syntenies. The pipeline automates pairwise whole genome analysis by parallelizing the construction of blastz alignments, their subsequent consolidation into chains and nets, and their coalescence into syntenic regions. The algorithms employed identify highly similar regions between two large sequences while allowing for segments without similarity, thus highlighting gene movement or genomic rearrangement within syntenic blocks. The tetraploid nature of maize and its history of whole genome duplications suggest that much of its genome should have at least two blocks that align to the same region of rice. Preliminary analysis using a pilot 22 megabase maize assembly spanning maize chromosome 4 exhibits synteny to a comparably sized region on rice chromosome 2. In agreement with marker-based syntenic studies, we show that this rice chromosome has a duplicate homologue on maize chromosome 5. We address the challenges of applying this pipeline to the maize genome in its partially assembled state.

P43

Using association rules in the QTL mapping of complex quantitative traits

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Currently in the plant community, much of the research concerning complex quantitative traits deals with QTL mapping. Because most available software packages are seen as "black boxes" by most plant researchers that use them, outputting only signal strength values at each marker, we are developing an approach for QTL mapping using association rules that will provide additional information to aid the researcher in determining actual QTLs. The types of rules that will be informative for mapping are those that link a set of chromosomal regions to an expression score or a range of expression scores. Because of this linking, these rules should be useful in determining modes of inheritance as well as establishing QTL significance for sections of the genome flanked by markers. We believe this additional information will help to more accurately locate QTLs. Also, we have developed techniques using computer vision and image processing algorithms to objectively and consistently score the expression of quantitative traits, specifically those that are difficult to quantify and which are typically manually scored using a rubric. To study this, experiments were conducted on the quantitative trait of disease resistance to Southern Leaf Blight of maize. Disease severity scores were obtained manually in the field by subjective scoring based on a semi-quantitative 17-point scale as well as automatically using our computational approach by processing images of diseased leaves. Both sets of scores are then used for QTL mapping by an available software package that employs traditional QTL mapping techniques as well as by using our association rules approach. Comparisons of the results of these four mappings will be presented. JG is supported by NLM Grant # 2T15LM007089-16, and CRS by NSF Grant #DBI-0447794.

P44

What's new at MaizeSequence.org

(submitted by Shiran Pasternak <shiran@cshl.edu>)

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The Maize Genome Sequencing Project, a multi-institutional collaboration, is in its 3rd and final year of sequencing the maize genespace to a finished quality. An automated pipeline framework at CSHL provides ongoing analysis and visualization of the maize genome sequence. Maize sequences are periodically retrieved from GenBank, thoroughly annotated, and made immediately available for public access on <http://maizesequence.org>, a comprehensive genome browser based on Ensembl. Primary maize annotations include peptide-classified ab initio gene predictions, alignments to known cereal data sets, predicted protein domains, repeat features, and mined sequence-based motifs. New secondary and comparative annotations are being integrated into the framework, including cereal orthologues, synteny-builds driven by sequence whole-genome alignment to other cereal genomes, extended gene ontologies, evidence-based gene builds, and tRNA analysis. Year 2 of the project has witnessed four major upgrades to the browser, with a burgeoning growth in depth and breadth of analyzed maize sequences and related data sets, as well as extensive software enhancements. The browser now provides integrated physical and sequence maps for a more informed genome view, new graphical tracks such as quality scores and maize insertion lines, alternative methods and formats of data access, consisting of a comprehensive FTP site, region-based export functions, web services, RSS syndication, and open access to the framework's relational databases. It also provides greater integration with other online maize resources such as Gramene, MaizeGDB, and PlexDB. Major performance and visual improvements provide a smoother and more intuitive user experience. In Year 3 the framework will deliver several higher-level analyses, including comparative and phylogenetic analyses between maize and other cereal genomes, BioMart functionality, the incorporation of vast expression data, and enhanced usability. This work was funded by the NSF/DOE/USDA "Sequencing The Maize Genome" project (NSF #0527192).

P45

dsyCS and *segII*: unique mutants help understand homologous pairing

(submitted by Christopher Bozza <cgb25@cornell.edu>)

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Pairing of homologous chromosomes during prophase of meiosis is essential for accurate segregation of genetic material and successful gamete production. While other mechanisms of meiosis, e.g. recombination, are well explored, homologous pairing remains the least understood meiotic activity. We are studying two novel homologous pairing-defective mutants, *dsyCS* and *segII*, from the collection of meiotic mutants in maize. We examined the two mutants by fluorescent in situ hybridization (FiSH) for pairing at the 5S rRNA locus and found each to have unique pairing defects. The overall level of pairing in the two mutants is distinguishing, 40% in *segII* vs. 0% in *dsyCS*. This investigation also yielded information about the unique dynamics of pairing for each mutant, where *dsyCS* appears to delay pairing and *segII* enters non-homologous associations with a slight delay. These data suggest that univalent chromosomes observed both mutants at metaphase I are due to an absence of homologous pairing. To further understand each mutant's defect, we examined the initiation of meiotic recombination (formation of double strand breaks), telomere bouquet, and installation of a protein hypothesized to be involved in the homology search (RAD51). *dsyCS* presents chromosome bridges at anaphase I and a complete absence of pairing which indicate that it may interface between recombination and homologous pairing. *segII*'s 60% deficiency in pairing compared to both its 2% RAD51 installation and absence of chromosome bridges indicate that *segII* may act specifically on pairing activity. We are currently working on cloning *segII*. The phenotypes of the *dsyCS* and *segII* mutants are similar, although not identical. Along with *phsI*, *dsyCS* and *segII* form a trio of genes that are specifically impaired in the early steps of homologous pairing. Differences between *segII* and *dsyCS* provide clues to their potential functions within the network regulating pairing.

P46

A conserved role for bHLH transcription factors in maize light signaling

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We have characterized 21 members of the maize bHLH gene family that are orthologous to the PIFs, HFR1 and PIL1-like genes involved in light signaling in Arabidopsis. The mRNAs of subclasses of the family of 21 genes respond to red or far-red light in etiolated seedlings and to simulated canopy shade in de-etiolated plants. Yeast two-hybrid and co-immunoprecipitation studies have shown that at least one of these bHLHs interacts directly with the photoreceptor phytochrome B, demonstrating a conserved signaling mechanism across over 100 million years of evolution. Results will be presented on protein-protein interactions between bHLH proteins and maize phytochromes, and on the phenotypes of plants with mutations in at least one bHLH gene.

P47

A role for *pan1* in cell polarization during maize stomata development

(submitted by John Humphries <jhumphries@ucsd.edu>)

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Asymmetric cell divisions play a critical role in numerous processes in plant development. In maize, asymmetric divisions involving polarization of the mother cell prior to division are vital for the formation of the stomata. During stomata development, two subsidiary mother cells (SMCs) are recruited into the stomatal complex by a guard mother cell (GMC), and subsequently divide asymmetrically to each produce a subsidiary cell and an unspecialized pavement cell. A mutator screen has been carried out to identify genes which participate in epidermal cell patterning in the maize leaf, and a gene designated *pangloss1* (*pan1*) appearing to play a role in SMC division has been identified. Characterization of the *pan1* mutant phenotype has revealed altered actin localization and depolarization of the nucleus in the SMC, leading to subsidiary cell defects. The *pan1* gene has been cloned, and encodes a putative leucine-rich repeat receptor-like kinase. To obtain further information of the role of *pan1* in the polarization of maize SMCs, an analysis of PAN1 activity and expression patterns has been performed. Antibodies raised against the PAN1 protein demonstrate that PAN1 localizes to the region of contact between the GMC and SMC in the developing stomata, suggesting it may play a role in transmitting a signal to the SMC for correct polarization.

P48

Anatomical differences in the bundle sheath and mesophyll cells of maize seedlings across a leaf developmental gradient

(submitted by Janelle Jung <jkj4@cornell.edu>)

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Many C4 grasses, including maize, sorghum, miscanthus, and switchgrass are important agricultural crops and potential biofuel feedstocks. While C4 photosynthesis is thought to have independently evolved at least 50 times from a common C3-type ancestor, the vast majority of C4 plants share basic structural and functional traits, such as Kranz anatomy, photosynthetic specialization of the bundle sheath and mesophyll cells, and high plasmodesmatal density at the bundle sheath/mesophyll junction. Detailed analysis of anatomical and physiological differences between C3 and C4 plants is still lacking, as is an understanding of the endogenous and environmental factors that influence the development of C4-associated traits. As part of a larger project (<http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=0701736>) to compare the differences of C3 and C4 leaf development in rice, maize, and sorghum, we examined the vascular anatomy and plastid development of intermediate veins in maize across a developmental gradient as defined by the source/sink transition. Sections were taken from the leaf tip, directly above and below the source-sink boundary, as determined through C14 labeling assays, and leaf base of the third leaf of 9-day-old maize seedlings. Results indicate minimal development of bundle sheath plastids in the leaf base, as well as in both sections adjoining the source-sink boundary

P49

Genetic analysis of telomere length regulation

(submitted by Amber Brown <brown@bio.fsu.edu>)

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Telomeres are specialized nucleoprotein complexes at the ends of linear chromosomes. They have essential functions in genome stability, meiotic chromosome behavior, and solving the end-replication problem. Telomere length control is known to be important for eukaryotic genome stability. We are exploring the genetic control of telomere length in maize in order to (1) identify the genes that regulate telomere length and (2) examine the functional significance of telomere length regulation in plants. We are using QTL mapping combined with association mapping to achieve these goals. For the QTL mapping project, we are using the well-defined IBM population, which consists of 302 RILs, and more than 2,000 markers. The quantitative trait is average telomere length, measured in absolute base pairs or relative abundance. We are comparing several telomere length quantification assays including terminal repeat fragment (TRF) analysis via Southern blot hybridization, slot-blot hybridization, and quantitative FISH. The best combination of these methods will be used to obtain telomere length data to identify QTL and potential underlying candidate genes. For the association mapping study we are measuring telomere length in a diverse set of maize inbreds. Candidate genes will be initially examined by RT-PCR in lines from both the IBM population and association mapping panels in order to establish the relationship between candidate gene expression and telomere length. These studies take advantage of the vast allelic diversity available in maize and combine the power of QTL mapping with that of association mapping. This will allow us to identify genes involved in telomere length homeostasis and investigate the biological significance of telomere length variation in a model plant species.

P50

Identification and characterization of Mu-inserts in genes potentially affecting cell wall biosynthesis

(submitted by Brent O'Brien <bob2373@ufl.edu>)

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The maize cell wall is a composite of numerous carbohydrate-based compounds. Understanding genes that control how these components are synthesized and integrated will be invaluable to our efforts to better utilize maize for grain, fiber, and renewable energy. The transposon-mutagenic Uniform Mu population allows new mutations to be identified, localized to a specific gene, and studied in a uniform, inbred background. Here, we employ two approaches based the Uniform Mu population to investigate cell wall biosynthesis. First, plants with visible phenotypes were used to construct Mu-flank libraries for sequence-based identification of potentially causal Mu-inserts. In addition, a database of randomly-sequenced Mu-flanks was used to identify maize lines carrying mutations in genes potentially involved in cell-wall biosynthesis. Co-segregational analyses are being used to determine which lines produce a phenotype that could be associated with a Mu-insert in a cell-wall biosynthetic gene. Thus far we have identified heterozygous lines that each carry a different cellulose synthase (CESA) gene, and segregate for severe kernel (embryo) phenotypes. In addition, we have developed homozygous mutant lines for a CESA, a polygalacturonase, a -galactosidase, a glucose-4-6-dehydratase, and an arabinogalactan protein coding gene. We have also identified mutant genes that are present only in heterozygous form, suggesting lethal homozygotes. These genes include a pentatricopeptide, a heme oxygenase, a cytochrome-c-synthesis protein, a putative coated-vesicle membrane protein, and an arabinogalactan protein. These have been, or are being tested for phenotypic associations with knockout genes under field conditions, and more thorough appraisals are in progress. It is especially interesting that some putative cell wall mutations also show abnormal kernel phenotypes. Understanding which cell wall genes affect seed development will greatly contribute to work aimed at enhancing kernel quality features and/or use of other tissues as sources of bio-energy.

P51

Identification and characterization of maize inner kinetochore protein MIS12

(submitted by Xuexian Li <xli@plantbio.uga.edu>)

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Centromeres are found at the primary constrictions where kinetochores assemble and microtubules attach. A collection of foundation proteins, including CENH3, CENP-C make up an inner kinetochore domain that directly associates with centromere DNA. MIS12 is a critical protein that is required for maintaining kinetochore structure, biorientation of kinetochores, and proper chromosome segregation. In yeast and animals, MIS12 depletion results in chromosome missegregation and loss of CENP-C. Here we identified maize Mis12 as duplicated genes in maize. Polyclonal antibodies against two MIS12 isoforms reveal that both are present on kinetochores throughout the mitotic and meiotic cell cycles. Extended fiber assays show that MIS12 closely associates with CENH3 and CENPC on chromatin fibers. Cross-linked chromatin immunoprecipitation shows MIS12s interacts with centromeric DNA. Further, two mis12 RNAi mutants were generated to better understand its function. Preliminary data suggests that mis12 knockdown leads to chromosome segregation defects. Our work reveals that maize MIS12 is a member of the centromere-binding inner kinetochore complex, and has essential roles in proper chromosome segregation.

P52

Mutations in nuclear genes alter post-transcriptional regulation of mitochondrial genes

(submitted by Terry L. Kamps <kampsuf1@yahoo.com>)

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Nuclear gene products are required for the expression of mitochondrial genes and elaboration of functional mitochondrial protein complexes. To better understand the roles of these nuclear genes, we exploited the mitochondrial encoded S-type of cytoplasmic male sterility (CMS-S) and developed a novel collection of nuclear mutations affecting mitochondrial functions in maize. These mutations gametophytically restore fertility function to the pollen of CMS-S plants. Although the restorer mutations rescue CMS-S pollen, many are homozygous lethal for maize kernel development and might, therefore, disrupt essential mitochondrial functions. For our nine molecularly characterized CMS-S restorers, genetic complementation testing indicates allelism between only a spontaneous *rfl2* mutant and one recovered from a transposon tagging population. We have profiled the transcripts and protein products of mitochondrial genes in developmentally staged pollen from these CMS-S restorer lines and an isogenic MO17 line having normal cytoplasm with no restorer genes. The profiles revealed that five restorer mutations conditioned global loss of mitochondrial gene products through post-transcriptional mechanisms. These mutants must therefore control mitochondrial protein accumulation via other post-transcriptional processes. We further tested this mechanism by examining RNA editing, a post-transcriptional feature of plant mitochondrial gene expression. The editing pattern of the maize mitochondrial *atp6* gene was first determined by sequence analysis of *atp6* RT-PCR products from microspores of non-restorer carrying normal and CMS-S cytoplasm isogenic lines. A high frequency of RNA editing caused codon changes, as well as the generation of several new restriction enzyme recognition sites. Restriction enzyme analysis of the *atp6* RT-PCR products demonstrated that RNA editing generated the three sequencing predicted cleaved amplified polymorphism (CAPs) sites in the developing pollen of all our CMS-S restorers.

P53

Pollen development in male-fertile and S male-sterile maize

(submitted by Christine Chase <ctdc@ifas.ufl.edu>)

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Cytoplasmically inherited male sterility (CMS), the maternally inherited failure to produce functional pollen, results from mutations that condition novel mitochondrial signaling pathways, culminating in the degeneration or homeotic transformation of male reproductive organs. In CMS-S maize, developing pollen collapses shortly after the microspore mitosis by a process displaying features of apoptotic programmed cell death. To better understand the mechanisms of pollen collapse, the cytology and molecular biology of pollen development were investigated in CMS-S and male-fertile (normal) maize. The two genotypes had similar cytological features through the late microspore stage. Following the microspore mitosis, the nuclei of normal bi-cellular pollen contained condensed, central chromatin, whereas the nuclei of CMS-S bi-cellular pollen contained fragmented chromatin at the periphery of the nuclear envelope. DNA sequence analysis of PCR and reverse transcriptase (RT) PCR products from normal and CMS-S ears and microspores uniquely associated the mitochondrial open reading frame *orf17* with pollen collapse. Although *orf17* predicts a hydrophobic peptide related to ATP synthase subunit 9, bioenergetic differences did not account for CMS-S pollen collapse. Mitochondrial respiratory complex subunits were absent from both CMS-S and normal microspores, and ATP levels did not differ between the two genotypes at the microspore or early bi-cellular pollen stage. Respiratory complexes re-accumulated only during later stages of normal pollen development. Accumulation patterns of the electron carrier cytochrome *c* and antioxidant Mn superoxide dismutase support models of mitochondrial cell death signaling mediated by ORF17 or by ORF17 in combination with mitochondria-produced reactive oxygen species in CMS-S pollen collapse.

P54

The Miniature-1 (Mn1) gene product, cell wall invertase-2 (INCW2), is associated with wall-in-growths (WIGs) in basal endosperm transfer cells (BETCs) in developing seeds of maize

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Cell wall invertases (CWI) are ionically bound to the cell wall in plant cells. A major CWI, INCW2, encoded by the Mn1 gene, provides the gateway to sucrose metabolism in developing maize seeds as it is entirely and exclusively localized to the BETCs that juxtapose the pedicel. The loss of INCW2 protein is the causal basis of the mn1 seed phenotype with a loss of > 70% seed weight at maturity. A ubiquitous feature of all transfer cells is labyrinth wall, the WIGs, that increases the plasma membrane area a feature believed to confer greater solute transport capacity to these cells. To better understand the roles of the INCW2, the WIGs and various cellular details of transport functions in the BETCs, 12 DAP Mn1 and mn1 kernels were examined by confocal, microscopy SEM and TEM approaches with the following noteworthy observations:

Direct fluorescence by Alexafluor 488, a marker for intracellular membranes, showed that WIGs were unique to the BETCs, and no major differences were seen in the architecture of WIGs in the two genotypes. Indirect immuno-fluorescence using the same dye conjugated to a secondary antibody and maize INCW2 as the primary showed, as expected, a positive signal in the Mn1 but not in mn1 kernels. More importantly, the signal was intensely localized to WIGs inside the BETCs and very little or no immuno-signal was seen on the primary cell wall. Confocal imaging of the Mn1 RNA after in situ hybridization and backscatter SEM imaging of INCW2 immuno-gold particles confirmed the immuno-fluorescence experiment results. Localization of the INCW2 protein in high-pressure frozen samples by immuno-electron microscopy is in progress. Possible significance of the collective data in sugar transport and a potential role of INCW2 in metabolic signaling in seed development will be discussed.

P55

xChIP combined with deep sequencing to reconcile the functional maize centromere with its DNA sequence

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Despite the precise, conserved function of every eukaryotic centromere, centromeric DNAs are widely divergent in sequence, size, and structure. However, a functional centromere absolutely requires the presence of a unique "centromeric chromatin" domain upon which the kinetochore attaches during cell division. Centromeric Histone H3 (CENH3), Centromeric Protein C (CENPC), and MIS12 comprise the highly conserved core of centromeric chromatin in nearly all species, yet their interactions with one another and their distributions along centromeric DNA are poorly understood. For example, do these three proteins interact as reinforcing repetitive units spread evenly over centromeric DNA, or do they exist as discreet domains that interact as an ultrastructure during cell division? We have developed a robust method for analyzing centromeric chromatin protein - DNA interactions using a crosslinked chromatin immunoprecipitation (xCHIP) method designed for maize. This method has allowed us the unprecedented opportunity to analyze protein-DNA complexes immunopurified by all three core centromere proteins. By independently deep sequencing the DNA precipitated with antibodies against ZmCENH3, ZmCENPC, and ZmMIS12, we are mapping the functional components of the centromere onto its linear DNA sequence. This strategy will help us to understand the relationships among the core components of the functional centromere and with its rapidly evolving DNA.

P56

Dissecting the formation and function of the meiotic telomere bouquet using the *plural abnormalities of meiosis1 (pam1)* mutant of maize

(submitted by Moira Sheehan <mjs224@cornell.edu>)

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The meiotic telomere bouquet describes the attachment of telomeres to the nuclear envelope (NE) followed by their clustering at a single location on the NE, generally juxtaposed to the microtubule organizing region. The bouquet is formed at the end of leptotene, extends through zygotene and dissolves in early pachytene. Coincident with the bouquet are the processes of chromosome pairing, recombination and synapsis where the bouquet is thought to play a role. Although subject to debate, it has been proposed that the bouquet maintains meiotic progression and facilitates chromosome movement. The maize mutant *plural abnormalities of meiosis1 (pam1)* is the best characterized telomere bouquet mutant in higher eukaryotes. In *pam1* mutant meiocytes, telomeres attach to the nuclear envelope but fail to cluster. The failure to form a bouquet causes asynchrony starting at zygotene that persists to the pollen stage, producing multiple-stage overlaps, and variably sized pollen and microspores. We are using the *pam1* mutant to address key questions about the function of telomere clustering and its impact on meiotic chromosome dynamics in maize. After crossing the *pam1* mutation into several inbred backgrounds, we observed that the *pam1* mutants in late flowering maize inbreds show a less severe phenotype than *pam1* in the early flowering inbreds. We are now examining the relationship between inbred background and duration of meiosis. To observe whether *pam1* mutants have altered chromosome dynamics, we are establishing a living observation system using multiphoton excitation (MPE) microscopy, which will allow us to examine nuclear dynamics in meiocytes in intact cultured anthers. We are also pursuing cloning the *pam1* gene. Because the *pam1* mutation was generated by NMS, and is likely a point mutation, positional cloning is the only option to identify the gene. Currently, we have mapped *pam1* to the middle portion of bin 1.05.

P57

Homologous synapsis revealed by ultrahigh resolution structured illumination (SI) microscopy

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Homologous chromosome pairing, recombination, and synapsis occur during meiotic prophase and are essential for the reductional division required to generate haploid gametes. During leptotene, each chromosome develops a linear proteinaceous structure called an axial element (AE). Around the same time, the homology search is initiated by the formation of double strand breaks. In zygotene, the homologues synapse via the polymerization of a central element between the two homologous AEs, forming the synaptonemal complex (SC). During pachytene, synapsis and recombination are completed. These events are well known cytologically, and have been explored with light microscopy and EM. However, little is known about the kinetics of synapsis and changes in the organization of chromosome axes during synapsis. Structured illumination (SI) is a method of ultrahigh resolution light microscopy developed by John Sedat at UCSF. It overcomes the 250 nm limit of resolution of conventional light microscopy, and it has a resolution less than 100 nm in the xz and z axes. We used SI to explore the organization of axial elements during synapsis by monitoring the distribution of two axial element antibodies, AFD1 and HOP1. The staining pattern of AFD1 on the two lateral elements is bilaterally symmetrical. The two lateral-element strands twist around each other and have a left handed helical pitch mostly along chromosomes. The pitch increases as chromosomes pair. About 80% of the unsynapsed regions in late-zygotene are associated with interlocks, implying that the resolution of interlocks between chromosomes may be a rate limiting step to complete synapsis. In addition, those unsynapsed regions are very different in length, suggesting that the lateral element and chromosome itself are dynamic structures and that pre-synapsis adjustment in length occurs during synapsis.

P58

Increasing the copy number of minichromosomes derived from the B chromosome

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Supernumerary B chromosomes possess an array of useful properties for genetic engineering, such as being basically inert and exhibiting an accumulation mechanism consisting of nondisjunction at the second pollen mitosis followed by preferential fertilization of the embryo by the B containing sperm. Minichromosomes, the product of telomere truncation of a maize B chromosome, have lost the nondisjunction property, because the tip of the B long arm required for this function is lost. However, this property can be restored to the minichromosome in the presence of a normal B chromosome. Using FISH, we have initiated a program of minichromosome accumulation. We are testing the accumulation limits of different sizes of minichromosomes with and without transgenes. With a GUS gene incorporated in the telomere truncation construct, we will be able to determine whether there is an onset of silencing at high copy number by analyzing the number of minichromosomes present in a plant. If silencing occurs, we will attempt to lower the number of minichromosomes to elicit re-expression. Minichromosomes will also be used to test the biological limits of chromosome number in plant cells. In maize, about 15 B chromosomes can be accumulated without affecting vigor, which could be due to dosage effects of the B on A chromosomes or large increases in chromatin that does not contribute to cellular constituents. Minichromosomes have significantly less chromatin than a normal B chromosome, so saturating the spindle or metaphase plate may be a more significant issue, which may allow even greater numbers of minichromosomes. We present initial results of accumulating minichromosomes with this procedure.

P59

Investigation of the mitochondrial DNA insertion site on maize chromosome 9L

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Mitochondrial DNA (mtDNA) insertion sites have been identified in the nuclear chromosomes of many eukaryotic species. Our lab has located mtDNA insertion sites in 10 maize inbred lines using fluorescence in situ hybridization (FISH). The 570 kb maize NB mitochondrial genome was divided into 20 overlapping pieces (in cosmids). The cosmids were used as FISH probes and hybridized to maize metaphase root tip chromosomes. One site on chromosome 9L in B73 hybridized to a majority of the NB mitochondrial genome. This site was observed in eight other inbred lines examined but not with the same intensity, suggesting that the mtDNA insertion site in B73 is recent. This mtDNA insertion site was investigated in detail on B37, Mo17, and M825 lines using individual cosmid-containing mtDNA FISH probes. B37 was analyzed because it is closely related to B73. Mo17 was chosen because it is often bred with B73 to make hybrids with heterosis. M825 is a sweet corn line unrelated to B73 that was recently found to contain a mtDNA insertion site on chromosome 9L with a similar hybridization strength to B73. A 19 cosmid-mix FISH probe was also used on B73, B37, Mo17, and M825 to look at overall mtDNA insertion site similarity. The B37, Mo17, and M825 karyotypes using individual cosmid probes and the 19 cosmid-mix probe were compared to the B73 karyotypes to examine the evolution of this mtDNA insertion site.

P60

Maize meiosis and meiotic genes

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Meiosis is the specialized cell division required to produce gametes with a haploid chromosome content in all eukaryotes with a sexual cycle. For a reductional segregation of chromosomes to occur, one round of chromosome replication is followed by two cell divisions. To ensure separation of homologs, before the first division homologous chromosomes pair and synapse and undergo homologous recombination to form chiasmata, regulation of sister chromatid cohesion is altered so cohesion of sister centromeres is maintained until the second division, and sister centromeres become monorientated. A suite of specialized meiotic genes are required to provide for proper reductional segregation.

Powerful forward genetics screens have led to the identification of a large number of maize meiotic mutants, representing over 50 mutants and at least 35 genes, although only a few of them have been cloned. Here we present the mutant collection. Their phenotypes are classified for defects in key meiotic events and characteristic cytological features for several maize meiotic genes including *afd1*, *sgo1*, *dys2*, *mtm10*, and *mtm25*, are presented.

P61

Maize nonhomologous chromosome segments likely interact throughout the genome in a dosage sensitive fashion to affect plant development

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We have used maize B-A-A translocations to simultaneously vary the dosage of two nonhomologous A chromosome segments. We tested 48 B-A-A chromosome stocks (each with a unique combination of chromosome segments) and have identified 23 B-A-A chromosomes that affected plant development when present in an extra copy in the genome. Measurements were made on hyperploid (containing an extra copy of the two chromosome segments) and euploid plants. In most cases the plants with the additional copy of the two chromosome segments (hyperploid) were significantly reduced in height as compared with their euploid counterparts. Leaf length generally paralleled the effect on plant height, while leaf width was increased in plants hyperploid for some B-A-A chromosomes and decreased in others. In several cases, ear height, internode length, stalk circumference, and tassel branch number increased or decreased significantly with the presence of an extra copy of the chromosome segments. Because these observations reveal much stronger effects than those seen when simple B-A chromosome hyperploid plants are compared with their euploid counterparts, these results suggest that the two chromosome segments present in the B-A-A chromosomes exhibiting these effects are interacting in a dosage-sensitive fashion with each other and with their counterparts on the normal chromosomes bearing these segments. Furthermore, because 10 of the 20 chromosome arms are represented in this non-exhaustive sample, it appears likely that there are regions on all of the chromosomes that interact with (talk to) regions on other chromosome arms, as well as on their own chromosome arm, in a dosage sensitive fashion. It is unlikely that these interactions could be readily detected without the use of B-A-A translocations to simultaneously vary the dosage of two nonhomologous chromosome segments. This research was supported by the NSF Plant Genome Research Program.

P62

Primed in situ labeling (PRINS) of maize somatic chromosomes

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Primed in situ labeling (PRINS) is a technique for the physical mapping of sequences on chromosomes in addition to FISH. As an advantage, PRINS can discriminate closely related sequences including subtypes of repeats; it also can be used for fast, large scale screening of chromosomal rearrangements that are undetectable by PCR or FISH. PRINS is based on the hybridization of a short unlabeled oligonucleotide to a denatured chromosomal DNA and synthesis of complement DNA catalyzed by a Taq polymerase in the presence of labeled nucleotides. Depending on the type of modified nucleotide used, labeled chromosomal sites can be detected by means of a specific fluorescent antibody (indirect PRINS) or directly visualized using fluorescence microscopy (direct PRINS). The polymerase chain reaction can be used to amplify the sequence that has undergone primed synthesis on the chromosome (cycling PRINS), which results in an increase of signal intensity. In human chromosomes, PRINS has been used to identify repetitive sequences, allelic differences and subtle chromosomal rearrangements in tumor cells, as well as for screening of mutations and karyotyping. PRINS has been successfully used in some plant species mostly for repetitive DNA labeling of microsatellites, telomeres and ribosomal genes but has not yet reached FISH sensitivity in detection of single copy sequences. We designed oligonucleotide primers and used direct and indirect PRINS and C-PRINS to locate sequences on maize metaphase mitotic chromosomes and to differentiate closely related sequences.

P63

Reactivation of inactive centromeres in maize

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In maize, a high frequency of stable dicentric chromosomes occurs by inactivation of one centromere under the appropriate circumstances. The inactive centromere together with a functional centromere can be transmitted from one generation to the next. Centromere inactivation is an epigenetic process, so the question arises: can it be reversed? A new dicentric chromosome, referred as #15 (Dic-15), containing one large B chromosome centromere (active) and another smaller B centromere (inactive), was recovered via recombination in plants containing one B-9Dp-9 and one centromere misdivision derivative, Telo 3-5, the source of the small centromere. In the plants containing one Dic-15, chromosome breakage was observed at anaphase I indicating that the small inactive centromere recovered its function. This reaction process is apparently specific to anaphase of meiosis I in which intrachromosomal recombination can occur because of a reverse duplication. In plants containing two Dic-15, chromosome bridges and new dicentric chromosomes occurred in anaphase I. Homologous chromosome pairing in the same orientation reduces the frequency of intrachromosome recombination and thus decreases the reactivation of inactive centromeres. Histone H3 phosphorylation at ser-10, CENH3 and CENPC antibodies were applied to this material and confirmed the respective inactivation and reactivation. These findings indicate an epigenetic component to centromere specification that is reversible.

P64

Towards the molecular cloning of meiotic telomere behavior mutants in maize

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Meiosis is the process by which sexually-reproducing organisms reduce their genomes from diploid (2n) to haploid (n) during the formation of gametes. Meiosis requires that homologous chromosomes pair, synapse, recombine, and finally segregate. These widely conserved processes are under genetic control yet the exact molecular mechanisms that regulate, carry out, and coordinate them remain elusive. The initial pairing and subsequent synapsis events are immediately preceded by the clustering of telomeres on the nuclear envelope in a widely conserved structure referred to as the telomere bouquet. We have investigated the functional significance of the telomere bouquet by analyzing genes that control its formation in maize pollen mother cells. The maize *desynaptic* (*dy*) and *desynaptic1* (*dysl*) mutations exhibit altered telomere behavior and chromosome pairing at mid-prophase-I of meiosis, resulting in either partial or complete male sterility (Bass *et al.*, 2003. *J Exp Bot* 54:39). We plan to further characterize the meiotic nuclear phenotype of these two mutants by immunolocalization analysis of the synaptonemal complex using an antibody raised against the Arabidopsis axial element protein ASY1. We are also working towards the genetic mapping and molecular cloning of the normal and mutant alleles of the *dy* and *dysl* genes of maize. Here we describe our positional cloning strategy using bulked-segregant analysis with an SSR mapping kit developed by the Maize Mapping Project and commercialized by Sigma (Maize SSR Primer Set, M4193). Map positions will be used to identify candidate genes for further testing and eventual molecular cloning of these genes. Isolation and analysis of *desynaptic* genes may contribute to a better understanding of telomere-mediated processes of meiosis.

P65

***sparse inflorescence 1* encodes an auxin biosynthesis gene which functions in axillary meristem initiation in the inflorescence**

(submitted by Solmaz Barazesh <sxb944@psu.edu>)

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The highly branched architecture of the maize inflorescence is produced by a series of axillary meristems. Phenotypic and genetic analysis of the barren inflorescence class of mutants of maize has provided insight into the role of the plant hormone auxin in the development of these structures. Previous work has shown that barren inflorescence2 (*bif2*) encodes a serine-threonine kinase that regulates auxin transport. Recent work on Barren inflorescence 1 (*Bif1*) suggests that this gene also functions in the regulation of auxin transport. Here, we introduce *sparse inflorescence1* (*spi1*). Mature *spi1* plants have a barren inflorescence phenotype with reduced numbers of tassel branches and spikelets in the tassel, and reduced numbers of kernels in the ear. Detailed analysis of immature *spi1* mutants using scanning electron microscopy and histology shows that this phenotype results from defective axillary meristem initiation during inflorescence development. In addition, *spi1* mutants fail to maintain the apical meristem, with spikelets growing over the tip of the inflorescence. Furthermore, *spi1* mutants produce fewer vascular bundles in the inflorescence stem than normal. *spi1* was cloned by chromosome walking, and encodes a YUCCA-like auxin biosynthesis gene, providing further evidence of the role of auxin in the development of the inflorescence. The phenotypic analysis of double mutants between *spi1* and other maize mutants provides new information on the pathways regulating inflorescence development. Of particular interest is the double mutant between *spi1* and *bif2*, which displays a synergistic interaction with severe vegetative and reproductive phenotypes. These results illustrate the importance of both auxin biosynthesis and transport in the regulation of organogenesis during vegetative and reproductive development.

P66

Analysis of meristem size regulation in maize

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The shoot apical meristem has the remarkable ability to regulate its size during development, by balancing stem cell proliferation with the incorporation of daughter cells into primordia. We are particularly interested in studying this process in maize, where a large number of meristem proliferation, or "fasciated" mutants are available. We cloned the FASCIATED EAR2 (FEA2) gene and found that it encodes a leucine rich repeat (LRR) receptor-like protein, orthologous to Arabidopsis CLAVATA2. We also isolated the thick tassel dwarf1 (td1) gene as a homolog of the LRR kinase CLV1. In Arabidopsis, these proteins form a receptor complex that is activated by the CLV3 ligand and represses the stem cell-promoting transcription factor WUSCHEL. Interaction of fea2 and td1 mutants suggests the simple CLV1-CLV2 co-receptor model is more complex in maize, since double mutants are much more severe than either single mutant alone. To address the molecular nature of these effects, we are characterizing the FEA2 receptor complex, using native FPLC and various immunoprecipitation approaches. We are currently using a transgenic 35S::FEA2-GFP line in combination with a commercial α -GFP antibody to isolate the FEA2 receptor complex. In addition to our biochemical approach, we are also pursuing a genetic approach to isolate the compact plant2 (ct2) mutation. Ct2 mutants develop abnormally enlarged inflorescence meristems, indicating that this gene also affects meristem size regulation. Using ~900 ct2 F2 mutants, we have narrowed down the chromosomal location of ct2 to an interval of 250kB on chromosome 1 covered by 2 BAC clones representing 8 genes. Subsequent sequencing of these genes in the ct2-Muszynski allele led to the identification of a Mutator-element insertion in a lipoxygenase-encoding gene, providing a possible link between jasmonate synthesis/signaling and meristem size regulation. To further substantiate this, we are pursuing a targeted EMS screen to isolate additional alleles. In addition, our analysis of td1/ct2 double mutants suggests that ct2 identifies a pathway for regulation of meristem size different than the CLAVATA signaling pathway.

P67

Analysis of molecular interactions during shoot-borne root formation in maize

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Plant roots serve important functions among which water and nutrient uptake and anchorage are the most important ones. The complex root system of maize consists of the embryonic primary root and seminal roots, and an extensive postembryonic, shoot-borne root system composed of crown- and brace-roots which make up the major backbone of the root system. Crown roots are formed at consecutive underground stem nodes, while brace roots are formed late in development from aboveground stem nodes. The monogenic recessive mutant *rtcs* (*rootless concerning the crown and lateral seminal roots*) completely lacks all shootborne roots including crown-, brace- and seminal roots. The mutant *rtcs* is affected at an early stage of root development before the initiation of the concerned roots (Hetz et al., 1996). The *rtcs* gene encodes a LOB domain protein (Taramino et al., 2007), which localizes to the nucleus. Gene expression and protein accumulation profiles of wild-type and *rtcs* coleoptilar nodes were compared and differentially accumulated proteins were identified (Sauer et al., 2006). Goal of this study is the analysis of the molecular networks involved in shoot-borne root formation in maize.

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P68

Auxins & pin1 proteins: Role during maize kernel development and membrane targeting analysis

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PIN proteins play a rate-limiting role in the catalysis of auxin efflux from cells, and their asymmetrical localization determines the direction of cell-to-cell auxin flow. The establishment of a PIN mediated apical-basal auxin gradient is fundamental for Arabidopsis embryo formation and polarity. Applications of auxin transport inhibitors, alteration of PIN polarity (*pid* mutant) and block of the exocytic step in the constitutive PIN cycling (*gnom* mutant) led to embryo abnormal polarity. To confirm the important role of PIN mediated auxin transport in monocots, we investigated on the behavior of ZmPIN1 proteins and auxin accumulation patterns during maize kernel development. Transcripts and proteins localization patterns were analyzed by *in situ* hybridization and immunolocalization assays during embryo and endosperm development. Moreover IAA accumulation sites were determined using an anti-IAA antibody. These different approaches let us to confirm that PIN1-mediated auxin transport and accumulation play a central role also during maize embryogenesis. In particular PIN1 proteins and auxin seem to be fundamental for protoderm differentiation, meristems definition, primordia initiation and vasculature differentiation. In addition a switch of PIN1 protein polarization from apical to basal membranes in scutellum pro-vascular cells correspond to the establishment of basipetal auxin fluxes towards the root pole. During endosperm differentiation PIN1 proteins were expressed in BETL and ESR and both these domains show high IAA levels. This auxin accumulation is the result of two different proteins expression patterns: while in transfer cells the proteins marked all the cell plasma-membrane without any evident polarization, in the ESR domain ZmPIN1 proteins exclusively localized in the endomembranes, suggesting a lack of auxin efflux from these cells. Analysis of cell-membrane targeting of ZmPIN1::GFP fusion proteins in heterologous systems let us hypothesize that the three ZmPIN1 proteins have different plasma-membrane insertion ability and may be subjected to different regulation pathways.

P69

Axillary meristem development of a variable penetrance maize mutant, grassy tillers1

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The ability to increase plant biomass is an ever-growing concern as the biofuel industry continues to grow. Lateral branch formation is one important method to increase biomass. Grassy tillers 1 (*gt1*) has been shown to play a role in lateral branch development in maize. The penetrance of *gt1* is highly dependent on genetic background. The goal of this study is to characterize the phenotype and expression pattern of *gt1* in backgrounds with varying penetrance, to better understand how it can be utilized in breeding programs to increase biomass production per unit of area. Isogenic lines, W64A, W64A*gt1/gt1*, W117Ht, W117Ht*gt1/gt1*, W182BN and W182BN*gt1/gt1* were evaluated with fluorescent microscope imaging to determine the timing of meristem activation in each background. Mature plants were evaluated to determine the level of penetrance of *gt1* in each genetic background. These isogenic lines were also evaluated using long oligonucleotide spotted arrays 48 hours prior to initial elongation of the lower axillary meristem. Differentially expressed genes unique to each genotype and common among all genotypes will be presented. Determining which genes are uniquely differentially expressed in backgrounds with varying levels of penetrance as well as genes that are always differentially expressed will provide insight into the genetic mechanisms controlling this gene.

P70

BIF2 interacts with bHLH transcription factors belonging to a monocot specific phylogenetic clade

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Axillary meristems are produced in the axils of leaves and give rise to the vegetative branches and inflorescence structures of plants. Therefore, the production and growth of axillary meristems determines the overall architecture of plants. In maize, several orders of axillary meristem develop resulting in highly branched tassels. Mutants that disrupt axillary meristem development exhibit a barren inflorescence phenotype with few branches and spikelets produced in the tassel. One barren mutant, barren inflorescence2 (*bif2*), is defective in a gene that encodes a serine/threonine protein kinase co-orthologous to PINOID which regulates auxin transport in Arabidopsis. Another mutant, barren stalk1 (*ba1*), is defective in a gene that encodes an atypical bHLH transcription factor belonging to a clade of monocot specific bHLH genes. Here we show that BIF2 and BA1 interact with each other and co-localize in the nucleus in planta. BIF2 also interacts with a second bHLH transcription factor similar to BA1. Moreover, these two bHLHs can homodimerize and heterodimerize with one another. We present evidence to support a model in which BIF2 plays multiple roles throughout organogenesis by directing polar auxin transport through PIN localization at the membrane as well as influencing axillary meristem initiation through interaction with transcription factor(s) in the nucleus.

P71

Cell fate acquisition and maintenance in maize anthers

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We have just started a joint project with Zac Cande and his lab to elucidate the genes and cellular processes responsible for cell fate setting and maintenance in maize anther locules. The Stanford component of the project currently focuses on transcriptome profiling and gene tagging and cloning and in working out methods for microdissection and analysis of individual cell types from maize anthers. Locules contain 5 cell types (epidermis, endothecium, middle layer, tapetum, meiotic cells) organized in concentric rings. Although cell division patterns are relatively stereotyped within the locule and lineage is sometimes invoked to explain cell fate acquisition, it is more likely that late cell-cell signaling is responsible. To initiate the studies transcriptome profiling experiments have been conducted with a number of male-sterile mutants with defects in cell fate setting: *msca1*, *ms23*, *mac1*, *ms8*, *ms32* (in progress), *am1* (in progress). Using directed Mu tagging multiple new alleles of *msca1*, *ms23*, *ms25*, *ms8*, and *mac1* have been obtained in large screens conducted at Cal Poly-SLO in collaboration with Matt Ritter and Jeff Wong; these screens are also a major investment in undergraduate research training. Traditional co-segregation analysis using DNA blot hybridization and new methods of inverse PCR to recover and sequence Mu ends are being used in attempts to clone these 5 genes efficiently. Populations were just established for summer 2008 tagging of *ms32* and 5 new cell fate mutants identified by the Cande lab in a 2007 cytological screen of *ms* lines obtained from the Coop. This research is supported by the NSF.

P72

Characterization and mapping of tassels replace upper ears1 in maize

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Plant architecture is determined in large part by the activity of axillary meristems. In maize these processes are regulated by networks of genes such as the ramosa pathway in the inflorescence and teosinte branched1 (tb1) in the vegetative shoot and by plant hormones. The tassels replace upper ears1 (tru1) gene regulates a sharp distinction between tassel and ear identity for the axillary branch in maize. We are characterizing the recessive allele tru*-99.507.2 from the maize stock center, presumably an allele of the previously reported tru1. In strong tru1 mutants, the branch that would form the upper ear is derepressed and transformed to a long branch tipped by a staminate inflorescence, giving it the appearance of a tassel. Similarly, the ramosa1 and ramosa2 mutants sometimes produce staminate spikelets in the ear. In tru1 mutants, successive axillary meristems are less derepressed. By contrast, tb1 mutants derepress all axillary branches. To clone tru1, we have begun a map-based approach. In our mapping population (1,556 individuals) we have localized the mutant allele to near the centromere on chromosome arm 3L, in an interval of 51 BACs between contig 126 and contig 128. To isolate additional tru1 alleles, we used EMS- and Mutator mutagenesis. In the EMS-M1, 12 plants showed a strong mutant phenotype and 8 plants a weak phenotype. No mutants were observed in 25,000 Mutator M1 plants. Double-mutant analyses for tru1 with ramosa, ts2, bif2 and kn1 mutants is underway to study potential interactions among these genes. Future work will include cloning and expression analysis of tru1, sequencing of different tru1 alleles and detailed analysis of the tru1 mutant phenotype, to eventually elucidate TRU1 function in regulating the fate of meristems and inflorescence development in maize.

P73

Characterization of the function of the RAMOSA3 gene in maize

(submitted by Namiko Satoh-Nagasawa <satoh@cshe.edu>)

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To elucidate the genetic and molecular mechanism of inflorescence branching, a major yield traits in crops, we are analyzing the ramosa3 (ra3) mutant of maize. By the observation of single and double mutant phenotypes, we found that RA3 functions upstream of another RA gene (RA1) to establish the correct identity and determinacy of axillary meristems in maize inflorescences. RA3 was cloned and found to encode a functional trehalose-6-phosphate phosphatase (TPP) (Nature441 (2006)). TPP is an enzyme which catalyzes the metabolic conversion of trehalose-6-phosphate (T6P) to trehalose. We also found that RA3 is expressed at the base of axillary meristems in young maize inflorescences.

From these studies, we developed two hypotheses for the molecular mechanism of RA3 function. First, T6P or trehalose may act as a mobile sugar signal during axillary meristem development in maize inflorescences. Second, RA3 protein itself may have a regulatory function to control the expression of other genes important for the determinacy of axillary meristems. To test which of these hypotheses are correct, we have started several experiments. Those include RA3 protein localization using fluorescent protein fusions and immunolocalization. The co-localization of RA3 protein and mRNA appears to rule out the possibility that it acts as a mobile protein signal. Transient bombardment assays of a RA3-YFP fusion protein showed that it was localized in cytosol and nuclei. Its nuclear localization supports the hypothesis that it may have a regulatory function. We also measured the concentration of trehalose and T6P in young inflorescences of wild-type and ra3mutants, and found no differences, suggesting the sugar signal hypothesis may not be correct. Several additional experiments using transgenic plants to regulate the expression of RA3 and of trehalase in developing inflorescences are also in progress, and these results will also be presented. Funding was provided by the NSF and USDA.

P74

Characterization of the maize mutant Developmental disaster1

(submitted by Kimberly Phillips <kap262@psu.edu>)

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Hormones regulate many different areas of growth and development in plants. Auxin is a particularly important plant hormone that is known to control such factors as shoot elongation, phyllotaxy, tropisms, and inflorescence development. A large number of mutations have been observed which affect the regulation and function of auxin. The semi-dominant maize mutant Developmental disaster1 (Dvd1) has been identified as a potential disruptor of auxin signaling. Dvd1 mutant plants exhibit numerous vegetative and reproductive developmental abnormalities including shortened internodes, flat and twisted stems, altered phyllotaxy, a barren inflorescence in the tassel, and reverse germ orientation in the ear. Dvd1 mutants have been studied in both B73 and Mo17 genetic backgrounds, and results show that mutants in Mo17 are more severely affected in all aspects of development with the exception of the tassel. Dvd1 heterozygotes typically resemble wild-type plants and show minor mutations such as reduced height and fewer tassel branches. Homozygous plants are much more severe, however, as they are often dwarf-like in stature and typically do not produce any ears or functional spikelets in the tassel. Quantitative analyses of Dvd1 phenotypes have been performed using data for plant height, leaf number, internode length, spikelet/tassel branch number, and ear/kernel number. In addition, histology and Scanning Electron Microscopy have been used to analyze developing tassel and ear meristems, and auxin transport assays have confirmed that auxin transport is reduced in Dvd1 mutants. Double mutant analyses were performed to further characterize the role of Developmental disaster1 in different maize signaling pathways, and have revealed that Dvd1 plays a key role in branch initiation and vegetative axillary meristem development. Finally, chromosome walking has been used in two mapping populations to narrow the location of Dvd1 to within one BAC contig on chromosome 5, and a candidate gene search is currently underway.

P75

Comparison of conventional, modified single seed descent, and double haploid breeding methods for maize inbred line development using GEM breeding crosses

(submitted by McDonald Jumbo <mjumbo@udel.edu>)

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Good choice of germplasm, breeding methods, and careful evaluation are essential for maize inbred line and hybrid development (Goodman, 1985). Breeding crosses from the Germplasm Enhancement of Maize (GEM) project are a good germplasm source for broadening and improving Corn Belt maize (www.public.iastate.edu/~usda-gem). Three GEM breeding crosses ANTIG01:N16DE4, AR16035:S0209, & DKXL212:S0943b, previously evaluated for general adaptability were utilized in this study. Four breeding methods, conventional mass (CM), conventional GEM (CG), modified single seed descent (MSSD) and double haploid (DH) were used. Using the CM and CG breeding methods, 160 and 250 S1 ears, respectively, were selected within the S0 generation from each of the three GEM breeding crosses in 2005 and advanced to the S2 stage in 2006. The MSSD method utilized a three kernel bulk from each of the selected 160 S1 ears from the CM method to advance to the S2 stage in the 2005 winter nursery. In 2006, 250 S2 lines from the MSSD method for each of the three GEM breeding crosses were grown for observation. Similarly, 229, 133, and 117 DH inbreds from ANTIG01:N16DE4, AR16035:S0209, and DKXL212:S0943b, respectively, were grown for observation. Fifty lines from each method were evaluated in yield trials using a reps within set design with the four methods nested within each set. Statistical analysis was done using SAS 9.1 version. Results showed more uniformity in the MSSD S2 lines derived from stiff-stalk perhaps due to greater genetic variability in non-stiff stalks. There was no significant breeding method effect for Y, Y/M, and M for the ANTIG01:N16DE4 breeding cross. The breeding method effect for the AR16035:S0209 breeding cross was highly significant ($P < 0.01$, $P < 0.001$) for Y, Y/M, and M. Similarly, the breeding method effect for the DKXL212:S0943b breeding cross was highly significant ($P < 0.001$) for Y/M, and M. The Y/M advantage of the DH method may reflect selection either during the DH process or per se evaluation of the DH families. DH lines are uniform and distinguishable. Ten S3 lines from each breeding method (CM, CG & MSSD) and 10 inbred lines from DH, for each population have been selected based on grain yield (Y) and yield/moisture (Y/Y) performance. This was a result of selecting one top performing hybrid per method from each set. In addition to the ten set winners, five additional lines per method were selected based on overall performance across the sets. These additional selections included both high performing families not previously selected as set winners and second ears selected within set winners for CM, CG and MSSD methods. The lines were placed in winter isolation blocks to produce testcrosses for further yield testing. Marker analyses will be utilized to determine the relative germplasm contribution of the exotic versus adapted germplasm for the four breeding methods.

P76

Differential gene expression of SBP-box genes in inflorescence development

(submitted by Judd Hultquist <judd.hultquist@mu.edu>)

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The transition from the vegetative to reproductive growth in plants is highly regulated by multiple inputs from various environmental and internal pathways to ensure proper timing and optimal conditions for successful reproduction. In *Zea mays*, this involves the differentiation of male and female tissues through selective abortion of either pistillate or staminate primordia respectively. This pathway is compromised in individuals homozygous for the recessive, nonfunctional *mediator of paramutation 1-1* mutation as evidenced by their display of a variety of abnormal phenotypes at the tassel including barrenization and feminization. It has been a focus of our lab to identify genes influenced by *mop1* in the sexual differentiation pathway by searching for differential gene expression of assorted candidate genes among the variant tassel phenotypes. We are currently studying numerous genes with high sequence similarity to the SPL (*SQUAMOSA Protein Like*) gene family in *Arabidopsis*. This family is characterized by the presence of their unique DNA-binding motif called an SBP-box (*SQUAMOSA promoter Binding Protein*). Extensive sequence mining has yielded a possible 36 SBP-box genes in maize. Based on the intron-exon structure and phylogenetic analysis of those genes with complete EST and genomic data, five subgroups were identified. Expression analyses were carried out for these genes using Real-Time RT-PCR on a number of immature tassel samples dissected from individuals of a family segregating for the *mop1-1* mutation. Individuals displaying a feminized tassel due to loss of *mop1* function exhibit upregulation of five Group II type genes: *tga1* (*teosinte glume architecture1*), *SBP5*, *SBP6*, *SBP7*, and *TC325927*. Expression analyses repeated on feminized tassel tissue derived from *tasselseed1* individuals yielded the same upregulation pattern. Current experiments are focusing on the expression of members of the miR156 family to which all of the above genes have a putative responsive element.

P77

Discolored1 (DSC1) function in maize kernel development

(submitted by Elizabeth Takacs <emt32@cornell.edu>)

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ADP-ribosylation factor GTPase activating proteins (ARF-GAPs) are a conserved group of proteins found in mammals, *Drosophila*, yeast, and plants. ARF-GAPs function in endomembrane trafficking and actin remodeling through the hydrolysis of the active GTP-bound ARF to the inactive GDP form. In maize, there are predicted to be at least 23 genes encoding ARF-GAPs based on the current sequence of the maize gene space. One of these genes that putatively encode an ARF-GAP is *discolored1* (*dsc1*). *dsc1* mutants were identified in a screen of defective kernel (*dek*) mutants as being brown, misshapen kernels. Detailed investigations of the *dsc1* mutant embryo and endosperm phenotypes reveal that *dsc1* mutant kernel development is severely delayed, albeit normal, up through 16 days after pollination (DAP). However, 20 DAP *dsc1* mutant embryos and endosperm tissues have begun to degenerate. Previously, partial sequence of the *dsc1* locus had been obtained through Mu transposon tagging and used in 3RACE RT-PCR to generate a full length cDNA. Genomic sequence in BAC AC197554 aligns to both the E1 cDNA sequence and the partial genomic sequence from the *dsc1* locus. Based on the full length *dsc1* cDNA sequence, the DSC1 protein is an 823 aa ACAP-type ARF-GAP with a putative orthologue in *Arabidopsis*, SFC/VAN3. SFC/VAN3 functions in endomembrane trafficking to transport PIN1 from the plasma membrane to an endosomal-like compartment. Thus, SFC/VAN3 is required for polar auxin transport and vascular patterning. Phenotypic analyses paired with the putative identification of DSC1 as an ARF-GAP suggests that DSC1 function in endomembrane trafficking could serve an important role in embryo and endosperm interactions. To better investigate this possibility, we will generate additional mutant alleles through reverse genetics, identify cargo trafficked in vesicles regulated by DSC1 function, inspect the role of DSC1 in auxin efflux, and execute complementation studies of *Arabidopsis* *sfc/van3* mutants.

P78

Elucidation of the cis and trans regulation of teosinte branched 1 (tb1)

(submitted by Anthony Studer <studer@wisc.edu>)

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The domestication of maize has resulted in striking morphological differences between maize and its wild progenitor, teosinte. These dramatic differences are partially controlled by the gene teosinte branched 1 (tb1). Our work is focused on studying regulatory elements that produce a two fold higher expression of tb1 in maize when compared to teosinte. tb1 is regulated by both a cis element upstream of tb1 on the long arm of chromosome 1 and a trans acting factor on the long arm of chromosome 3. The cis regulatory region is located ~58-69 kb upstream of tb1. We observed a large insertion in this region that is present in most teosinte but absent in maize. To identify whether this insertion is linked to the cis regulatory element, recombinants between maize and teosinte haplotypes in the region upstream of tb1 were generated. Recombination events were recovered that flank the insert which have allowed us to assess the affect of this insert on teosinte-like phenotypes. The trans acting factor maps to the domestication QTL on the long arm of chromosome 3 and causes an epistatic interaction with tb1 which affects inflorescence architecture. To investigate the epistatic interaction, three introgression lines were previously generated in a W22 background; one containing the teosinte long arm of chromosome 1 (T1L), one containing the teosinte long arm of chromosome 3 (T3L), and one containing both T1L and T3L. We are analyzing the expression profile of candidate genes on the long arm of chromosome 3 in each introgression line using quantitative PCR to pinpoint the trans acting factor and possibly aid in the positional cloning of the domestication QTL on chromosome 3. By studying the regulation of tb1 we hope to use the domestication of maize as a model to better understand the evolution of regulatory elements controlling complex traits.

P79

Exploring the mechanisms underlying the critical period for weed control in *Zea mays* (L.)

(submitted by Eric Page <epage@uoguelph.ca>)

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Previous research has demonstrated that early season weed interference can significantly affect maize (*Zea mays* L.) growth, development and yield. It has been hypothesized that early detection of weeds through reflected light quality (i.e. the ratio of red to far-red light or R:FR), may be an important mechanism affecting the onset and outcome of crop-weed competition. The objectives of this research were to quantify the impact of early season weed interference on maize growth and development during the critical period for weed control. A maize hybrid was grown in a greenhouse under ambient and reduced R:FR conditions, simulating weed-free and weedy conditions, respectively. These light quality environments were established by planting maize seeds in pots surrounded by turf (a baked clay medium with high or ambient R:FR) or commercial sod (low R:FR), such that there was no below ground competition. Using these two light environments, a classical critical period experiment was conducted with weed-addition and -removal series. Five weedy or weed-free durations were used in each series (E.g. Weed removal series: 0, 3, 6, 9, 12 or 15 days weedy). Maize seedlings were harvested 15 days after emergence. Seedling biomass and leaf area decreased linearly as the duration of weed competition increased in the weed-removal series. In contrast, weed addition following a weed free period had little impact on either of these parameters. These results suggest that the detection of weedy competitors occurs during the very early stages of maize development (~3-4 leaf tips). Furthermore, effective weed control at or near the time of crop emergence may reduce the impact of subsequent weed emergence events.

P80

Expression and functional characterization of the putative floral regulator, *conz1*

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Maize was domesticated from teosinte, a plant requiring short day photoperiods to flower. Post-domestication breeding included efforts to grow maize in a broad range of latitudes. Thus, modern maize is often characterized as day-neutral because time to flower is relatively unaffected by photoperiod. We identified *constans of zea mays1* (*conz1*; formerly *zmco1*), a gene highly similar to *CONSTANS* (*CO*) in *Arabidopsis* and syntenous to *Heading date1* (*Hd1*) in rice, both key regulators of the photoperiod response to flowering. To begin to understand the function of *conz1*, we are characterizing its expression. *conz1* exhibits diurnal expression patterns notably similar to its *Arabidopsis* and rice homologs yet expression of the maize gene is distinct in long-days compared with short days. Thus maize is able to discern variations in photoperiod, despite the idea that it is day-neutral. Furthermore, the expression of *conz1* in teosinte grown in non-inductive long-day photoperiods is more similar to the expression of *Hd1* in rice than of *conz1* expression in B73. This suggests the possibility of altered transcriptional regulation.

In addition to characterizing *conz1* gene expression, current experiments are focused on ascertaining the function of the CONZ1 protein with regards to flowering. Complementation experiments are being completed in *Arabidopsis co* mutants and rice NIL *hd1* using 35S::*conz1* cDNA and a 9.4kb *conz1* genomic clone. Flowering time of the transformants will reveal if CONZ1 functions as a floral regulator under different photoperiods in heterologous systems. Additionally we have identified amino acid differences in CONZ1 relative to other CO-like proteins in numerous plant species and are exploring if these changes have a functional significance. These expression and functional assays will allow for characterization of *conz1* and will further our understanding of the role, if any, of *conz1* in maize flowering.

P81

Expression differences between normal and indeterminate1 maize suggest downstream targets of ID1, a floral transition regulator in maize

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We have profiled the molecular differences between normal flowering maize and the severely delayed flowering mutant indeterminate1. INDETERMINATE1 (ID1) encodes a putative transcription factor that is a key regulator of the transition to flowering in maize. Loss-of-function *id1* mutants make many more leaves than normal plants and produce aberrant flowers, however they exhibit no obvious developmental defects in early growth stages. ID1 is expressed exclusively in immature leaves, suggesting that it controls the production or transmission of leaf-derived florigenic signals. We used a maize oligonucleotide microarray to assess the molecular differences between immature leaves of normal flowering and *id1* mutant plants just prior to the floral transition. This analysis revealed 55 genes with a significant 2-fold difference in expression. Among the most prominent differences is a novel family of three maize β -glucosidase genes with similarities to sorghum dhurrinases. These genes, which we designate *Zmdhr1*, *Zmdhr2* and *Zmdhr3*, are undetectable in *id1* mutants and are expressed in normal immature leaves in a pattern identical to the ID1 gene. Based on structural motifs, ZMDHR proteins are predicted to target to chloroplasts. Furthermore, a significant proportion of genes up-regulated in *id1* mutant immature leaves have potential roles in photosynthesis and carbon fixation substantiating a possible connection between floral induction and assimilate partitioning. Finally, the expression analysis of these genes in florally induced vs. uninduced teosinte, a short-day photoperiod sensitive progenitor of day neutral maize, showed few expression differences, suggesting that ID1 acts in an autonomous floral induction pathway that involves novel components not present in the photoperiod induction pathway.

P82

Expression of chimeric ATP synthase genes in maize CMS-C mitochondria

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Analysis of the complete CMS-C mitochondrial genome (Acc#) was undertaken to identify possible CMS-associated regions. Previously, chimeric genes for three essential proteins (COX2, ATP6 and ATP9) had been identified in Sam Levings' laboratory (Curr. Gen., 1991). In our analyses, an additional 11 chimeric open reading frames were discovered; however, their expression patterns suggested that none was a strong CMS candidate. Hence, attention was brought back to the previously characterized chimeric genes. Comparing CMS and fertility-restored plants, Levings et al. had reported no differences in transcription patterns of the chimeric *atp6*, *atp9*, or *cox2* genes and no protein expression changes of the chimeric *cox2* gene. The chimeric *atp9* gene had been the least studied because it is chimeric only in its 5' UTR, not in its protein-coding region. However, an additional "normal" copy of the *atp9* gene was discovered in the sequence data. Both the normal *atp9* (*atp9-1*) and chimeric *atp9* (*atp9-2*) genes are expressed and both produce a 4 kb transcript in CMS-C and restored CMS-C. In normal plants, there are four *atp9* transcripts, 2, 1, 0.8 and 0.6 kb. Analysis of the *atp9* transcripts using real-time PCR identified no significant differences in overall *atp9* levels between normal and CMS-C plants. However, using primers specific to *atp9-1*, a decrease of over 20 fold was observed in CMS-C samples when compared to B37N. Introduction of the restorer nuclear background had no effect on the *atp9* transcripts. In order to determine the cause of altered expression levels of *atp9-1*, the upstream sequence was examined closely and compared among the sequenced maize mitochondrial genomes. This identified two conserved indels and one conserved SNP corresponding to the apparent 5' ends of the 2, 1, and 0.6kb transcripts. To determine if the chimeric *atp6* or *atp9* genes are producing an extended chimeric protein, western analysis was performed. No chimeric protein was identified; however there was a tassel-specific decrease of ATP9 levels in CMS-C that does not occur in the restored background. This decrease may be due to lower translation efficiency of the *atp9* transcripts in CMS-C tassels, which could decrease the number of fully formed ATP synthase molecules, resulting in pollen abortion.

P83

Expression profiles of cytokinin (CK) genes in the *miniature-1* (*mn1*)-associated genotypes with variable levels of cell wall invertase (CWI) activity in developing seeds of maize

(submitted by Tomaz Rijavec <tomaz.rijavec@bf.uni-lj.si>)

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The two major hormones, auxins and cytokinins, essential for normal cell division and development are present in high amounts in developing maize kernels. They are presumed to be involved in sink strength formation, either directly by affecting cell divisions, or indirectly through sugar-hormone signaling. The maize *mn1* seed mutant is of much interest, because it shows pleiotropic changes both at cellular and metabolic levels due to a loss of the *Mn1*-encoded cell wall invertase (CWI) that controls the flux of sucrose in developing endosperm during seed development. In this study, we used previously described *mn1* alleles that show gene-dose dependence for the levels of CWI activity, sink strength and seed mass to check transcription profiles of hormone metabolism related genes. Using real-time quantitative PCR (qPCR) we obtained expression profiles of five CK metabolism related genes, isopentenyl transferase (*Ipt*), cytokinin oxidase (*Ckx*), N-glucosyl transferase (*Cngt*), histidine kinase (*Hk*) and cis-zeatin glucosyl-transferase (*Czog*) and an auxin biosynthesis gene *Yucca* in developing kernels during the 6 to 28 DAP (days after pollination) period. Of these six genes, *Ipt*, *Cngt* and *Yucca*, showed peak levels of expression that coincided with the expression of the *Mn1* gene during cell division and elongation phase, whereas, *Ckx*, *Hk* and *Czog* genes showed a rather uniform pattern of expression throughout seed development. Overall, the developmental profiles of expression of the genes studied here were similar between *mn1* and *Mn1*, but the absolute levels in terms of number of transcripts for each gene, varied widely between the two genotypes. Significance of these results in relation to CWI activity and seed mass will be discussed.

P84

Functional and genetic analysis of bladekiller1

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BLADEKILLER1 (BLK1) is necessary for meristem maintenance and leaf development in maize. Plants homozygous for the *blk1-R* mutant allele show a progressive reduction in SAM size that often leads to premature termination of the shoot. The meristem phenotype is also correlated with a successive reduction and eventual deletion of the leaf blade, although leaf sheath is unaffected. Inflorescence structures are often absent from *blk1-R* mutant plants and when formed, the ears and tassel show reduced numbers of branch and/or floral meristem populations. Taken together, these phenotypes suggest BLK1 functions to maintain meristem populations throughout the shoot. Accordingly, several genes with known roles in meristem development, including *tdl*, *fea2*, *abph1*, and *kn1*, have reduced transcript accumulation in mutant axillary meristems. Genetic analyses indicate that *blk1* is epistatic to or required for the function of *tdl*. Preliminary data also suggest that *blk1* may interact with *abph1*. In order to characterize BLK1 function at the molecular level, a map-based cloning project is underway. The *blk1* locus has been mapped to a 250kb interval between BACs b0569K03 and c0287O15 on chromosome 3L. Five candidate genes, all of unknown function in maize, are currently being screened for mutations that may alter normal gene expression or protein function. Identification of the *blk1* gene product and analysis of its function will provide insight into poorly understood mechanisms of meristem maintenance in maize and can potentially uncover mechanistic links between leaf and meristem development.

P85

Functional characterization of OCL1, an epidermis-specific HD-ZIP IV transcription factor, by identification and characterization of its target genes

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Epidermis differentiation is a key step in plant embryogenesis and a condition for a normal development of the plant embryo. HD-ZIP IV transcription factors including FWA, GL2, AtML1 or PDF2 in Arabidopsis and OCL 1 to 5 (Outer Cell Layer) in maize, seem to play crucial roles in the differentiation and maintenance of the epidermal cell fate. The majority of them show epidermis-specific expression patterns and in several Arabidopsis mutants, the differentiation of epidermal cells is affected. In particular, the *atml1/pdf2* double mutant embryo completely lacks epidermal cells in the shoot and is seedling lethal. In maize, OCL1 is specifically expressed in the epidermis of the embryo, the endosperm and young organ primordia. Plants over-expressing OCL1 have a pleiotropic phenotype. To identify direct or indirect target genes of OCL1, the transcriptome of 18 day old plantlets over-expressing OCL1 was compared to that of wild-type plantlets using the maize 70mer micro-array. Of 35 candidate genes, 12 were confirmed as being up- or down-regulated by Q-RT-PCR. Expression patterns of the 12 genes in the maize plant were established by Q-RT-PCR and/or in situ hybridization. Bioanalysis of putatively complete cDNA sequences revealed that several target genes encode proteins involved in lipid metabolism, defense or cuticle biosynthesis. Whenever available, the promoter sequences were scanned for the presence an 8bp motif called L1-box, which has been identified as the cis-element of HD-ZIP IV transcription factors in Arabidopsis. The promoters of three genes with L1 box were cloned and fused to a GUS reporter gene to check for trans-activation by OCL1 by transient expression in maize kernels. The promoters of a lipid transfer protein and an ABC transporter but not a TPR domain protein seem to be direct targets of OCL1. This hypothesis is strengthened by co-expression of OCL1 and the LTP gene in the epidermis.

P86

Functional characterization of the *rum1* gene in maize

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Plant roots serve important functions among which water and nutrient uptake and anchorage are the most important ones. The complex root system of maize consists of the embryonic primary root and seminal roots, and an extensive postembryonic, shoot-borne root system composed of crown- and brace-roots which make up the major backbone of the root system. A common feature of all root types is the formation of lateral roots.

The monogenic mutant *rum1* (*rootless with undetectable meristems 1*) is deficient in the initiation of the embryonic seminal roots and the postembryonic lateral roots at the primary root. For none of these root types primordia are initiated (Woll *et al.*, 2005).

The *rum1* gene is located on chromosome 3L. Progress in cloning the *rum1* gene and results of comparative proteome analyses of wild type versus *rum1* primary roots will be presented.

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P87

Functional genetic analysis of *ragged seedling2*: a gene required for mediolateral leaf development in maize

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Ragged seedling2 (RGD2) plays a role in establishing and maintaining mediolateral development in maize leaves. Plants homozygous for the recessive mutation *rgd2-R* display a range of mutant phenotypes, including radial leaves. In contrast to other radial leaf mutants, *rgd2* radial leaves preserve both adaxial and abaxial epidermal identity and collateral vascular structure is maintained. qRT-PCR and *in situ* hybridization analyses reveal that RGD2 may play a role in establishing and/or maintaining transcript accumulation of adaxial/abaxial identity genes in maize shoot apices. The morphology of *rgd2* and leafbladeless1 (*lbl1*; which encodes a maize SGS3 ortholog and is necessary for the biogenesis of ta-siRNAs) mutants are similar. qRT-PCR has shown that *lbl1* transcript levels are increased in *rgd2* mutants and *rgd2-R/lbl1-rgd1* double mutants exhibit synergistic shootless phenotypes, suggesting that RGD2 and LBL1 function in separate but overlapping genetic pathways. Interestingly, *auxin response factor3A*, which is targeted for degradation by ta-siRNAs, has elevated transcript levels in both *rgd2* and *lbl1* mutants, suggesting a possible convergence point for the two pathways. A positional cloning approach is being utilized to identify the *rgd2* gene. *rgd2* is currently localized to a ~450 kbp region containing six candidate genes in bin 1.04 on the short arm of chromosome 1. Analyses of genomic sequence and transcriptional polymorphisms among the candidate genes are progressing, in order to identify genetic lesions within the *rgd2-R* allele.

P88

Genetic control of floral development in maize

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Flowers are the product of floral meristems. To identify key regulators in floral meristems, we are studying mutants that affect meristem identity, determinacy, and organogenesis. bearded-ear (bde) mutants are defective in multiple aspects of floral development. bde mutants produce extra florets in both the tassel and ear. In addition, bde floral meristems produce the incorrect number of floral organs, as well as fused, or partially transformed organs. Interestingly, the upper and lower floral meristems in bde mutant tassels are differentially affected. The lower floral meristem produces axillary meristems, and carpel abortion often fails. In contrast, the upper floral meristem does not produce axillary meristems or carpels, but does exhibit defects in organ number and identity. We cloned bde and found that it encodes the MADS-box transcription factor, zag3. MADS-box transcription factors are a key family of regulators that control floral meristem patterning and organogenesis [1]. zag3 belongs to the AGL6 subfamily of MADS-box genes [1]; no loss of function phenotypes have been reported for this subfamily, and bde provides insight into the role of AGL6-like genes during development. We have examined zag3 expression by RNA in situ hybridization and found that it is restricted to floral development. zag3 is expressed in both upper and lower floral meristems, but not in developing lemma, palea and stamens. Later in development, zag3 is expressed in developing carpels and the inner integument. We are also mapping a second mutant, fuzzy tassel (fzt), that affects multiple aspects of inflorescence development including floral development. We have mapped fzt to Chromosome 1, bin 1. Progress towards cloning fzt will be reported at the meeting.

1. Becker, A., and Theissen, G. (2003). The major clades of MADS-box genes and their role in the development and evolution of flowering plants. *Mol Phylogenet Evol* 29, 464-489.

P89

Heterosis in early kernel development

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Heterosis is the superior performance of hybrids compared to their parental inbred lines. The crossbreeding advantage is obvious by comparing adult plants showing the highest degree of heterosis. We already found clear heterotic traits in hybrid embryos six day after pollination (dap) using different maize inbred line combinations (flint /dent). Caused by enhanced growth rate, detectable by an increased embryo length and higher cell number of the F1 hybrid embryos, heterosis takes shape early after crossbreeding. To characterize heterosis and associated gene expression pattern in early kernel development of maize we microdissected the kernel and isolated the embryo and endosperm tissue separately to avoid contaminations by maternal tissue. To analyze gene expression at six dap we generated cDNA populations enriched for differentially expressed genes between hybrids and the corresponding inbred lines by suppression subtractive hybridization. By microarray analysis using the subtracted cDNA libraries, a cDNA collection of chromatin modifying genes (www.chromdb.org) and cDNAs representing active genes or stored transcripts of female gametes as probes we identified differentially expressed genes between 6 dap hybrids and the parental genotypes. Quantitative RT-PCR analysis revealed predominant additive, but also dominant and overdominant expression patterns in hybrid embryos. Association of heterosis in embryos with genes related to signal transduction and other regulatory processes was implied by the enrichment of these functional classes among the identified gene set. Allele specific expression analyses of 25 differentially expressed genes refer to enhanced trans-regulatory interactions in early F1 hybrid embryos. Our phenotypical data confirm that the manifestation of heterosis starts early after combination of genetically-distant genomes. Overall our molecular analyses indicate that the formation of early heterosis involve altered gene expression of regulatory genes and enhanced gene regulatory interactions among the parental alleles.

P90

Hsp101 prevents adventitious root formation in maize seedlings

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Adventitious roots (AR) constitute the prevalent root system of adult maize plants. AR arise from stem nodes and little is known about their inducing factors. We found that a single heat shock triggered the formation of AR in maize seedlings grown in the dark. Under certain conditions, the viability of the primary root showed a negative correlation with the emergence of AR. However, surgical experiments showed that the loss of viability of the primary root or the root cap were not the primary signals for AR formation. Young (36 and 60 h) seedlings required higher heat-shock temperatures to achieve maximal induction of AR than old seedlings (84 h). This observation paralleled the higher basal heat-shock resistance of young seedlings and correlated positively with the levels of embryo-accumulated heat shock protein Hsp101. Moreover, the higher resistance of the coleoptile, relative to the primary root, also correlated with the levels of Hsp101, which persisted for a longer time in the coleoptile during post-germinative growth. To study the role of Hsp101 in this process, we compared the response of wild type and null Hsp101 mutants. In a homozygous *hsp101-m5::Mu1* mutant, AR emerged in seedlings grown at optimal temperature. At 36 and 60 h, AR production was maximal in *hsp101-m5::Mu1* seedlings after heat shocks between 40 and 45°C. In wild type seedlings maximal response was between 45 and 49°C. At 84 h, AR induction, in both mutant and wild type, occurred between 28 and 45°C. When exposed to light, wild type seedlings developed AR at optimal temperature. Under these conditions, Hsp101 protein levels were undetectable in adventitious root primordia, but present in the remaining cells of the coleoptilar node. We suggest that Hsp101 assists a developmentally-regulated switch that represses the appearance of AR at the coleoptilar node in young seedlings and that light can abrogate this negative effect.

P91

Isolation of *Tripsacum dactyloides* genes using putative apomixis genes from *Pennisetum ciliare*

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In the present study, we will show the isolation of gene sequences pertaining to apomixis in *Tripsacum dactyloides*. *Tripsacum* is an ancient wild relative of maize, and therefore, identification and trait transfer of the multiple genes associated with apomixis to its relatives could be accomplished once the genes have been identified and characterized. PCR primers have been developed for two candidate genes for apomixis in *Pennisetum ciliare*, and used to isolate homologous gene sequences in both diploid ($2n=36$) and tetraploid ($4n=72$) *Tripsacum dactyloides*. Amplified products have been obtained from both the diploid and tetraploid samples with variable numbers of bands indicating that not only are homoeologous sequences for these putative apomixis genes from *Pennisetum ciliare* present in the *Tripsacum* genome, but also that there are significant differences between amplified products of diploid and tetraploid *Tripsacum*. Purified, cloned and sequenced products are being used for bioinformatics analysis of ORFs, and genomic comparisons, mapping analysis, FISH/GISH, and hybridization studies with developmentally staged cDNA libraries of female gametophytic tissues to determine the stage of gametophytic development associated with expression. Confocal microscopy analysis of the three distinct ovule developmental stages of the *Tripsacum dactyloides* cDNA libraries has identified distinct morphological variations in preparation for future in situ hybridization studies.

P92

Male sterile 8 (ms8): Transcriptome profiling, proteome and cytological analysis

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Maize male gametophyte development is a highly coordinated process that requires cooperative gene expression among 5 cell types. Because of its biological significance and commercial use in hybrid seed production, maize male sterility has been investigated intensively. male sterile 8 (ms8) plants do not disperse any pollen and the spikelets are shrunken by the time pollen is shed by normal sibs. Compared with yellow and plump fertile anthers, the ms8 anthers are brown in color and wizened in shape. In order to characterize this mutant comprehensively, upper floret anthers at the 1.0 mm (mitotic proliferation stage), 1.5 mm (pre-meiosis stage) and 2 mm (mid-meiosis) stages were dissected from ms8 plants for comparison to stage-matched fertile siblings. Transcriptome profiling identifying several hundred differentially expressed transcripts was performed on custom 4 by 44 K Agilent in situ-synthesized microarrays. In progress is a proteome analysis of mutant anthers from these stages; selected proteins will be identified by mass spectrometry. In addition, structural analysis of ms8 anthers is being carried out to detect the cell type differences from normal development. In summer 2007 five ms8-Mu tagged alleles were recovered in a screen at Cal Poly-SLO in collaboration with Jeff Wong and Matt Ritter; cloning is in progress. The molecular, proteomic, and cytological data will be analyzed with regard to several questions. What cell types are affected by ms8, and when? What anther transcriptome and proteome differences occur before and during meiosis? Once cloned we can address when and where ms8 is expressed. Ultimately analysis of ms8 and other mutants should help define cell-type specific gene expression patterns that establish and maintain anther cell fates. This research is supported by a grant from the NSF.

P93

Molecular control of egg activation in maize and *Tripsacum dactyloides*: Transcription profiling using cDNA microarrays

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Sexual and asexual modes of reproduction are complex and highly regulated developmental phases in plants. Our special interest is directed towards egg activation through parthenogenesis, one of the pivotal steps in apomictic reproduction. Using sexual maize and its wild apomictic relative *Tripsacum dactyloides* as model plants, we are looking for key regulators of parthenogenesis. Analysis of various *T. dactyloides* accessions showed that diploid plants reproduce sexually and polyploids through apomixis of the pseudogamous type (Bantin et al., 2001, *Sex. Plant Reprod.*). We have established microdissection techniques for both, maize and *T. dactyloides*, that enable the manual isolation of intact embryo sac cells providing access to sexual and parthenogenetic egg cells as well as defined stages of zygote and very early embryo development. In polyploid *T. dactyloides* lines, we found that parthenogenetic embryos develop precociously making the isolation of parthenogenetic egg cells a great challenge. However, due to the high gene sequence homology of both plant species (e.g. Cordts et al., 2001, *Plant J.*), isolated single embryo sac cells provide now an ideal basis to compare gene expression pattern of egg cells during parthenogenesis and after fertilization. We have generated 988 ESTs from maize egg cells representing 544 different genes and identified 120 fertilization induced ESTs from *in-vitro* zygotes representing 41 different genes. Expression profiles of sexual and parthenogenetic egg cells of maize and *T. dactyloides* as well as different developmental stages of maize zygote have now been compared after hybridization to cDNA microarrays containing these ESTs. In addition, we have compared the transcriptome of maize and polyploid *T. dactyloides* egg cells on a 46,000 maize oligo chip. First predictions can be made about the timing of maternal to zygotic transition in early embryo development in maize. Based on this approach, we also have detected a number of genes suggesting that parthenogenetic embryo development partly mimics zygotic gene expression pattern. Our long term goal is to identify master regulators required to induce parthenogenetic embryo development from sexual egg cells.

P94

Molecular dissection of heterosis manifestation in young seedling roots of maize

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Heterosis describes the superior vigor of F1 hybrid plants derived from crosses of genetically different homozygous inbred lines. Heterosis in maize (*Zea mays* L.) has been shown to be manifested already during early plant development. Despite its agronomic importance, the molecular mechanisms that underlie the establishment of heterosis remain elusive. Goal of this study was the identification of genes and proteins in 3-day-old maize primary roots that are expressed different from additivity. Experiments were performed on the German flint line UH002, the dent line UH301, and their reciprocal hybrids. The identification of single nucleotide polymorphisms (SNPs) in these inbred lines will allow for the determination of the allele specific contribution to gene expression of individual genes.

First results of these studies revealed non-additive expression of several genes in F1 hybrid plants, for example a [Cu-Zn] superoxide dismutase (SOD) gene, which showed increased transcript levels in all analyzed F1 hybrid roots compared to the roots of the corresponding parents. Since SODs contribute to the detoxification of reactive oxygen species (ROS) it was hypothesized that hybrid F1 plants might be able to deal with higher ROS levels than their parents. To test this hypothesis, several morphological, physiological and molecular analyses will be conducted on young maize seedlings grown under stress conditions such as high light or high temperature.

P95

Molecular evolution of bearded-ear orthologs in the grass family

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Several genes have recently been identified that are involved in the control of floral development and sex determination in maize. Among these, bearded-ear (bde), a MADS-box transcription factor that belongs to the AGL6 clade, is required for floral meristem determinacy, organ identity and carpel abortion in the tassel. Moreover, bde orthologs have been reported to be preferentially expressed in flower tissues in several species more or less closely related to maize. Little is known about the evolution of AGL6-like genes in the grass family and other monocots. Here we report progress in characterization of the evolution of AGL6 orthologs from 27 grass species and 6 species of non-grass monocots. Based on sequence comparisons, the C-terminal region of AGL6-like genes exhibits considerable heterogeneity among grasses, suggesting possible variation in the control of transcriptional activation, gene interactions, DNA binding specificity and/or localization. Interestingly, a glutamine-rich domain in the C-terminal region characterizes the subfamilies Pooideae and Chloridoideae, but is absent in gymnosperms, dicots, other grasses, and non-grass monocots. The preliminary phylogeny for the AGL6-like genes in grasses largely agrees with the species phylogeny. At least three different duplication events were recovered within grasses: a) at the base of the grasses (OsMADS6 and OsMADS17), b) inside the BEP Clade and, c) at the base of *Zea* and *Tripsacum* lineage (ZAG3 and ZAG5). Despite multiple efforts at PCR with various combinations of primers, we have not detected an OsMADS17-like sequence in grasses other than rice; all sequences recovered so far are homologs of OsMADS6. Our data suggest that perhaps OsMADS17 has been lost in the grasses.

P96

Molecular genetics of the arbuscular mycorrhizal symbiosis in maize

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The most widespread and successful strategy of plants to improve the uptake of phosphate, nitrogen and other minerals is to form symbioses with arbuscular mycorrhizal (AM) fungi. These fungi explore the soil substratum, take up nutrients from far beyond the depletion zone of the root, and transport and deliver them to their host plant by intracellularly colonizing the root cortex. Recognition, development and maintenance of the symbiosis must be the result of a coordinated signaling cross-talk between the plant and the fungus. The identification of plant-encoded factors essential for the development and/or functioning of the symbiosis represents our central research interest. A forward genetic screen on a Mutator-mutagenized population of maize has resulted in the discovery of a set of seven mutants perturbed at different stages of the interaction. Gene cloning is currently ongoing. A second approach addresses traits related to functionality of the symbiosis by examining a collection of genetically diverse maize lines for their responsiveness to mycorrhizal colonization. It has been widely documented that, under mineral-limiting conditions, mycorrhizal plants grow bigger than their mock inoculated counterparts. Responsiveness to mycorrhizal colonization varies among plant species or even cultivars and has been defined as the difference in performance between colonized and non-colonized plants at a given level of nutrient availability. A novel screen has been initiated that makes use of a population of diverse maize lines. Dry weight and total phosphorus content serve as an expression of responsiveness to AM colonization. Genotypes displaying high responsiveness to the AM symbiosis in the absence of dependence have been identified and are now subjected to further characterization.

P97

MuExpress: isolation of key genes for kernel development through the identification, in a collection of 300 mutant lines, of Mutator insertions in genes expressed in the maize seed

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The size and shape of the two seed compartments (embryo and endosperm) is determined during seed development. The process involves an estimated 1000 genes and their identification and characterisation presents a major scientific and agronomic challenge. The MuExpress project aims at the identification and functional analysis of genes involved in maize seed development via the molecular characterisation of 300 Mutator induced maize seed mutants. The mutants were selected from a much larger initial collection based on a clean 3:1 segregation of the mutant phenotype, stability of the mutant phenotype over at least 3 generations, and defects concerning mainly endosperm rather than embryo development. The novelty of the experimental approach lies in a transposon display technique that uses cDNA rather than genomic DNA as starting material. Mutator-containing kernel transcripts are amplified from both ends using 5' and 3' RACE with primers located in the terminal inverted repeats. This transcript based approach should reduce the complexity of the system and the number of displayed bands. Mutant kernels from two segregating ears and wildtype kernels from a single wildtype ear were harvested at 13 DAP. After the first year of this 3 year project, RNA extraction has been completed for the great majority of mutants, RACE reactions have been performed for 118 mutants, and 95 FSTs have been obtained. Approximately 25% of the FSTs showed the expected profile after PCR with a Mu-primer and a flanking primer on cDNA and gDNA of the original samples. So far only 2 FSTs have been tested in large scale co-segregation analysis using genomic DNA of approximately 100 plants segregating for the respective mutation. Both FSTs showed moderate linkage to the mutation, suggesting that the insertions were located on the same chromosome as the respective mutations, but that they were not causal for the phenotype.

P98

Mutants that alter phyllotaxy in maize

(submitted by Robyn Johnston <johnston@cshl.edu>)

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The maize plant exhibits a very ordered pattern of growth. Leaves of the main axis are initiated singly in an alternate pattern, generating a distichous phyllotaxy. *aberrant phyllotaxy 1 (abph1)* mutants have an altered phyllotaxy leaves are initiated in pairs in a decussate pattern, rather than the normal distichous pattern. *abph1* encodes a cytokinin-inducible type A response regulator and is involved in the negative regulation of cytokinin signaling, indicating a role for cytokinin in phyllotactic patterning (Giulini *et al.*, 2004). Recent data indicate that PIN1 signaling and auxin levels are reduced in *abph1* SAMs. To investigate this interaction further, *abph1* embryos were cultured on auxin and the effects on meristem size were examined. These results will be presented.

To further understand the regulation of maize phyllotaxy, we are characterizing a second mutant, *Abph2*. *Abph2* is dominant mutation that alters phyllotaxy. However, unlike *abph1*, *Abph2* phyllotaxy is initially normal and changes to decussate at about leaf 5. The change to decussate phyllotaxy is generally preceded by the production of a fused leaf. Examination of embryonic shoot apical meristems (SAMs) indicates that the *Abph2* SAM is wider than normal, but there is no significant difference in height. The identity of *Abph2* remains unknown. However, *Abph2* has been mapped to a region of two BACs on chromosome 7, and fine mapping is in progress. Characterization of the *Abph2* phenotype is ongoing.

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Giulini, A., Wang, J. and Jackson, D. (2004). Control of phyllotaxy by the cytokinin-inducible response regulator homologue *ABPHYLL1*. *Nature* 430, 1031-1034.

P99

New maize mutants with leaf polarity defects

(submitted by Hector Candela <hcandela@nature.berkeley.edu>)

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The many cell and tissue types make maize leaves an excellent experimental system for studying the genetic basis of pattern formation mechanisms. We will present data on the phenotypic and genetic characterization of several dominant and recessive mutants that exhibit patterning defects along the abaxial/adaxial and proximal/distal axes. *Rough sheath4 (Rs4)* is an EMS-induced dominant mutation that causes extra cell divisions in the abaxial domain of maize leaves. *Wavy auricle in blade1 (Wab1)* dominant mutations cause proximal cell fates, such as sheath and auricle, to appear at more distal blade locations. *Morph** is a new EMS-induced dominant mutation that causes the outgrowth of leaf tissues, as manifest by sectors that run along the sheath and the blade. To further our understanding on the molecular basis of these phenotypes, we have mapped these genes and are attempting the positional cloning of *Rs4* and *Wab1*. In a *Mutator* family, we identified a new recessive mutant that resembles and complements *milkweed pod1 (mwp1)*, a mutant that fails to specify or maintain abaxial identity. We have named this new gene *mwp2*. In a different non-complementation screen for *mwp1* alleles, we also found a mutant with a distinct recessive phenotype consisting in ectopic outgrowths at the sheath-stem junction.

P100

OCL4, an HD-ZIP IV transcription factor involved in macrohair distribution and anther development

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OCL4 (Outer Cell Layer4) belongs to the plant-specific HD-ZIP IV family of transcription factors, which are almost exclusively expressed in the epidermal cell layer. Functional data from *Arabidopsis* reinforce the idea that HD-ZIP IV genes are important for the differentiation of epidermal cells. For example, the *gl2* and *hdg11* mutants present trichomes (specialised epidermal cells) with a decreased and increased number of branches, respectively.

OCL4 is expressed in the epidermis of the endosperm, the embryo, the vegetative meristem, the inflorescence and the female flower. An *ocl4::Mu* mutant showed two phenotypes: (1) A deficiency in anthesis of varying severity depending on growth conditions and (2) an extension of the zone of the leaf bearing macrohairs from the tip to the middle of the leaf. The latter phenotype was also found in an RNAi line targeting OCL4 and, surprisingly, in a line over-expressing OCL4. A model explaining the common phenotype between *ocl4::Mu*, OCL4-RNAi, and OCL4-OE lines and their effect on the transcription of direct target genes will be presented.

The function of transcription factors is to activate or repress the transcription of target genes. To identify target genes of OCL4, a microarray study was carried out comparing the transcriptome of OCL4-RNAi and wildtype kernels from 15 DAP segregating ears. For 12/37 candidate genes differential expression was confirmed by Q-RT-PCR. Putative functions based on sequence analysis will be proposed.

We also checked for genetic association of certain polymorphisms of OCL4 with agronomic traits. Significant associations were found for flowering time and vitreousness. Introgression of a late, non-vitreous allele from inbred CM174 into the FV2 background carrying originally an early, vitreous allele did not confirm the effect on flowering time, while the data on vitreousness are not yet available.

P101

Polycomb genes controlling endosperm development in rice

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Genes of the Polycomb group family (PcG) have been implicated in the maternal control of endosperm development in the model species, *Arabidopsis thaliana*. Wild type PcG proteins assemble in a chromatin remodeling complex and repress transcriptional activity of target genes. Genetic studies in *Arabidopsis* have identified four PcG genes, MEA, FIS2, FIE and MSI1 that are required for normal endosperm proliferation and patterning. In maize, FIE and three orthologs of MEA, *Mez1*, *Mez2*, and *Mez3* have been identified. Although no functions are known for these genes in maize, some maize PcG genes are known to be expressed from only one parental allele during endosperm development while the other allele is silenced typically through methylation. We are investigating the potential functions of candidate orthologs of the PcG genes in seed development in rice. Sequence database searches have identified rice homologs of the *Arabidopsis* FIS2, MSI1, and maize *Mez* and FIE2 genes and the phenotypic effects of insertions in several of these rice genes are being examined. Preliminary observations indicate seed abortion in lines carrying T-DNA insertions in MSI1, *Ez1* and DME suggesting these mutations are lethal. These data indicate that PcG genes might play an essential role in seed development in cereals.

P102

Population variation for microRNA expression in maize

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Prior molecular genetic studies have established that microRNA156 and microRNA172 act antagonistically, but in a common pathway, to regulate the expression of the Glossy15 transcription factor, juvenile versus adult leaf identity, and vegetative phase change in maize. The maize inbred lines W23 (three juvenile leaves) and NC61 (10 juvenile leaves) represent phenotypic extremes for the number of juvenile leaves, and a population of recombinant inbred lines has been derived from the cross of these two lines. Genetic mapping experiments identify regions harboring *gl15* (a molecular target of miR172) and a SPL transcription factor (a likely target of miR156) as being major QTL for leaf identity traits. Sequence comparisons of the *gl15* alleles from W23 and NC61 suggest changes in regulatory sequences as the basis for the *gl15* QTL. We also investigated the expression of miR156, miR172, and *G115* mRNA via qRT-PCR in the W23 and NC61 parents, as well as a subset of derived recombinant inbred lines. Expression variation for each of miR156, miR172 and *G115* differ greatly between NC61 and W23 and is also associated with leaf identity phenotypes observed among the progeny recombinant inbred lines. Our results to date indicate that expression variation the miR156-miR172-*G115* pathway is predictive of leaf identity phenotypes in the population examined, and suggests avenues for using knowledge of miRNA expression variation in the characterization of quantitative traits and their modification via breeding.

P103

Regulation of sink strength in developing female florets: a transcriptome-wide assessment using microarray and sequencing of 3'-UTRs

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The pre- and early post-pollination phases of maize reproductive development are critical for optimal seed set and subsequent grain yield. During this time, increased sensitivity of female reproductive tissues to drought stress can lead to inefficient pollination and/or abortion. Such effects can be tightly coupled to whole plant source/sink balance or regulation of carbohydrate allocation. In the present work, we investigated the processes underlying growth and sucrose use during normal progression of maize ovary development. A pre- to post-pollination shift in sink strength from silks and subtending floral parts (lemma, palea, and glumes) to the developing ovary was consistent with changes in isoform-specific activities of sucrose-metabolizing invertases. We profiled genome-wide transcriptional changes during ovary growth and development using parallel microarray and 454-based sequencing approaches. In addition, biochemical analyses were used to support transcript-based hypotheses. Key genes were identified that showed significant changes in expression over a four-stage time course which included pre-silking, silk emergence, pollination, and fertilization. We used a series of clustering methods to predict relevant metabolic and regulatory pathways involved in carbohydrate assimilation and sink expansion in developing ovaries. A 3'-UTR profiling strategy was used to generate gene-specific, sequence-based expression profiles and enabled resolution of complex gene families and other near-identical paralogs. The significance of this work is two-fold. 1) The potential of 454-based 3'-UTR profiling as an effective method for genome-wide transcript profiling was evaluated in parallel to a microarray approach. 2) High-resolution transcriptome analyses provided insight into important metabolic and regulatory processes that contribute to normal female reproductive growth. These data serve as a foundation for further functional analyses, particularly under genetic or environmental perturbation.

P104

Screening male-sterile mutants in Berkeley for anther development mutants

(submitted by Ljuda Timofejeva <ljuda_timofejeva@yahoo.com>)

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We are interested in identifying mutants that have specific defects in early anther development that occur in the window of developmental time between initiation of the anther and initiation of meiosis. Studying these mutants will allow us to identify processes involved in anther cell fate acquisition, including acquisition of meiotic cell fate. In order to identify appropriate mutants, we are screening all known male sterile mutants that we can find, including those at the Maize Genetic Stock Center, the majority of which contain RescueMu, and other resources. This screen is part of a new NSF sponsored plant genome research project, Cell Fate Acquisition in Maize which we call "The Anther Project" (PI: Virginia Walbot, CoPI: Zac Cande). Please send us your uncharacterized male sterile mutants so we can include them in our survey.

From 49 uncharacterized mutants screened so far, 7 segregate for classic male sterile pollen development defects, 3 have defects in meiosis, and 5 have defects in early anther development (the class we want). Thus, of the 49 uncharacterized mutants examined, 5 warrant further analysis in our study; almost 10%. The rest appeared normal in the acetocarmine squashes, probably indicating post meiotic defects. Cross-sectioning allowed us to distinguish mutants with abnormal tapetal and/or middle layers. Some mutants exhibit unique phenotypes while others have similarity with known maize or Arabidopsis male sterile mutants. After we complete complementation tests, we will know how many new genes these 5 mutants represent.

P105

Suppressor of sessile spikelet (Sos) and its role in the spikelet meristem initiation and determinacy

(submitted by Xianting Wu <xzw104@psu.edu>)

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Maize produces male and female inflorescences called the tassel and ear. Organs produced on the tassel and ear are initiated by axillary meristems. There are four types of axillary meristems initiated during maize inflorescence development: branch meristem (BM), spikelet pair meristem (SPM), spikelet meristem (SM) and floral meristem (FM). Each SPM produces a sessile SM before itself converting to a pedicellate SM. As a result, maize produces paired spikelets, which is a unique characteristic of the Andropogoneae family, a group of 1000 grasses to which maize belongs. The Suppressor of sessile spikelets (Sos) mutant is a semi-dominant of maize that produces single instead of paired spikelets in both the tassel and ear. Dosage analysis suggests that the Sos mutant is an antimorph. Phenotypic analysis and Scanning Electronic Microscopy (SEM) analysis show that the sessile spikelet fails to initiate in Sos mutants. Therefore, Sos mutants are more determinate as they produce fewer spikelets than normal. By contrast, in other mutants such as ramosa1 (ra1) and ramosa2 (ra2), SPM determinacy is delayed and more spikelets are initiated by each SPM. To determine the genetic interaction between Sos and the ramosa pathway, double mutants were constructed. In Sos/Sos;ra1/ra1 double mutants, the highly branched ear phenotype of ra1 was inhibited by Sos in severe examples. On the other hand, Sos/Sos;ra2/ra2 double mutants had a more extreme branched ear phenotype. These results suggest that the interaction between these three genes to determine SPM determinacy is more complicated than expected. A model for the genetic interactions is proposed. Considering the importance of Sos in the evolution of grass inflorescence architecture, we are attempting to clone Sos by chromosome walking. Sos has been mapped to the short arm of chromosome 4. SSR markers on either side of the gene have been identified and the area where Sos maps has been narrowed down to one BAC contig. Further efforts to identify the Sos gene are ongoing.

P106

Survey of gametophyte-specific EMS-induced mutants in maize

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Mutation analysis remains an essential tool to determine gene function. This poses a problem in the study of plants. The life cycle of plants involves an alternation between the sporophyte and the gametophyte generations. Gametophyte plants are haploid, since they are the products of meiosis. For diploid plants, such as maize, this means that the gametophytes are monoploid and so it is expected that mutant alleles of many genes are lost in the gametophyte because they cannot be complemented. These gametophytic-sensitive genes may include essential housekeeping genes as well as genes that are specific to the functioning of the gametophyte. Because both the female (embryo sac) and male (pollen) gametophytes are able to utilize products provided by parental (sporophytic) tissue, it is unknown how often mutations affect viability of the pollen, the embryo sac or both. A simple genetic test was made using a classical genetic marker (*bz2*). Mature pollen from a *bz2/bz2* was treated with ethyl methane sulfonate and applied to silks of a *Bz2/Bz2* female parent. The surviving pollen grains delivered a potentially mutagenized sperm to a normal egg producing a *bz2/Bz2* F1. The F1s were grown and crossed reciprocally as both male and female with *bz2/bz2* testers. The resulting test-crossed ears were screened for ratios of dark (*bz2/Bz2*) and light (*bz2/bz2*) kernels. A reduced rate of *bz2* transmission was taken to indicate that *bz2* was linked to some factor with reduced transmission through the gametophyte. The results of this test indicate that such mutations are common. It was also observed that these mutations often specifically affect either only male or female transmission, but that there are a substantial number of mutations that appear to reduce transmission in both gametophytes.

P107

The fused leaves gene affects shoot apex organization and coleoptile opening in maize

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The fused leaves (*fdl*) mutation is detectable during the early phases of maize seedling growth, starting from germination to the four-leaves stage. Two *fdl* related defects have been investigated. The first one is the presence of adhesion regions between the coleoptile and the first leaf. Light microscopic analysis demonstrated that the epidermal cells of the two organs are fused together in these regions. Further electron microscopy analysis revealed that the fusion implies the absence of the cuticle between the two epidermis that appear joined by a single cell wall. The second *fdl* specific trait consists in the presence of a thicker and shorter coleoptile whose opening is delayed and occurs with an irregular lateral fracture in contrast to the clear-cut hole that is formed in the wild-type. As revealed by cytological analysis, the origin of these coleoptile defects can be traced back to earlier events that take place prior to germination. Mutant and wild-type embryos have been compared for the occurrence of the programmed cell death (PCD) process. Results obtained with the TUNEL method demonstrate that in wild-type embryos both the scutellum and the coleoptile undergo PCD whereas this does not occur in the central portion of the shoot, including the leaf primordia. By contrast, in the mutant embryos the TUNEL positive signals were not detected in the coleoptile, although they were visible in the scutellum. The *fdl* gene thus seems to be involved in different aspects related to embryo and seedling development. Since the *fdl* mutant was identified in an active Suppressor-mutator (*Spm*) line, the co-segregation analysis was performed. On the basis of this, the mutant phenotype can be linked to an *Spm* tagged sequence containing a region of homology with a Myb R2R3 motif. We are currently investigating the correspondence between this sequence and the *fdl* gene.

P108

The indeterminate spikelet1 and sister of indeterminate spikelet1 genes are necessary for floral meristem initiation

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The indeterminate spikelet1 (*ids1*) gene functions to control spikelet meristem determinacy. *ids1* mutant spikelet meristems are indeterminate, and initiate extra palea/lemma-like bracts, each of which contains fertile florets in their axils. The *ids1* gene encodes an APETALA2 (AP2) transcription factor that contains a binding site for a MIR172 microRNA. A candidate microRNA is the tasselseed4 (*ts4*) gene, which corresponds to a unique member of the MIR172 gene family, *zma-MIR172e*. Through a combination of double mutant analysis and immunolocalization, we showed previously that *ts4* negatively regulates *ids1* at the level of translation.

A closely related paralogue of *ids1* exists that arose before the divergence of maize from rice. This gene is expressed in a similar pattern as *ids1*, and is named sister of indeterminate spikelet1 (*sid1*). Five Mutator transposon insertions were isolated into *sid1*, all of which showed no effect on development when made homozygous. However, in *ids1/sid1* double mutants a strong enhancement of the *ids1* mutant phenotype was observed. *ids1/sid1* double mutant spikelet meristems continuously initiate lemma-like bract leaves in the both the male and female inflorescences. These bracts are sterile in the tassel, but in the ear the meristem terminates in an unusual floret-like structure that can be fertilized at low frequency. These results show that *ids1* and *sid1* function redundantly to confer spikelet meristem determinacy, and in addition, are both necessary for floral meristem initiation.

Double mutants between *sid1* and *ts4* partially suppress the *ts4* mutant phenotype, indicating that *sid1* is a target of *ts4*. In support of this, molecular evidence demonstrating the increased stability of the *sid1* transcript in a *ts4* mutant background will be presented.

P109

The regulation of expression of *Teosinte branched1* gene by light signals perceived by phytochromes in maize and Teosinte

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The number and position of vegetative axillary branches (tillers) is an important component of the shoot architecture of grasses and has been the target of domestication and improvement of maize. With the recent interest in grasses as biofuel feedstocks, shoot architecture has also become a target for enhancing biomass yield. Tillers develop from buds that are formed in the axils of leaves whose subsequent growth is determined by complex signaling networks of endogenous and environmental signals that are not well understood. *Teosinte branched1* (*Tb1*) is one of the few known genes in maize that contributes to shoot architecture of modern maize and has played an important role in the domestication process. Recent studies in sorghum indicate that the expression of *SbTb1* gene is responsive to red (R) and far-red (FR) light signals perceived by phytochromes. The R/FR is an indication of vegetative shade and an important environmental signal that regulates tiller number in grasses. To further explore the role of the phytochrome signaling pathways in the regulation of tiller development we are examining the molecular response of axillary buds to vegetative shade in maize. We are exploiting phytochrome mutants, branching maize varieties such as sweetcorn and popcorn and teosinte together with expression profiling and quantitative RT-PCR technologies. Here, we present our initial studies of bud dormancy and growth in maize and teosinte grown under simulated shade.

P110

The *sml* and *dgr* phenotypes in maize: genetic and microscopy analysis

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Shootmeristemless (*sml*) and distorted growth (*dgr*) genes are involved in maize organogenesis. *Sml* is required for SAM establishment. Its location on the long arm of chromosome 10 has been defined by traditional B-A translocation mapping followed by linkage analysis with visible as well as molecular markers. The introgression of the *sml* mutation in different genetic backgrounds has highlighted the epistatic interaction between *sml* and the unlinked *dgr* gene. Seedlings homozygous for both *sml* and *dgr* loci have a shootless phenotype whereas *Dgr*/*-sml*/*sml* seeds give rise to seedlings exhibiting defective morphogenesis (*dgr* phenotype).

Many developmental abnormalities are associated with the *dgr* phenotype. *dgr* mutants display a variety of leaf phenotype and the severity of leaf defects may also vary widely within a single mutant plant, including half leaf, thread leaf and narrow leaf. Histological analysis has revealed that even in the most severe phenotypes, xylem and phloem show a normal polarized organization and that the abaxial/adaxial leaf polarity is maintained. Also the inflorescence is affected by the mutation. The male flower is sterile and the female flower often develops secondary ears in husk leaf axils at the base of the main ear. In addition the female flower shows extra silk.

These abnormalities might be attributable to a defective organization of the mutant SAM. Histological analysis of the shoot reveals that in the mutant L1 outer layer, the cell shape is less regular than in the wild-type. It is interesting to note that the L1 layer plays a key role in shoot development: it has been shown to be necessary for maintenance of indeterminacy in the underlying meristem layers. The cloning of *dgr* and *sml* gene will lead to a better understanding of their function in plant development.

P111

Training undergraduate students in genetics: mapping maize genes involved in meristem development

(submitted by Irina Makarevitch <imakarevitch01@hamline.edu>)

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Undergraduate biology students are introduced to genetics and molecular biology in core courses during first two years and usually understand genetics processes and networks involved in regulation of organism function and appreciate how molecular genetics techniques are used to investigate gene function and interaction. However, they are frequently unable to utilize and develop this knowledge in a research environment. Our project allows undergraduates practice their skills in experimental design and data analysis as well as receive training in molecular genetics techniques through mapping maize mutations. A large collection of unique maize mutant lines with different developmental abnormalities was developed as a part of TILLING project (Till et al., 2004) and was made available to us by Dr. Springer. Here, we present the initial mapping of two genes involved in meristem development performed by two undergraduate students during an independent research study. Maize plants, homozygous for mutant alleles of these two genes are short, exhibit undeveloped meristem, abnormalities in leaf structure, and sterility. The approximate location of these two genes was determined by Sequenom technique (Springer et al., submitted). To increase the mapping resolution, we chose five linked molecular markers for each of the genes and scored mutant plants produced from F2 cross between B73 (originally containing the mutant allele of the investigated gene) and Mo17. The percent of Mo17 alleles in these F2 plants indicates the distance between the marker and the mutated gene. Using this approach, we were able to confirm the positions of both of the genes and to further refine their map position. Students involved in the project during next semesters will build on the current results to map these two genes down to a BAC and to identify their nucleotide sequence using a candidate gene approach.

P112

Transcriptional and metabolic analysis of the effects of cold and drought on the cellular growth processes in maize leaves

(submitted by Bart Rymen <bart.rymen@psb.ugent.be>)

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Limiting environmental conditions, such as low temperature and limiting water availability lead to growth retardation and reduced maize yield. To study the effects of these environmental factors, we quantified leaf growth of maize seedlings at low night temperature (4C) and at limited water supply (soil water potential of -1 MPa). Both treatments resulted in a 25% reduction of the steady-state leaf elongation rates. Although this growth reduction was similar at the organ level, a kinematic analysis revealed that different cellular dynamics played a role. Both treatments inhibited meristem activity, but in a different manner: low temperature reduced the production of meristematic cells by extending the cell cycle duration, while drought stress reduced the size of the meristem. In addition, cell expansion was affected by drought leading to reduced mature cell size, while low temperature had no effect on this parameter. These results show that these stresses induce a similar macroscopic phenotype through contrasting effects on cell division and cell expansion. To investigate this complex interplay between cell division and expansion at a molecular level, we sampled dividing, elongating and mature cells of stressed and non-stressed leaves. Transcript profiling by micro-array experiments and real-time PCR profiling of cell cycle genes revealed expression profiles that are strongly associated with the different cellular growth processes under the environmental conditions tested. These expression profiles were complemented with a quantitative metabolite profiling by GC-MS. This analysis enabled the identification of approximately 300 metabolites with characteristic profiles along the leaf growth zone and changes in response to the environmental conditions. Together these results provide a comprehensive insight into the molecular and cellular responses of maize leaf growth to limiting environmental conditions and provide a framework for the identification of candidate genes for improved stress tolerance.

P113

Transposon mutagenesis to determine the role of EPF genes in the development of maize

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The EPF gene family encodes Cys2-His2 zinc finger proteins that are putative transcription factors. Several intronless, single-finger EPF genes, including *ramosa1*, are expressed in or near meristems. Learning the specific functions of these genes may be useful in elucidating mechanisms of meristem function. We will discuss a few EPF genes we have begun to analyze for their roles in maize development. The first gene, tentatively named EPF261, is being investigated because of its possible role in tassel development. ESTs for EPF261 have been recovered only from young tassel primordia. We mapped the gene to bin 2.04, using the IBM mapping population. To determine this gene's function, we obtained a potential knockout mutant using the maize-targeted mutagenesis (MTM) system. Two MTM lines appear to have the same MuDR transposon inserted 175 bp upstream of the most 5-prime end contained in EST sequences. Homozygous individuals in both active and inactive Mutator backgrounds appear normal. In further attempts to generate knockout mutants we have begun to screen active Mutator lines for MuDR-induced deletions. Additionally, we have begun to study a paralogous gene, tentatively named EPF110, to test for redundancy. Like EPF261, this gene is expressed in young tassels. However, its expression in ear tissue as well may suggest a divergent role. Lastly, we will present some preliminary data for an EPF gene for which we detected a nearby insertion through the AcDs project. Flanking Ds sequences show that the allele possesses a Ds insertion roughly 1kb downstream of the coding sequence. Through remobilization of the Ds, we will attempt to create mutant alleles of this gene and use the data to determine the gene's function.

P114

A distinct RNA-dependent DNA methylation mechanism regulates p1 paramutation

(submitted by Lyudmila Sidorenko <lyudmila@ag.arizona.edu>)

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Paramutation at the maize p1 (pericarp color1) gene involves two epialleles; a highly expressed P1-rr (red pericarp and red cob) and a low expressed P1-rr' (patterned pericarp and pink cob). Interaction of these epialleles in a heterozygote heritably reduces P1-rr expression to that of P1-rr'. Initial studies by Sidorenko and Peterson (2001, *Plant Cell*: 13, 319-335) localized cis-acting sequences required for establishment and maintenance of paramutation to the 1.2 kb repeat structure harboring the P1.2 enhancer fragment.

Subsequent experiments in transgenic plants demonstrated that a 603 bp sub-fragment of P1.2 mediated both establishment and maintenance of paramutation. Elevated siRNA production and extensive cytosine methylation in all contexts (CG, CNG, CHH) within the cis-acting sequence in silenced P1-rr' individuals suggests involvement of RNA dependent DNA methylation (RdDM) in p1 paramutation. Further support for a role of RdDM comes from experiments with mop1. A mutation in the mop1 (mediator of paramutation 1) gene encoding an RNA dependent RNA polymerase (RDRP) disrupted establishment of P1-rr' silencing. MOP1 was also required to maintain P1-rr' silencing, although the effects were not immediate; only after two generations of exposure was sporadic reactivation observed. Experiments with other mutations affecting paramutation demonstrated that the RdDM mechanism involved in regulation of p1 paramutation has distinct characteristics. After two generations of exposure, maintenance of p1 paramutation was disrupted by a mutation in the mop3 gene, while mutations in the mop2, rmr1, and rmr2 (required to maintain repression1, 2) genes had no effect. This is different from the immediate effects these mutations have on maintenance of paramutation at the b1 (booster1) and pl1 (purple plant1) loci. Taken together these results suggest that the unique required for paramutation cis-acting regulatory sequences specify differences in the RdDM mechanism involved in p1 paramutation.

P115

Epigenetic asymmetry of imprinted alleles in maize

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Imprinting occurs in plants and mammals, and results in the differential expression of maternal or paternal alleles. However, we remain ignorant of the exact epigenetic mechanism(s) regulating this peculiar mode of allelic gene expression. We previously showed that methylation is one such epigenetic mark associated with imprinted gene expression in maize. We have investigated cytosine methylation at a number of maize imprinted loci in gametes and in the products of double fertilization the embryo and endosperm. For some imprinted loci, differential methylation is already manifest in the gametes, while for others, differential methylation is only apparent post-fertilization between parental alleles in the endosperm. Moreover, we show that these imprinted loci exhibit methylation in different cytosine contexts, which are most likely regulated by different mechanisms. We are currently investigating other epigenetic mechanisms that might be associated with the differential methylation observed at these imprinted loci.

P116

Epigenetic regulation of p11-blotched

(submitted by Kyungju Shin <ksgw3@mizzou.edu>)

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P11-Blotched is a stable epiallele of the anthocyanin regulatory gene purple plant1 (p11). Plants with P11-Blotched show variegated pigmentation in vegetative and floral plant tissues. However, the amount of pigmentation in plants with P11-Blotched can be increased in the presence of a modifier called Suppressor of plant blotching1 (Spb1). At the molecular level, the phenotypic effect of Spb1 is due to increased P11-Blotched mRNA levels and correspondingly higher levels of mRNAs for the structural genes encoding the anthocyanin biosynthetic enzymes. In addition, Spb1 leads to changes in DNA methylation at methylation-sensitive restriction sites in the promoter of P11-Blotched. These features led us to hypothesize that Spb1 alters the epigenetic state of P11-Blotched by changing its chromatin organization. To test this idea, we used DNaseI sensitivity and chromatin immunoprecipitation (ChIP) assays to examine the chromatin structure of P11-Blotched in the presence and absence of Spb1. Results show that Spb1 causes P11-Blotched to have a more relaxed chromatin configuration with histone modifications typical of active genes. Together, these data support the conclusion that Spb1 modifies P11-Blotched expression through an epigenetic mechanism.

P117

Epimutants in maize

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The mop1 gene encodes an RNA-dependent RNA polymerase that is required for paramutation and transposon silencing in maize. Our previous work has demonstrated that it takes multiple generations in a mop1 mutant background for a previously silenced MuDR element to be reactivated, and additional generations for that element to become heritably active even in the presence of the wild-type Mop1 product. The progressive nature of this process suggests that MOP1 primarily acts to "remind" the silenced element to remain inactive. In the absence of this reminder function, it takes multiple generations for the heterochromatic nature of the element to be lost. We have found similar phenomenology with respect to developmentally important genes. In a line maintained as a mop1 homozygous mutant for five generations, we have observed a wide variety of morphological variants, from ectopic ears to Lesion-mimics to polarity defects, loss of apical dominance and ectopic nodes. Each of these epimutant phenotypes is dependent on the mop1 mutation and are absent in wild-type siblings. We suggest that the loss of the Mop1-encoded reminder function in these lines has gradually resulted in the ectopic release of a variety of genes important for development. We present a model to explain these results that suggests that a subset of genes in maize are preset as "default off" using similar machinery to that used to keep transposons and paramutant alleles inactive. When the Mop1 reminder function is lost, these genes, like MuDR, become progressively more active when they should be off, resulting in ectopic expression.

P118

Mutations affecting transcriptional transgene silencing in maize

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Previous work led to the isolation of a line, referred to as Btg-s, bearing a stably and transcriptionally silenced transgene. The transgene includes the coding region for a transcription factor that activates the anthocyanin biosynthesis pathway, enabling easy visual identification of plants with a transcriptionally active transgene. By crossing with known mutants and observing transgene reactivation, five genes were demonstrated to be required for silencing of this locus in the Btg-s line (McGinnis et al., 2006). Recent molecular cloning of two of the five tested genes suggests that at least one mechanism involved in this regulation is the RNA-mediated heterochromatin pathway (Alleman et al., 2006; Hale et al., 2007). These results suggested that the Btg-s line provides a powerful assay for heritable, RNA-mediated epigenetic regulation of transcription in maize. To identify other genes involved in this process, a forward genetic screen was initiated using an EMS mutagenized population of plants with the Btg-s transgene. Two classes of phenotypes were observed in 380 M2 families, and a total of 11 putative mutants have been identified thus far. Complementation and allelism tests are in progress to determine which mutations represent novel genes. Future efforts will focus on detailed phenotypic characterizations of each mutant.

P119

Paramutation: RNA-mediated heritable silencing

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Paramutation is an interaction between alleles that sets up distinct epigenetic states that leads to a heritable decrease in gene expression of one allele. One classic example of paramutation is the b1 locus in maize, which encodes a transcription factor that activates the biosynthesis of purple anthocyanin pigments. Two alleles are involved in b1 paramutation: the paramutable, darkly pigmented and highly expressed B-I allele, and the paramutagenic, lightly pigmented and extremely stable B allele. When B and B-I are crossed together, paramutation always occurs. The key sequences required for paramutation are noncoding tandem repeats located ~100 kb upstream of the b1 transcription start site. An RNA-dependent mechanism is critical for paramutation in maize. Transcription occurs on both strands of the tandem, which may lead to the production of double-stranded RNA (dsRNA). In addition, an RNA-dependent RNA polymerase (RdRP), mediator of paramutation1 (mop1), which is most similar to RDR2 and which has been implicated in the production of siRNA (short interfering RNA) molecules in *Arabidopsis thaliana*, is absolutely required for paramutation at several maize genes, including b1. We show that the presence of tandem repeat siRNAs depends on mop1, yet they are observed in all genotypes, even those that do not undergo paramutation. We hypothesize that mop1 is required to maintain a threshold level of repeat RNA, which functions in cis to regulate transcription of b1 and in trans to establish and maintain the heritable chromatin states associated with paramutation.

P120

RIF1 (R Interacting Factor1) links pigment formation and chromatin functions

(submitted by Antje Feller <feller.11@osu.edu>)

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One of the largest transcription factor families in plants is characterized by the presence of a basic-helix-loop-helix (bHLH) domain. The first identified plant bHLH factor corresponded to the product of the maize R gene, which functions as an essential co-activator of C1 (R2R3-MYB) in the activation of maize anthocyanin biosynthetic genes. Although the bHLH domain is highly conserved, its role in transcriptional regulation is not well established. By comparing the activation of endogenous genes and promoter-reporter constructs, we show that the bHLH domain of R is necessary for the activation of the former but dispensable for the regulation of the latter, suggesting a possible involvement in chromatin functions. We identified RIF1, a partner for the R bHLH region, as a novel, AGENET containing, EMSY-like factor. In humans, EMSY is a partner of BRCA2 and plays a role in histone modifications during DNA repair. Knock-down experiments in maize Black Mexican Sweet cells showed that RIF1 is required for regulation of endogenous flavonoid genes, but not for activation of transiently expressed genes. Furthermore, we established by ChIP experiments that RIF1 is part of the C1/R regulatory complex on at least one of the flavonoid gene promoters. The specific role that RIF1 plays in that complex will be discussed.

P121

Role of chromatin modifiers in exonic methylation-mediated transcriptional silencing of *Arabidopsis phyA'* epiallele

(submitted by Gulab Rangani <grangani@uark.edu>)

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The role of exonic methylation in gene expression is not well understood. We isolated a transcriptionally suppressed epi-allele of *Arabidopsis thaliana* phytochrome A gene termed *phyA'*, which shows methylation only in symmetric CG sites (^mCG) resident to exonic regions. These exonic modifications confer a strong *phyA'* mutant phenotype, characterized by elongated hypocotyl in seedlings grown under continuous far-red light. De-methylation of *phyA'* in the DNA methyltransferase I mutant (*met1*) background resulted in its reversion to the wild-type phenotype and expression level, confirming the pivotal role of the ^mCG in *phyA'* silencing (Chawla et al., 2007). In the present study, we sought to determine roles of chromatin modification genes in *phyA'* silencing. For this purpose, both chromatin genes and RNAi genes were chosen. CHROMOMETHYL TRANSFERASE (CMT3) and DOMAINS REARRANGED METHYLASE (DRM 1/2) that are responsible for maintaining CNG methylation and de novo methylations, respectively, had no role in *phyA'* silencing. This confirms the importance of ^mCG maintenance in *phyA'*. Further, RNA-DEPENDENT RNA POLYMERASES (RDR2 and RDR6), ARGONAUTE 4 (AGO4) and SGS3 were dispensable for *phyA'* silencing, suggesting that *phyA'* silencing is not dependent on RNA species. Additionally, weak alleles of *ago1* and *ddm1* (SALK lines) were unable to cause phenotypic reversion of *phyA'*. The study on determining the role of KRYPTONITE (H3-K9 methyltransferase) is underway. The details of these genetic analyses will be presented.

References:

Chawla R, Nicholson SJ, Folta KM, and Srivastava V (2007) Transgene-induced silencing of Arabidopsis Phytochrome A gene via exonic methylation. *Plant Journal* 52: 1105-1118.

P122

Sequence composition of functional maize centromeres provides insight into maize genome evolution

(submitted by Gernot Presting <gernot@hawaii.edu>)

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We have identified the functional centromere regions of all ten maize chromosomes by chromatin immunoprecipitation with an antibody to the centromeric histone CENH3. BAC clones from unanchored centromeric FPC contigs were mapped to the maize genetic map (IBM) using novel centromere markers derived from repeat junctions and retrotransposon display. Custom "JunctionViewer" software was used to identify repeat junctions. Singleton BACs containing the centromeric satellite sequence CentC were subjected to 1-2x survey sequencing in an attempt to close gaps between centromeric FPC contigs. Examination of centromeric satellite repeats and retrotransposons (CRM) with respect to sequence evolution and chromosomal distribution provide first insights into maize centromere evolution.

P123

Submergence stress responsive microRNA genes in maize

(submitted by Zuxin Zhang <nxzzx@hebau.edu.cn>)

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MicroRNAs (miRNAs) play critical roles in regulating gene expression at the post-transcriptional level. Several recent studies showed important functions of miRNAs in response to adverse abiotic stresses. For exploring the role of miRNAs in submerged maize roots, a custom microfluidic array (LC Sciences, USA) containing version 10.0 plant MicroRNA probes was used to assay differential expressed miRNAs. Small RNAs isolated from 0 h, 12 h, 24 h and 36 h treated roots were respectively hybridized with the microarray, each sample data were collected from 3 independent hybridization replications. Real Time PCR and RT-PCR respectively were employed to examine expression of specific miRNAs and target genes. Microarray data revealed that the expressions of miRNAs were significantly altered at the p

P124

Unlocking heterosis: Implications of epigenetic polymorphism by considering maize as both a polyploid and a diploid

(submitted by Josphert Kimatu <josphert@yahoo.com>)

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Although heterosis has perhaps been exploited to the fullest extent in maize than in any other crops, the molecular basis of this phenomenon still remains largely elusive even in this crop. Maize had long been regarded and studied as a diploid, but recent sequencing and genomic data clearly indicated its polyploid origin. The intrinsic nature of genetic and epigenetic liability often associated with polyploidy as demonstrated in other plants may bear relevance to novel gene expression pattern, and hence, heterotic phenotypes, in interstrain hybrids of maize, given the exceptionally nonsyntenic property between maize inbred lines. Thus, the new knowledge and understanding of maize as a structurally and functionally diploidized ancient polyploid may promote our understanding on the molecular basis of heterosis in this important crop from a new perspective.

P125

A guardian of grasses: specific origin and conservation of a unique disease resistance gene in the grass lineage

(submitted by Satya Chintamanani <satya@purdue.edu>)

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The maize Hm1 gene provides protection against a lethal leaf blight and ear mold disease caused by *Cochliobolus carbonum* race 1 (CCR1). Although it was the first disease resistance (DR) gene to be cloned, it remains a novelty because, instead of participating in the plant recognition and response system as most DR genes do, Hm1 disarms the pathogen directly. It does so by encoding an NADPH-dependent reductase, whose function is to inactivate HC-toxin, an epoxide containing cyclic tetrapeptide, which the pathogen produces as a key virulence factor to colonize maize. While CCR1 is strictly a pathogen of maize, orthologs of Hm1 as well as the HC-toxin reductase activity are present in the grass family, suggesting an ancient and evolutionarily conserved role of this disease resistance trait in plants. Here we provide proof for such a role by demonstrating its involvement in nonhost resistance of barley to CCR1. Barley leaves in which expression of the Hm1 homologue was silenced became susceptible to infection by CCR1, but only if the pathogen was able to produce HC-toxin. Phylogenetic analysis indicated that Hm1 evolved exclusively and early in the grass lineage. Given the devastating ability of CCR1 to kill maize, these findings imply that the evolution and/or geographical distribution of grasses may have been constrained if Hm1 did not emerge.

P126

Analysis of nucleotide diversity near a maize domestication locus: Implications on the evolution of ramosal1

(submitted by Brandi Sigmon <bsigmon@iastate.edu>)

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The maize ramosal1 (ra1) gene encodes a transcription factor involved in the branching architecture of maize inflorescences. ra1 is a hypothesized domestication gene because its nucleotide diversity is reduced in maize inbreds. Statistical tests indicate that selection for specific versions of the ra1 gene occurred at some point since maize diverged from its common ancestor with *Tripsacum*. To further investigate the hypothesis, nucleotide diversity of ra1 was sampled in a broad panel of teosintes and maize landraces. HKA tests showed significantly reduced nucleotide diversity for the landraces, but not teosintes, supporting the hypothesis that ra1 may be a domestication locus. In the landraces, the 3' noncoding sequence showed the greatest reduction in nucleotide diversity, but both 3' and 5' noncoding sequence showed greater reduction than the coding sequence. Also, reconstructions of the maize-teosinte haplotype tree for each of the three regions show a more neutral topology for the 5' noncoding and the coding sequence but a more domestication-like topology for the 3' region, suggesting the target of selection may be located in the 3' direction of ra1. Initial diversity surveys of genes close to ra1 suggest the selective sweep extends at least 370kb in one direction. Additional diversity surveys of other linked genes will be needed to assess the full extent of the selective sweep in the ra1 region of maize chromosome 7. This region shows conserved synteny with regions in rice and sorghum and with a homeologous region on chromosome 2 in maize. Deviations in synteny include an absence of ra1-like homologs in rice and the homeologous region of maize chromosome 2. These observations suggest ra1 was gained once following the divergence of the Andropogoneae from rice followed by the loss of sequence from one homeologous region in maize which would have occurred following the tetraploidy event.

P127

Bioinformatic selection of syntenic sorghum BACs with maize core bin markers for use as FISH probes in the development of a cytogenetic map of maize

(submitted by Katherine Beckham <kdb05e@fsu.edu>)

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The overall goal of this project is to develop a cytogenetic map of the maize genome using maize marker-selected sorghum BACs. Here we describe our progress toward cytogenetically mapping the maize Core Bin Marker (CBM) loci or nearby surrogates on chromosomes 2, 7, and 10. We have manually added the locations of the CBMs onto maize-sorghum and maize-maize synteny maps as displayed by SyMAP (<http://www.agcol.arizona.edu/symap>). Visualization of the syntenic regions between maize and sorghum allows us to choose corresponding sorghum BACs for use as fluorescence in situ hybridization (FISH) probes. For each locus to be mapped we choose 4-8 overlapping Sorghum propinquum BAC clones. The clone homology is verified by Southern blot analysis of the sorghum BACs using maize RFLPs as DNA hybridization probes. A single representative BAC clone is then selected for FISH mapping as described by Koumaris and Bass (2003, Plant Journal, 35:647) and Amarillo and Bass (2007, Genetics, 177:1509). The development of this cytogenetic map will contribute to our understanding of the structure and evolution of the maize genome. The project is described at cytomaize.org and the resulting FISH mapping data will be released by MaizeGDB using the format developed for the FSU Cytogenetic FISH 9 map.

P128

Comparative sequence analysis of the maize *rfl* locus

(submitted by Roger Wise <rpwise@iastate.edu>)

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Comparative genome analysis is a useful tool for identification of conserved gene regions and analysis of genome evolution across organisms. Comparing closely related germplasms of a single species can show areas that experience mutation and recombination within short periods of evolution. We have sequenced and annotated two contigs totaling 1.6 Mb near the centromere of maize B73 chromosome 3 in order to cover the locus of the cytoplasmic male sterility restoration gene *rfl*. This region contains 54 genes and numerous retrotransposon clusters, the largest of which spans 600 Kb. To provide a comparative genome analysis of the *rfl* locus we have constructed a BAC library of the *Rfl* containing genotype, Wf9-BG. Overgos were developed across the 1.6 Mb B73 contigs and PCR primers were designed in the annotated genes, these were used as Southern probes against the Wf9-BG BAC library. The hybridizations identified approximately 1,000 Wf9-BG BACs that are homologous to the B73 region. These BACs were high information content fingerprinted (HICF) to develop a physical map, FPC formed several BAC contigs. These contigs will be joined with PCR mapping and Southern hybridizations designed off the B73 contig sequence. Two overlapping Wf9-BG BACs corresponding to two B73 gene clusters have been sequenced and annotated. The two contigs share six genes in this region, with order and orientation retained across genotypes. One retrotransposon cluster is conserved between the two sequences while a second repetitive region contains differing sequences, suggesting a recombination break point near the *rfl* locus.

P129

Constructing a cytogenetic map of maize in oat addition lines using sorghum BACs as FISH probes

(submitted by Debbie Figueroa <figueroa@bio.fsu.edu>)

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We are currently producing a new cytogenetic FISH map of using maize RFLP marker-selected sorghum BACs as described by Koumbaris & Bass (2003, *Plant J.*, 35:647). Here we describe findings from our recently developed high-density map of chromosome 9 (Amarillo & Bass, 2007, *Genetics*, 177:1509) and progress towards a Core Bin Marker (CBM) FISH map of chromosomes 1, 3, 4, 5, 6, and 8. The new maize 9 FISH map consists of 32 cytogenetic loci, each derived from a single sorghum BAC. We found that the markers appeared in the same order on linkage and cytogenetic maps, but their distribution revealed three large regions of maize genome hyperexpansion. These over-expanded regions are found in bins 9.02, 9.04, and 9.06. Interestingly, none of these correspond to known heterochromatic regions such as those found at centromeres or knobs. In addition to chromosome 9, we are FISH mapping pairs of chromosome segments using single sorghum BAC probes to detect duplicated regions, such as those between chromosomes 1 and 5. To demonstrate the feasibility of this approach we used sorghum BACs mapped onto chromosome 9 to detect corresponding duplicate loci on chromosome 1, thus increasing the value of these BACs for structural genomics. Preliminary FISH mapping of CBM loci chromosomes 1 and 4 is also presented. The resulting cytological maps will provide a means for integrating the physical, genetic, and cytological maps of maize with an added dimension of genome-wide cross-anchoring to the maps of sorghum. This project is described at cytomaize.org and mapping and image data are released through MaizeGDB.

P130

Mapping maize centromeres reveals a genetic basis of centromere evolution

(submitted by Jinghua Shi <jshi@plantbio.uga.edu>)

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Centromeres are typically composed of highly repetitive DNA sequences that diverge rapidly. This repetitive feature makes it difficult to construct either genetic or physical maps. Therefore, centromeres remain mysterious and little is known about the mechanism that drives centromere evolution. By combining CENH3 (a fundamental kinetochore protein) chromatin immunoprecipitation (ChIP) and CRM (centromeric retrotransposons in maize) display, we precisely mapped all ten maize centromeres. The data suggests that double recombination events happen frequently around functional centromeres and provide a viable mechanism for centromere evolution.

P131

RFLP Full-Length Insert Sequence (RFLP-FLIS) data for use in the cytogenetic map of maize project

(submitted by James Davis <jdd03f@fsu.edu>)

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The maize genome is partitioned into ten individual chromosomes that can be navigated using a linkage, physical, or cytological map. The Cytogenetic Map of Maize project serves to integrate the linkage and cytological maps of maize (Koumbaris & Bass, 2003, *Plant Journal*, 35:647; Amarillo & Bass, 2007, *Genetics* 177:1509). The genome is being mapped with sorghum Bacterial Artificial Chromosomes (BACs) as fluorescence in situ hybridization (FISH) probes on maize pachytene-stage chromosome spreads. These sorghum BACs are selected by a laborious filter hybridization screening method using maize restriction fragment length polymorphism (RFLP) probes. To expedite this selection process, we are sequencing selected public RFLP probes to enable more rapid in silico BLAST-based screens. We determine the full length insert sequence of each probe using both-strand sequencing and manual assessment of chromatograms. Next we validate their identity via BLAST searches, annotate, and submit them to GenBank. Two groups of RFLP probes, all of the Core Bin Markers (CBM), and some of the framework markers from the UMC-98 map, have been sequenced. Links to the RFLP-FLIS data for maize core bin markers are available through the MaizeGDB Bin Viewer web page. In conjunction with PlantGDB curators, we designed a custom RFLP database (PlantGDB BLAST button "Zea mays RFLPs") to assist in our bioinformatic sequence assignment and analysis efforts. Resulting data are being released on public databases such as MaizeGDB, GenBank, and PlantGDB. A progress report on this effort will be presented.

P132

The Maize TILLING Project: Updates and EcoTILLING industrial germplasm

(submitted by Rita-Ann Monde <rmonde@purdue.edu>)

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The Maize TILLING Project (MTP) has a collection of ~14,000 EMS-mutagenized B73 and W22 lines that can be used as both a reverse and a forward genetics resource. We currently screen DNAs from ~3500 B73 and ~2300 W22 lines from these collections and continually add more lines of each. We are evaluating mutation density in three newly mutagenized populations of both inbreds, one that we have made and two that have been contributed by the community.

MTP has delivered 576 mutations (279 non-silent) for 105 gene targets, and find an average of 5.5 new alleles per target screened. An additional 37 screens are in progress. Mutation data is regularly updated at MaizeGDB and our website (<http://genome.purdue.edu/maizetilling/>).

We continue to provide EcoTILLING data on natural variation in targets submitted for TILLING. The number of Maize Diversity Lines evaluated has been expanded to 86 and we have added ten industry inbreds recently off-patent. Our evaluations of these industrial lines for ~20 genes indicate they contain the same amount of sequence variation as the other Diversity Lines, suggesting that at least these lines are no more depleted of genetic diversity than the rest of the germplasm worldwide.

Massively parallel sequencing methods have made resequencing specific genes in thousands of lines feasible. Purdue University now has an ABI SOLiD sequencer and we are developing protocols to switch mutation discovery from Cell based TILLING to Targeted Resequencing Using Massively Parallel methods (TRUMPing). The advantages include: 1) lower cost to users (

P133

A survey study of transcriptional factors from developing maize seeds

(submitted by Rentao Song <rentaosong@staff.shu.edu.cn>)

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The development of maize seed results from spatial and temporal expression of a series of genes, which are regulated by transcription factors (TF). In this study, we constructed a full-length normalized cDNA library from maize seeds covering different development stages. We randomly sequenced 10,848 clones from this library. These sequences were clustered into 6,330 unigenes. From these unigenes, we identified 57 TFs that belong to 7 TF families including bZIP, AP2/EREBP, Zinc-finger, MADS, bHLH, Homeodomain and MYB. We analyzed these TFs' expression status in different maize tissues and expression patterns during maize seed development.

P134

An Enhancer trap system for ploidy studies in maize

(submitted by Siva Chudalayandi <chudals@missouri.edu>)

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We are interested in determining cell and developmental gene expression changes in different ploidies and hybrid backgrounds in maize. To achieve this objective we have developed an enhancer trap system for maize with a GFP reporter. The enhancer trap vector consists of an m-GFP reporter driven by a minimal 35S promoter and a plant selectable marker (bar gene) cloned within a Ds element. This cassette disrupts a 35S-maize C1 gene (excision marker). All these elements were cloned within the right and left borders of a binary vector, which was used to transform Hi II maize by Agrobacterium mediated transformation. We have obtained transgenic plants from 15 different events. On crossing the transgenic plants with a line containing an immobile Ac element we have successfully shown that we can induce the Ds element to jump, thus restoring the C1 gene activity. The kernels from this cross are mosaic purple. Further development will entail identifying several lines in which germinal transposition of Ds has occurred, which will be screened for lines with GFP expression (indicating trapped enhancers) and self pollinated to remove Ac. In addition to ploidy studies, the enhancer trap lines will serve as an important tool for the maize community in functional genomics.

P135

An integrated expression profiling system for maize

(submitted by Roger Wise <rpwise@iastate.edu>)

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The sequenced maize genome offers the opportunity to conduct highly parallel expression profiling using "all genes" platforms. We describe a next-generation, integrated expression profiling system for maize, a 100K Affymetrix GeneChip built from evidence-based gene predictions of the B73 nuclear, chloroplast and mitochondrial genomes. The expression of each gene is assayed by 25 x 25nt oligo probes distributed optimally across the exon space, allowing quantification of transcript levels, interrogation of splicing, and maximized coverage for discovery of sequence polymorphisms. To add value to the generated data, statistical and visualization tools will be supported by the PLEXdb (Plant Expression) database (<http://plexdb.org/>). PLEXdb features MIAME-compliant experiment annotations as well as required Plant Ontology terms through PLEX Express, its user-friendly, web-based submission tool. For every probe set on the chip, links to genome resources in PlantGDB, Uniprot and NCBI annotations, as well as genome alignments on the Maize- (<http://www.maizesequence.org>) and Rice-genome browsers (<http://www.gramene.org>), promote the integration of genetic, physical, and expression resources for grass genomics. Experimental datasets can be visualized according to whichever factors are specified (genotypes, treatments, timecourses, etc.), or browsed by gene or expression pattern. Users may also import their own list of gene or probe set names from off-line analyses and compare them in various combinations: union of two gene lists, intersection of both gene lists and unique genes in either gene list. Further value will be added to this platform as MaizeGDB transitions to being a sequenced-based MOD, which will facilitate construction of interconnected modules in both databases.

P136

Autopolyploidy effects on leaf proteomes in a ploidy series of maize inbred Oh43

(submitted by Hong Yao <yaoho@missouri.edu>)

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Autopolyploidy alters the phenotypes of maize plants. In an Oh43 ploidy series of maize (2N, 4N and 6N), plants of higher ploidy exhibit decreased stature and growth rate, late flowering and reduced fertility, and increased size of stomata and pollen. To gain insight into the molecular basis of the phenotypic changes caused by autopolyploidy, we compared proteomes of adult leaf tissues among the 2N, 4N and 6N plants via 2D DIGE (Difference Gel Electrophoresis) analysis. Among the 1,098 spots present in at least 15 out of the total 18 gel images analyzed by using the DeCyder 2D software (version 6.5; GE Healthcare), the majority do not show significant differences (threshold: P-value < 0.05 in student's t-test) associated with ploidy change. Only 29 spots differ significantly in all three comparisons (2N vs. 4N, 4N vs. 6N and 2N vs. 6N) and 19 of them exhibit the same trend of change from 2N to 4N and from 4N to 6N. Protein abundances of 18 out of the 19 spots are up-regulated by the ploidy level. Relatively larger differences were detected in the 6N vs. 2N and 6N vs. 4N comparisons than in the 4N vs. 2N comparison. No qualitative variations were detected in our experiment. Mass Spectrometry revealed the identities of some ploidy-modulated proteins.

P137

Characterization of inaccurate repair of chromosomal double-strand breaks in maize

(submitted by Fang Lu <fanglu@uga.edu>)

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Chromosomal double-strand breaks (DSBs) occur frequently in somatic cells. The repair of DSBs is essential for cell survival and for the maintenance of genome integrity. In this study, we utilized I-SceI, an endonuclease from yeast with an 18 base pairs recognition sequence to introduce DSBs in maize cells. We then employed PCR to characterize the inaccurate repair events that removed the I-SceI recognition site. Of 96 inaccurate repair events characterized, 71 were associated with deletions, 16 were associated with both deletions and insertions, 6 were associated with insertions, and 3 were SNPs. Of the 71 deletion-only events, ~39% ranged in size from 1 to 9 base pairs. The sequences flanking these deletions and insertions have the hallmarks of illegitimate recombination. These results are compatible with models suggesting that inaccurate repair of double-strand DNA breaks is the major factor responsible for the rapid removal of unselected DNA from higher plant genomes.

P138

Comparative analysis of C3 and C4 leaf development in rice, sorghum and maize

(submitted by Timothy Nelson <timothy.nelson@yale.edu>)

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C4-type plants such as maize and sorghum possess complex traits that enhance their efficiency of carbon-fixation, water and nitrogen use, and performance in high temperature and light, in comparison to C3-type plants such as rice. The key C4 traits are (1) cooperation of two leaf cell types (mesophyll and bundle sheath) for carbon fixation and photosynthesis, (2) enhanced movement of metabolites between cooperating cells, and (3) high density of leaf venation. These appear to be regulatory enhancements of features present in less-efficient C3 plants. Although C4 plants have evolved at least 50 times independently in various taxonomic groups, the molecular basis of key C4 traits is insufficiently understood to permit their introduction into important C3 plants to enhance their performance as agricultural or biofuel feedstocks.

We are comparing the leaves of rice (a C3 grass), maize (a moderate C4 grass) and sorghum (an extreme C4 grass). Transcriptomes, proteomes and metabolites will be compared along a developmental gradient from immature tissues at leaf base to mature tissues at the leaf tip. To align the gradients of the three species, markers for developmental time points in gene expression, protein accumulation, and sink-source transition are used. Mesophyll and bundle sheath cells are obtained for comparative profiling from each leaf stage by laser microdissection. Two hypotheses will be tested by the comparative analysis of the corresponding C3 and C4 plant datasets: (1) To produce C4 traits, plants use networks of genes, proteins, and metabolites that are already present in C3 plants, and (2) C4 features are plastic and expressed in a degree that depends on environment and developmental stage.

Project outcomes will be available through a project-specific public website (C3-C4DB, <http://c3c4.tc.cornell.edu>), and curated into the Gramene public database (<http://www.gramene.org>). Supported by NSF award DBI-0701736

P139

Comparative analysis of divergent and convergent gene pairs, their expression patterns, and bidirectional promoters in rice, Arabidopsis, and Populus

(submitted by Wusirika Ramakrishna <wusirika@mtu.edu>)

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We present a study of the coexpression and inter-species conservation of pairs of adjacent genes with divergent and convergent arrangement in three plant species with complete genome sequences: rice (*Oryza sativa*), *Arabidopsis thaliana*, and *Populus trichocarpa*. Using genome-wide microarray and Massively Parallel Signature Sequencing (MPSS) expression data, strong coexpression, as determined by Pearson correlation, was observed in large numbers of divergent and convergent gene pairs in all three species. Furthermore, a correlation between intergenic distance in divergent and convergent gene pairs and the probability of coexpression was observed, in varying degrees, in all three species. Cross-species conservation of coexpressed gene pairs was also tested for, and it was found that, despite the high degree of coexpression observed among adjacent genes, divergent and convergent arrangement is conserved for only a small fraction of gene pairs. The frequency of gene pair conservation does, however, appear to be increased when the paired genes' expression is strongly correlated or when they belong to the same Gene Ontology categories. Furthermore, we explored novel bidirectional promoters shared by divergent genes. We present a study that compares putative bidirectional promoters shared by divergent genes in three plant species: rice (*Oryza sativa*), *Arabidopsis thaliana*, and *Populus trichocarpa* with three databases: PLACE, FGENESH NSITE and PLANT CARE. We identified three cis-regulatory motifs: SORLIP2AT, SITEIIATCYTC, UPIATMSD, which are overrepresented in putative bidirectional promoters in all the three genomes.

P140

Completing the expression catalog of the Arabidopsis transcriptome by quantitative real time PCR

(submitted by Yongli Xiao <yxiao@jcvl.org>)

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Gene expression in *Arabidopsis thaliana* has been studied extensively using a variety of platforms, including cDNA microarray, Affymetrix gene chip, whole genome tiling array, massively parallel signature sequencing. However, there are still many genes in the *Arabidopsis* genome that could not be profiled effectively by any of these methods mentioned, generally due to their low abundance in mRNA populations. We are generating expression profiles by quantitative real time PCR (qRT-PCR) for 4,000+ *Arabidopsis* genes for which expression data are currently unavailable. To date, we have performed qRT-PCR on about 2,000 genes that either lack reliable expression data from or are not represented on the ATH1 array using mRNAs from leaf, root and T87 cell culture and seedlings treated with IAA, SA and salt. Over 90% of the genes were expressed in at least one of our current cDNA populations and ~40% of them showed differential expression in at least 2 out of 6 conditions. We are using co-expression analysis to associate these low-expressing genes with functionally annotated genes and pathways. In addition, we have developed a high throughput pipeline to generate promoter-reporter constructs and transgenic *Arabidopsis* plants for 1,000 genes of unknown function. So far, promoters from 587 genes have been cloned into a GFP reporter construct, 495 have been transformed into *Arabidopsis*. All the GFP expression patterns detected in these transgenic plants are localized to small regions of tissues and cell types. Both the qPCR and reporter construct data can be found at www.tigr.org/tdb/e2k1/ath1/qpcr/index.shtml. Supported by NSF 2010.

P141

Construction of a sequence indexed transposon resource based on the UniformMu maize population

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As the sequencing of the maize genome nears completion, efforts to understand the functions and interactions of maize genes will benefit from new resources for systematically generating genetic mutations that disrupt every gene. Toward this end, several large collections of maize plants containing random Mutator transposon insertions, including the inbred UniformMu population, have been created that combined offer the prospect of saturating the genome with insertional mutations. To facilitate identification of mutations in genes of interest in the UniformMu-W22 population, a DNA sequence based index of the location of each insertion site in the genome will be created using massively parallel DNA sequencing technologies. For efficient indexing and mapping of insertion sites to individuals, plants will be organized and sampled using a series of 24 X 24 plant grids. We have implemented improved methods for efficient extraction of Mu flanking sequences that achieve unbiased representation of all germinal insertions in individual genomes. When the current project is completed, the sequence index database will contain annotations of up to 100,000 unique transposon insertion sites in a collection of at least 8,050 genetically stable, sustainable maize stocks. A public interface to the resource including annotation and phenotype data will be provided through the MaizeGDB.org web portal, which will enable individual researchers to identify mutations of interest and obtain seed of mutant stocks from the Maize Stock Center. We anticipate release of the first dataset in Spring 2008.

P142

Development of an inducible two-component gene expression system for maize

(submitted by Jose Gutierrez-Marcos <j.f.gutierrez-marcos@warwick.ac.uk>)

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An important step in understanding gene function is to be able to control the temporal and spatial expression pattern of any given gene of interest. Until now, this has proven difficult in maize and in most other monocots, due to the lack of suitable genetic tools available. In order to overcome this limitation we have developed a transgenic system that allows for the controlled expression - either downregulation or overexpression- of genes of interest. Here we describe a dexamethasone inducible two-component system that can be used to effectively manipulate gene expression in maize. Furthermore, this system can also be applied to other agronomically important monocot crop species, such as barley, rice and wheat.

P143

Distinctive transcriptome responses to adverse environmental conditions in *Zea mays* L.

(submitted by Darren Morrow <djmorrow@stanford.edu>)

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Maize seedling transcriptome responses to six abiotic perturbations (heat, cold, darkness, desiccation, salt, UV-B) were analyzed. Approximately 7800 transcripts are expressed in one or more treatments compared to light-grown seedling plus juvenile leaves from field-grown plants; about 5200 transcripts are expressed in one or more treatments and absent in the light-grown seedling. Approximately 2000 transcripts were unique to one treatment. Salt and heat elicited the largest number of transcript changes, however, salt resulted in mostly decreased abundance of transcripts while heat-shock resulted in mostly increased transcripts. A total of 384 transcripts were common to all stress treatments and not expressed in light grown seedling, and 146 transcripts were present in light grown seedlings and absent from all stress treatments. A complex pattern of overlapping transcripts was found and a significant pattern of congruence in the direction of transcript change between pairs of treatments was uncovered. From the analysis it appears that the scope of gene expression changes is determined by the challenge, indicating specificity in perception and response. Nonetheless, transcripts regulated by multiple responses are generally affected in the same manner indicating common or converging regulatory networks. The data are available for additional analysis through a searchable database.

P144

Evolution of maize defense gene expression altered by *Wolbachia*

(submitted by Georgia Davis <davisge@missouri.edu>)

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Western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte is a major pest in the maize fields of North America causing tremendous economic loss. WCR vectors *Wolbachia* spp., an obligate intracellular bacterium that has recently been shown to down regulate plant defense gene expression in maize (*Zea mays* L.). WCR originated as a maize pest in Mesoamerica and then moved northward through the United States. It was introduced to Europe in 1992. To date WCR has not spread to Africa. This experiment investigates the response to WCR feeding in relation to geographic origin of the inbred line and length of evolutionary exposure to WCR in relation to defense gene expression. Twenty-eight diverse maize lines were grown to the V3 stage in the growth chamber and subjected to one of three treatments: no treatment, mechanical wounding, or infested with WCR. Samples were collected for RNA preparation 24 hours after infestation or 5 hours after mechanical wounding. One step RT-PCR using SYBR green was performed with primers to several maize defense genes. Significant differences in gene expression were observed among lines, for lines x treatments, and among regions of inbred line geographic origin for the maize PR-1 gene. Similar results were obtained for the maize PR-3/4 (chitinase) gene. No significant differences among regions of origin were observed for the mechanical wounding control.

P145

Expression profiling of sixteen maize tissues reveals extensive overlap in gene expression between tissues as well as differential and tissue specific gene expression

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Sixteen maize tissues were selected for gene expression profiling using 70-mer oligonucleotide arrays that had been developed by the Maize Microarray Project. The tissues represented a range of developmental stages and tissue types including seedling roots, leaves (adult and seedling), immature tassels and ears, embryos (14 and 18 DAP), endosperm (14 and 18 DAP), silks, pollen, HiII callus, and black mexican sweet cell suspension cultures. For any given tissue, ~50% of the array elements reported gene expression using our expression cutoff criteria. For the 57,000 array elements, ~23% were expressed in all tissues examined while 27% were not expressed in any tissue. Not surprisingly, oligos designed to an EST singleton or AZM gene model were less effective in detecting expression relative to oligos designed to EST Tentative Contigs (TCs), consistent with EST TCs representing the more highly expressed fraction of the maize transcriptome. Pair-wise comparisons between tissues revealed a wide range in the number of differentially expressed (DE) genes between tissues, with immature ears vs. immature tassels having the lowest number of DE genes, and adult leaves vs. 18 DAP endosperm having the highest number of DE genes. An analysis of the sixteen tissues revealed that pollen and black mexican sweet suspension culture had the largest number of tissue specific genes.

P146

Expression variation associated with artificial selection for grain protein concentration in maize

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Evolutionary changes in mRNA expression are important contributors to phenotypic differences between species. Thus, documenting expression variation in response to artificial selection is likely to reveal genomic targets of directed evolution within species. The Illinois Protein Strains represent four related populations that since 1896 have been subjected to more than 300 combined cycles of divergent recurrent selection for grain protein concentration. This selection program has produced lines that span the known phenotypic extremes in maize for grain composition and a number of correlated traits, suggesting these populations may reveal mechanistic insights into responses of the maize genome to artificial selection.

This study tests the hypothesis that genes which show altered RNA expression among the Illinois Protein Strains are also likely to be functional targets of phenotypic selection. Microarray experiments comparing leaf and seed RNA expression profiles from the Illinois High Protein (IHP), and Illinois Low Protein (ILP) lines have found thousands of features that show significant expression differences ($FDR \leq 0.05$) between these divergently selected genotypes. Similar mRNA expression profiling experiments that with Reverse Illinois High Protein and Reverse Illinois Low Protein strains identified a subset of features for which expression patterns also reversed, suggesting functional association with protein concentration. These genes include those encoding the 19kDa and 22kDa α -zeins (endosperm storage proteins), genes involved in nitrogen assimilation within leaves and genes that participate in the remobilization of nitrogen from leaves to seeds. DNA sequencing and RT-PCR assays validated RNA expression differences for 37 out of 41 genes whose expression varied more than 10-fold between IHP and ILP in microarray analyses. Our results suggest that selection has altered multiple physiological pathways and the expression of their associated genes.

P147

Expressional profiling study revealed unique expressional patterns and dramatic expressional divergence of maize alpha-zein super gene family

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The alpha-zein super gene family encodes the most predominant storage protein in maize (*Zea mays*) endosperm. In maize inbred line B73, it consists of four gene families with 41 member genes. In this study, we combined quantitative real-time PCR and random clone sequencing to successfully profile the expression of alpha-zein super gene family during endosperm development. We found that only 18 of the 41 member genes were expressed, and their expression levels diverge greatly. At the gene family level, all families had characteristic zig-zag expressional patterns that diverged into two major groups. At the individual gene level, member genes showed dramatic divergence of expression patterns, indicating fast differentiation of their expression regulation. A comparison study among different inbred lines revealed significantly different expressed gene sets, indicating the existence of highly diverged haplotypes. Large gene families containing long gene clusters, e.g. z1A or z1C, mainly contributed the highly divergent haplotypes. In addition, allelic genes also showed significant divergence in their expressional levels. These results indicated a highly dynamic and fast evolving nature to the maize alpha-zein super gene family, which might be a common feature for other large gene families.

P148

Fluorescent protein tagged maize lines for cell biology and functional genomics

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Completion of the first draft of the maize B73 genome provides unprecedented sequence resources for functional studies. We are using the power of these genomic resources to develop high throughput methods for generating fluorescently tagged maize lines useful for cell biological, physiological and developmental studies. One of the outcomes of the project is to provide lines of maize that are stably transformed with fluorescently tagged proteins that specifically mark diverse intracellular compartments. Targeted proteins are selected for the tagging pipeline if they fit several criteria including the following: 1) full genomic sequence must be available for 2-3 kb upstream and 1-2 kb downstream of the gene of interest; 2) total tagged genomic sequence is limited to

P149

Gene expression analysis and physiological responses of Mexican maize landraces under drought stress

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In Mexico, most of the maize crop is grown under rainfed conditions and as in most tropical and subtropical regions, drought leads to severe losses in maize production. As a consequence, farmers have selected landraces for drought tolerance. Some have been chosen for the capacity to delay flowering under drought stress, others for the ability to rapidly recover following stress. Our objective was to analyze the gene expression and physiological responses of three contrasting maize landraces under different drought stress conditions. Cajete criollo and Michoacan 21 were chosen as drought tolerant genotypes and 85-2 as the susceptible control. To monitor gene expression changes, a microarray containing 56,600 maize gene oligonucleotides was used. Total RNA was extracted from leaves of maize plants under 10 and 17 days of drought stress, and after recovery irrigation. Significant differences were observed in the transcriptional responses of the three maize landraces analyzed. As drought stress became more severe an increasing number of genes were up or down-regulated. Interestingly a higher number of genes were differentially expressed in both tolerant lines than in the susceptible control under drought stress. Michoacan 21 showed more regulated genes than the other landraces at 10 and 17 days of stress as well as in the recovery irrigation. Leaf water potential, photosynthesis, transpiration rate, soil water potential and relative humidity were monitored throughout the experiment. Real-Time PCR was used to confirm the differential pattern expression of some selected genes. A correlation between gene expression profiles of the three landraces and their tolerance or susceptibility to the drought stress was also made.

P150

Genome-wide analysis of gene expression profiles during the kernel development of maize (*Zea mays* L.)

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Maize kernel is an important source of food, feed and industrial raw materials. The illustration of the molecular mechanisms of maize kernel development will be helpful for the manipulation of maize improvements. A microarray with approximately 58,000 probes was used to study the dynamic gene expression during kernel development from the fertilization to physiological maturity. By comparing six consecutive time-points, 3,445 differentially expressed genes were identified. These genes were then grouped into 10 clusters showing specific expression patterns using a K-means clustering algorithm. An investigation of function and expression patterns of genes expanded our understanding of the regulation mechanism underlying the important developmental processes, cell division and kernel filling. The differential expression of genes involved in plant hormone signaling pathways suggested that phytohormone might play a critical role in the kernel developmental process. Moreover, regulation of some transcription factors and protein kinases might be involved in the whole developmental process.

P151

High throughput linkage analysis of Mu insertion sites

(submitted by Philip W. Becraft <becraft@iastate.edu>)

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A number of large mutant collections have been derived from Mutator lines. The high copy Mu transposons are an effective mutagen, however, the copy number also poses a challenge for identifying the particular element responsible for a given mutant phenotype. Genetic linkage is the first criterion generally used to identify candidate elements; if a particular Mu insertion caused a mutation, then that element should show complete genetic linkage to the mutant phenotype. Methods to analyze the Mu elements present in an individual's genome include Southern hybridization and a PCR based transposon display known as AIMS (Frey, et al., 1998. *Plant J.*13: 717). These methods, including the subsequent cloning of elements of interest, are too laborious to be applied in a high throughput fashion. To more fully realize the payback on the investment made in developing these mutant collections, a high throughput method for detecting and recovering Mu insertion sites is required. Mu-TAIL is an efficient high throughput means of specifically recovering Mu insertion sites (Settles, et al., 2004. *Nucleic Acids Res* 32, e54), but it is not suitable for linkage analysis. We have combined aspects of Mu-TAIL and AIMS to create a protocol we call MuTA that allows high throughput identification and cloning of Mu insertions linked to mutations. The Mu primer is fluorescently labeled and PCR products are analyzed on an ABI3700. A segregating family is analyzed and if a product is linked to a mutation, the PCR products are cloned from one of the mutant reactions. Clones are sequenced and the clone of interest is identified by its size. In a trial of 12 mutants, the analysis was completed in under two weeks and linked candidate Mu elements were identified for six of them.

P152

Identification and analysis of SNPs on a large scale using high-throughput sequencing in maize based on reference sequences

(submitted by Martin Ganal <ganal@traitgenetics.de>)

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New sequencing technologies produce now up to 1 billion bases per run. The main difficulty is that with the produced short reads ranging between 25 and 40 bases direct de novo sequencing is difficult. We have used reference sequences generated for genic fragments for the identification of SNPs in pools and individual lines in maize. In experiments, we have amplified fragments with an average length of 560 bases from 4000 maize genes and sequenced these fragments using the Illumina/Solexa platform. Based on the reference sequences, it was possible to assign many of the reads back to the individual reference sequences. Methods were developed which permit the identification of SNPs in individual lines and for allele frequency estimations in pools of lines. The results were validated using available Sanger sequencing data. The results demonstrate that it is possible to simultaneously analyze a large proportion of the genes for the presence of SNPs and in the long term establish a genotyping by sequencing procedure for maize.

P153

Identification and characterization of lineage-specific genes within the Poaceae

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Using the *Oryza sativa* subspecies japonica (rice) genome annotation along with genomic sequence and clustered transcript assemblies from 184 species in the Plant Kingdom, a set of 861 rice genes have been identified that are evolutionarily conserved among six diverse species within the Poaceae yet lack significant sequence similarity with plant species outside of the Poaceae. This set of evolutionarily conserved and lineage-specific rice genes is termed Conserved Poaceae Specific Genes (CPSGs) to reflect the presence of significant sequence similarity across three separate Poaceae subfamilies. The majority of the rice CPSGs encode proteins with no known or putative function nor do they contain a functionally characterized protein domain. On average, the CPSGs have fewer exons, shorter total gene length, and elevated GC content when compared with genes annotated as either transposable elements or those genes having significant sequence similarity in a species outside the Poaceae. At the genome level, syntenic alignments with sorghum and rice CPSGs demonstrates an additional level of conservation for this set of genes within the Poaceae. The extensive sequence similarity in evolutionarily distinct species within the Poaceae family and an additional screen for transposable element-related structural characteristics and sequence discounts these CPSGs as being misannotated transposable elements. Collectively, these data confirm that we have identified a specific set of genes that are highly conserved within, as well as, specific to the Poaceae.

P154

Identification and cloning of QTL based on near isogenic introgression lines (NILs) of maize

(submitted by Zuxin Zhang <nxzzx@hebau.edu.cn>)

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Near isogenic Introgression Lines (NILs) derived by backcrossing are useful for QTL identification and gene cloning. A total of 91 link-up introgression lines were developed by BC-MAS with Zong 3 as the recurrent parent, HB522 as donor. The total length of introgressed segments covered about 86.2% genome of maize, average length was about 33.5 cM, and more than 97.0% average recovery of genetic background was evidenced by SSR screening. A total of 59 QTLs for 9 traits were identified in two locations, and only nine QTLs were simultaneously identified, showing that there were much of QTL Environment interactions. A F2 population was developed with a line containing bnlg1647-phi036 segment crossed with Zong3. Six new SSR markers were developed in the segment, and then a major QTL for plant height was finely mapped in 3.1cM interval. Meanwhile, six overlapping lines were constructed in target region to confirm this QTL. Furtherly, a potential candidate gene for the QTL, zm-GAox3, was cloned. The function analysis by association mapping and genetic transformation are in process.

P155

It's a knockout: sequencing the MTM population

(submitted by Robert Martienssen <martiens@cshl.edu>)

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The MTM (maize targeted mutagenesis) population comprises seed and tissue from 44,000 maize plants in which Mutator transposons have been mobilized and then stabilized genetically (May et al., 2003; <http://mtm.cshl.edu>). Tissue was harvested in 18 "grids" of 48x48 plants each, and genomic DNA has been prepared from 680 superpools that permit three-dimensional cross-referencing of any individual sample (May et al., 2003). Target sites flanking each Mutator insertion can be amplified from these pools by an anchored PCR method known as "GenomeWalker". From previous studies (Alleman and Freeling, 1986), we anticipate that there are at least 50 new insertions in each plant, along with a similar number of parental insertions. We have amplified products from 2 pools of 48 plants each, and sequenced them using the Illumina (Solexa 1G) instrument. In the two pools, more than 6,000 clusters of reads matched repeatmasked MAGI contigs, including a control contig identified by PCR selection as part of the MTM service. Using one lane of a flow cell per pool, between 50 and 600 reads matched the control contig at the same location, indicating several pools can be sequenced in each lane. More than half of the matches are in the promoter of annotated genes, as expected for Mutator insertions (May et al., 2003). This indicates that up to 60 new insertions can be identified by this method in each plant, or 150,000 per grid. We estimate the MTM resource has more than 1 million independent new insertions of Mutator elements in the 400Mb gene space, or more than one insertion per kb.

Alleman M and Freeling M (1986) *Genetics*. 112(1):107-19.

May BP, Liu H, Vollbrecht E, Senior L, Rabinowicz PD, Roh D, Pan X, Stein L, Freeling M, Alexander D, Martienssen R. (2003) *Proc Natl Acad Sci U S A*. 100(20):11541-6.

P156

Large-scale circadian clock regulation of maize transcription

(submitted by Sadaf Khan <s_khan@berkeley.edu>)

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In plants, the circadian clock orchestrates 24-hour rhythms in internal physiological processes to coordinate these activities with daily and seasonal environmental changes. The clock has a profound impact on many aspects of plant growth and development including biomass accumulation, cold tolerance, shade avoidance, and flowering. Despite recent advances in understanding of the circadian system in the model plant *Arabidopsis thaliana*, much remains to be learned about the contribution of the circadian oscillator to important agronomic traits in crop plants. Therefore, we are studying the circadian system in *Zea mays*, which serves as an excellent model for cereal crops. Since much of circadian clock control is exerted at the transcriptional level, we employed a genomics-based approach to identify circadian clock-regulated genes in the maize inbred B73. Seven day-old maize seedlings were entrained in day:night cycles of 12 hours each followed by release on the eighth day into constant light conditions for 48 additional hours. While in constant conditions, tissue samples were harvested at 4-hour intervals and the Affymetrix Maize Genome oligonucleotide array was used to determine the expression level of 14,850 *Z. mays* transcripts in each sample. Curve fitting analysis of these data with the COSOPT algorithm, which categorizes genes based on expression waveform, identified 980 transcripts that exhibited robust circadian expression. Thus, approximately seven percent of the transcripts represented on the array exhibit circadian expression patterns, which is comparable to the extent of genome-wide circadian regulation in *Arabidopsis*. Expression of *ZmGIGANTEA*, *ZmCATALASE 3*, and *ZmCHLOROPHYLL a/b BINDING PROTEIN 1* were found to exhibit the same circadian expression waveform as their *Arabidopsis* orthologs. Further analysis of this dataset will define the extent to which the maize circadian clock contributes to key physiological processes that influence agronomic traits such as yield, stress tolerance, and flowering time.

P157

Loci controlling gene expression and morphological UV responses: pleiotropy links levels of regulation

(submitted by Ann Stapleton <stapletona@uncw.edu>)

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It is especially important to understand regulatory cascades in order to engineer enhanced acclimation responses to stress. Co-ordination of acclimation responses to UV-B appears to involve multiple signal transduction cascades; thus far, changes in chromatin structure, intracellular calcium, calmodulin, serine/threonine kinases, and phosphatase activities have been implicated. A first step in ordering the various pathways is understanding the genetic architecture of UV responses.

We have measured whole-plant morphological and physiological effects in the IBM94 mapping population. To link whole-plant phenotypes with information about gene expression, we performed microarray analysis using seedling leaf tissue, with and without UV, in a subset of IBM94 lines. Extensive development of statistical methods was required. Our methods for exploiting pleiotropy to understand how various cellular and whole-organism traits are regulated will be generally applicable.

P158

Maize full-length cDNA project

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Maize is the most important US crop and the complete genome sequencing is in progress. Full-length cDNA sequences (FLcDNA) become crucial for the accurate annotation and future functional characterization of genes. NSF funded maize full-length cDNA project (NSF Award# 0501857) will finish 30,000 FLcDNAs, focusing on reproductive development and stress-induced transcripts. Full-length cDNA libraries, Gateway-compatible, were built using: 1) pooled RNA from 13- B73 reproductive tissues, highly enriched for full-length transcripts by cap and tail selection, and then normalized to increase representation of rare transcripts, 2) embryo, scutellum and 7-day seedlings with stress inducer treatments.

For the project, about 25,000 candidate FLcDNA clones for full-length sequencing were selected based on 360,000 5' and 3' EST assemblies generated using PAVE (software package designed for assembling coding sequences). Iterative primer walking was employed on each candidate FLcDNA to completely sequence the selected cDNA inserts with phred 40 quality as a finishing standard. Currently, assemblies from the 25,000 clones revealed 10,000 FLcDNA met the criteria for sequence finishing and are in the process of being characterized-- an additional 5000 candidate FLcDNAs are being analyzed for selection. Rearray of presumed large clones and additional primer walking is expected to increase the number of affirmed FLcDNAs toward the goal of 30,000.

In addition, we collected expression profiles by hybridization of RNA from stress induced seedlings and dissected reproductive tissues on two oligo-microarray platforms. We are currently in the process analyzing FLcDNA sequence data to link to expression profile data with the expectation of an interactive web display for public use of the data sets.

Information derived from this project will uncover gene regulatory networks involved with reproduction and environmental stresses. Maize full-length cDNA project has actively trained teachers and young undergraduate scientists from underrepresented groups through outreach efforts.

P159

Maize full-length cDNA sequencing - walking and primer design

(submitted by John Fernandes <john.fernandes@stanford.edu>)

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The Maize Full Length cDNA project, whose primary goal is to fully sequence 30,000 cDNAs, was funded by the NSF for 3 years and is currently in its last year. Library tissue samples were generated at Stanford and then normalized by Invitrogen. Sequencing and clone selection for walking were done at AGI (Arizona Genomics Institute at the University of Arizona). Two cDNA libraries were constructed: the first was a mix of reproductive and vegetative tissues and the second included 6 seedling stress treatments plus normal seedlings and field-grown leaves plus 3 stages of anthers. To ensure an efficient pipeline, processes were developed by project participants from UA's Arizona Genome Computational Laboratory to track the sequencing and walking of the clones, determine success rates, flag problem clones, scrub and do quality checks and assemble sequences, select clones for walking, determine completed clones, and determine completed plates. Sequences were aligned to check priming results and overlaps. Primer design was performed at Stanford using primer3. Primer design codes were automatically assigned to sequences to indicate the desired priming location (e.g. to generate either higher overlap or more new sequence) and to request a replacement for a failed primer. Because of the high frequency of repetitive sequences, of which several pairs were sometimes found in the 600+ bp sequences submitted for primer design, pre-design processing was added to exclude the repetitive sections. Sequences with no primers under the most stringent design parameters were automatically queued for up to 3 additional rounds with progressively less stringent conditions. On average, primers could not be designed for 1 sequence in 1000.

P160

Plant genomic resources at National Center for Biotechnology Information

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Plant genomics is a simple expansion of the scope of genomics at the National Center for Biotechnology Information (NCBI). In addition to the tools for storage of and analysis of nucleotide sequence such as, respectively, GenBank and BLAST, genomics at NCBI includes databases that enable 1) monitoring the progress of genome sequencing projects (Entrez Genome Projects), 2) datamining of probes (Entrez Probes), 3) datamining of primer sequences (UniSTS), 4) datamining of gene information (Entrez Gene) and 5) viewing genome units (MapView). These standalone tools are enhanced at NCBI by the capability to move among these and other databases as the data associations dictate. The pan-organism resources are supplemented by plant-specific resources: plant text search, PlantBLAST, and plant-EST BLAST. PlantBLAST provides organism-specific databases composed solely of the accessions associated with mapped loci visible through MapViewer. EST-BLAST provides plant-specific databases composed solely of the ESTs from those plants with more than 50,000 ESTs.

The scope of plants with maps - both sequence-based and nonsequence-based - visible in MapViewer has been increased from 26 to 41 organisms. The scope of organisms with mapped probes has increased from 32 to 53. The Entrez Gene records are gaining links to organism-specific databases.

This expanded scope of data will be used in examples of the developing capabilities of the genomic resources for plants at NCBI.

P161

Population structure and genetic diversity in Japanese rice cultivars

(submitted by Masanori Yamasaki <yamasakim@tiger.kobe-u.ac.jp>)

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It is essential to elucidate genetic diversity and relationships among individuals and populations for genetic analysis. Since Japanese rice breeding have been conducted to improve agronomic traits such as yield and eating quality to suit the needs and preference of the Japanese people, the modern Japanese rice cultivars are originated from narrow genetic resource and closely related. To resolve the genetic diversity and relationship in the Japanese rice population, we used a total of 127 Japanese rice cultivars composed of 102 improved varieties, 21 landraces and 4 upland rice varieties. These cultivars included the major Japanese rice cultivars and were used for breeding of modern Japanese rice breeding. The 140 simple sequence repeat (SSR) loci were assayed for polymorphism. The landraces exhibited greater gene diversity than improved lines, suggesting that the landraces can provide additional genetic diversity for future breeding. Phylogenetic analysis indicated that the improved varieties and landraces formed monophyletic clade and relationships among varieties were not fully resolved. A model-based clustering analysis revealed eight clusters and admixture situation, showing good agreement with pedigree information and the phylogenetic tree. We defined Japanese rice diverse sets that attempt to capture the maximum number of SSR alleles for given sample sizes. These sets are useful for a variety of genetic application in Japanese rice cultivars.

P162

Population structure and genetic diversity of New World maize landraces assessed by microsatellites

(submitted by Jeff Glaubitz <glaubitz@wisc.edu>)

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We analyzed the population genetic structure of maize landraces by genotyping 964 individual plants, representing nearly the entire set of ~250 maize landraces native to the Americas, with 96 microsatellites (SSRs). Using a Bayesian, model-based approach (STRUCTURE) we detected four main genetic clusters consisting of Highland Mexican, Northern U.S., Tropical Lowland, and Andean landraces. Furthermore, combined results of phylogenetic and model-based analyses indicated that landraces of Middle South America (Bolivia, Argentina, Paraguay, and Uruguay) were originally derived from Andean landraces and subsequently mixed with Northern South American germplasm that arrived via Brazil. Moreover, Southeastern U.S. landraces appear to contain mixed Tropical Lowland and Northern Flint ancestry. Of the four main landrace clusters, the highest genetic diversity occurs in Highland Mexican landraces, while diversity is reduced in the Andean and Northern U.S. clusters. Isolation by geographic distance appears to be the main factor underlying the historical diversification of maize. Several cases of recent (post-Columbian) movement of landraces were also detected. Northern Flint ancestry is apparent in some Cuban Landraces, and Southeastern US ancestry is apparent in landraces of Brazil; it is likely that post-Civil War emigrant farmers brought maize with them to these countries.

P163

QTL regulating seedling dry weight in IBM RILs and their F1 hybrids with B73 and Mo17

(submitted by Ruth Swanson-Wagner <swansonr@iastate.edu>)

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Heterosis (hybrid vigor) has been exploited in maize production for a century. Despite its importance, the molecular mechanisms underlying heterosis are not well understood. Heterosis for dry weight accumulation has been observed for maize inbred lines B73, Mo17, and their F1 hybrids at the seedling stage. These inbred lines were used to generate the IBM (Intermated B73 and Mo17) population of RILs (Recombinant Inbred Lines). Microarray comparisons of B73, Mo17, and their hybrids are consistent with the view that differential gene expression may contribute to heterosis. A subset of the IBM population was used to identify thousands of genes whose expression is regulated by hundreds of expression QTL (eQTL). In the current study, ~200 RILs were crossed onto both B73 and Mo17. In combination with the RILs *per se*, the resulting F1 lines provided a contrast of biomass accumulation for the heterozygous genotype and both homozygous genotypes across all loci polymorphic between B73 and Mo17. Seedling dry weight values were measured in four biological replications. Each RIL and its crosses onto B73 and Mo17 were planted using a randomized block design, with restrictions on randomization so that the three cross-types for each RIL were grouped together. Mid-parent and high-parent measures of heterosis were computed from the dry weight data. The subsequent phenotypic QTL (pQTL) analysis was conducted using an ISU genetic map of the IBM population containing over 1,000 SNP markers. A significant QTL interval exhibiting gene action consistent with heterosis was identified, where heterozygous lines exhibited significantly higher measures of heterosis than did homozygous lines. Additionally, the QTL interval overlaps with previously identified *cis*- and *trans*-eQTL. Based on these findings, we are testing the hypothesis that one or more eQTL in this interval is/are responsible for differential dry weight accumulation in hybrids.

P164

Quantitative high-throughput phenotyping of maize seeds

(submitted by Gertraud Spielbauer <gspielbauer@ufl.edu>)

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Kernel composition is an important target for developing improved grain for food, feeds, and various industrial processes. Genetic and environmental effects create significant variation in the amount and quality of seed constituents. Our goal is to identify maize mutants that have significant effects on the chemical composition or weight of seeds. To this end we are screening the UniformMu transposon-tagging population using single kernel near-infrared spectroscopy (NIR) and seed weights. So far, NIR spectral data and seed weights have been collected from individual seeds of more than 500 visibly normal M2 families and normal kernels from 1034 visible seed mutants using a custom-built automated single kernel grain analyzer. NIR spectroscopy enables the nondestructive determination of phenotypic traits of individual maize kernels in a high-throughput manner. It is a universal analytical tool that reports numerous seed components and by applying calibration procedures it can be used to predict the amount of individual chemical constituents. We developed partial least square (PLS) calibration models to predict the major seed components starch, protein, and moisture. To further exploit the full power of NIR spectroscopy the data are compressed by principal component analysis (PCA) and a Bayesian test for clusters was developed to identify ears segregating for differences in NIR spectral data, and hence chemical composition. Cumulative distributions of seed weights are being screened for mutants that segregate for distinct weight classes. Families with significant seed weight or composition changes will be selected for further biochemical and genetic analysis. We will focus on phenotypes that show dosage-dependent or parent-of-origin changes to the kernels based on the hypothesis that these genes are the best targets for modifying the seed with transgenes.

P165

Reverse genetics for cell-wall mutants in the UniformMu maize population

(submitted by Charles Hunter <ibe@ufl.edu>)

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Over the past four years, a reverse genetics effort has been underway utilizing the UniformMu maize population at the University of Florida. This population was generated by introgressing the Robertson's mutator line into the W22 inbred, optimizing the material for genetic studies. Eight reverse genetic grids representing over 15,000 individual UniformMu lines have been constructed for PCR-based screens to identify transposon-generated knockouts. Screening using primers for 50 unique cell wall biosynthetic genes has yielded 22 sequence-confirmed insertions thus far. During reverse genetic screening, two different methods were employed and compared. The standard screening procedure involved a single unidirectional PCR amplification with direct sequencing. This method was fast, cost-efficient, and yielded a ~7% rate of Mu-insert detection per grid screening. The advanced screening procedure was more time consuming and more expensive, but improved the detection rate of Mu insertions within a target gene. Coverage was increased by employing multiple primers, Southern blotting the original PCR products, and including a second round re-amplification PCR using nested primers. This was followed by direct sequencing. Each method has positive and negative aspects relative to throughput or return. Using these approaches we have identified Mu insertions in Cellulose Synthase-Like genes CslA6, CslA7, CslD1, CslD2, and CslD5. Homozygous lines have been generated for phenotypic analysis. A mutation in CslD5 leads to plants containing root hairs that fail to elongate, likely analogous to the *kojak* mutation (CslD3) in *Arabidopsis*. The CslD5 mutant phenotype has been verified in multiple alleles and does not appear to have any other physiological effects in field or lab conditions. Thus far, it is the only one of the above genes that has displayed a visible phenotype, but further analysis is in progress.

P166

Sequence-indexed Mutator transposon insertion sites using 454 sequencing

(submitted by Mark Settles <settles@ufl.edu>)

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Gene knockouts are an essential resource for the functional analysis of the maize genome. A major challenge for the maize genetics community is to identify mutations in every gene in the genome. Multiple maize transposable element systems have been used as endogenous mutagens to generate knockouts on a genome-wide scale. These mutations are most accessible when the insertion sites are indexed with Flanking Sequence Tags (FSTs) from individual seed stocks. Endogenous transposons exist as multicopy elements within the genome. Identifying novel insertions relies on either highly redundant FST sequencing to identify a mix of parental and unique mutations or labor-intensive experimental manipulations to purify novel flanking DNA prior to sequencing. Here we show that the 454 Life Sciences sequencing technology is effective for achieving the level of redundancy needed to associate novel Mutator (Mu) insertions to specific plants. We sequenced FSTs from 144 mutagenized plants from the UniformMu population. The insertion sites were amplified from 24 pooled DNA samples arrayed in a 12 x 12 grid. Each pool was sequenced with a molecular barcode to assign individual reads to pools. We recovered 437,199 reads containing signature sequences indicating a bona fide Mu FST. These FSTs had an average read length of 155 bp after removal of the pool barcode and Mu signature sequences. Less than 1% of the FSTs could not be assigned to a specific pool due to sequencing or primer synthesis errors. Recovery of the same insertion site from row and column pools allows specific insertions to be addressed to individual plants. We have used site-specific PCR based on the FSTs to validate this addressing system. More detailed bioinformatic analysis of these FSTs will be presented.

P167

Submergence stress responsive microRNA genes in *Zea mays* L.

(submitted by Yonglian Zheng <yonglianzheng@gmail.com>)

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MicroRNAs (miRNAs) are approximately 21-nucleotide-long, non-coding RNAs that play critical roles in regulating gene expression at the post-transcriptional level. Several recent studies showed important functions of miRNAs in response to adverse abiotic stresses, such as phosphate starvation, mechanical stress, cold and drought stress. A few of stress responsive miRNAs have been reported in plant. Submergence is a serious abiotic stress to maize seedlings during its development and growth season. The previous results in our Lab showed that aerobic metabolism of carbohydrate is switched off but glycolysis pathway is induced in maize roots under submergence condition, aerobic metabolism is converted to anaerobic metabolism. It is implying that genes involving in sugar metabolism are regulated on transcription level or post-transcription level by an unknown regulating mechanism. Whether the miRNAs involve into the conversion of metabolic pathway or not? For uncovering the potential role of miRNAs in submerged maize roots, a custom Paraflo microfluidic array (LC Sciences, USA) containing version 10.0 plant MicroRNA probes was used to explore differential expressed miRNAs in submerged maize roots. Small RNAs isolated from 0 h, 12 h, 24 h and 36 h treated roots were respectively hybridized with the Microarray, each sample data were collected from 3 independent hybridization replications. Microarray data revealed that the expressions of miRNAs were significantly altered under $p < 0.001$ level. Those differential expressed miRNAs mainly contain members of eleven maize miRNAs families and *ptc-miR474* family. Subsequently, these miRNAs were assigned on genome by query maize genomic sequence using pre-miRNAs sequences. Using Plant miRNA Target Finder, target genes modulated by submergence included miRNA were detected. Many of target genes encode a putative MADS, ZIP, Myb and zinc finger motif transcription factor, some function genes, such as serine/threonine protein phosphatase, starch synthase, beta-expansin 8, also were the target of miRNAs. In 5' upstream of *zma-miRNA* target genes, ARE and GC-motif, two cis-acting regulatory elements essential for the anaerobic induction were detected, implying that identified miRNAs involved in regulation of anaerobic metabolism. We suggested that *zma-miR159*, *zma-miR160* and *ptc-miR474* maybe regulate the open of anaerobic metabolism, whereas, *zma-miR156*, *zma-miR166*, *zma-miR168*, *zma-miR171*, *zma-miR319* and *zma-miR399* involve in the regulation of switch off aerobic metabolism.

P168

Syngenta maize allelic diversity platform

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Current routine deployment of genomics technologies, the complete sequencing of the rice genome (and soon the maize genome), and the success of statistical genetics methods for QTL identification, have enabled the maize community to identify large number of genetic factors and candidate genes affecting many important agronomic traits. Novel strategies for identifying genes and QTLs are proving successful; however quantitative validation of these discoveries remains difficult. Transgenic knock-outs and knock-downs, and over-expression have been successful in characterizing gene function; however subtleties of allelic variation in the context of genetic background are lost in these strategies. Precise introgression of discrete chromosomal segments into varied genetic backgrounds on a project basis takes several generations and consequently a considerable amount of time. Syngenta Maize Allelic Diversity Platform was developed to provide a catalogue of material in elite near-isogenic backgrounds that can be queried and evaluated for effects in phenotypic variation. We selected more than 100 diverse inbreds and landraces as allelic diversity donors. Two proprietary complementary inbreds (one stiff stalk and one non-stiff stalk) were selected as recurrent parents to cross with the donors. Each donor is being crossed to both recurrent parents and backcrossing has been continued to BC5 generation. Over 65,000 backcross-derived near isogenic lines (NILs) distributed in 266 populations are being generated. A subset of donors is also being crossed with B73, a public maize line, and will be available for distribution. Each of the BC5 populations is genotyped using an average of 128 evenly distributed SNP markers. Genotypic analysis of these populations and characterization of the donors will be presented.

P169

The Plant Ontology Database: A community resource for plant structure and developmental stages controlled vocabulary and annotations

(submitted by Pankaj Jaiswal <pj37@cornell.edu>)

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The main objective of the Plant Ontology Consortium (POC; <http://www.plantontology.org>) is to create, maintain and facilitate the use of controlled vocabularies (ontologies) for plants. The ontology allows users to ascribe attributes of plant structure (anatomy and morphology) and developmental stages to data types, such as genes and phenotypes, to provide a semantic framework to make meaningful cross-species comparisons. While the initial emphasis has been made on three plants: Arabidopsis, rice, and maize, support for several other angiosperm crop plants is now included. At MaizeGDB database, anatomical aspects of phenotypes have been associated with the Plant Ontology accessions; the phenotype-inferred associations to loci, stocks and alleles have been supplied to the Plant Ontology database. It's evident the application of the PO in phenotypic evaluation and characterization for useful traits from diverse maize germplasm facilitates the data retrieval and sharing. Currently, over 17,000 annotations from MaizeGDB, Gramene, TAIR and NASC have been documented in the Plant Ontology database, and can be used in conjunction with other useful information (such as their biochemical characterization from their source databases) to predict phenotypes, determine functions of gene products, and possibly initiate gene discovery through comparative genomics analysis. We expect the PO development to be an ongoing activity as we seek to create a robust resource that is responsive to new discoveries in our understanding of plant biology.

P170

Dwarf8 polymorphism associated with flowering time in maize

(submitted by Sara Larsson <sjl65@cornell.edu>)

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Flowering time is one of the most important adaptive traits for wild and cultivated plants. One of the pathways involved in flowering time is the gibberellin pathway. *Dwarf8* is one of the gibberellin pathway genes, for which nine polymorphisms were shown to be significantly associated with variation in flowering time by Thornsberry et al. (2001) in an association study with 92 diverse inbred lines. These results have been confirmed by Andersen et al. (2005) and Camus-Kulandaivelu et al. (2006). Here we report the results of association analyses between the 18bp indel in the non-coding promoter region of *Dwarf8* (one of the nine polymorphisms) and phenotypic data for flowering time scored as days-to-anthesis and days-to-silking, in an association panel of 282 inbred lines. Our purpose is to evaluate the results of the initial association study using another association population that is significantly larger compared to the initial study population, and with new statistical methods (e.g. Q+K model of Yu et al. (2007)). The association results generated here will be incorporated with the QTL mapping results from Maize Nested Association Mapping (NAM) panel consisting of 5000 recombinant inbred lines produced by crossing 25 diverse lines to B73. While genotyping the 18 bp indel in this population, we have also discovered a several kilobases long insertion within the promoter region of *Dwarf8* that may have an influence on the expression of the gene. Results to date are presented.

P171

A survey of diverse *Zea mays* germplasm identifies new sources of resistance to Western corn rootworm (*Diabrotica virgifera virgifera* LeConte)

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The rootworm is an important pest of maize that causes its most significant damage through feeding on the root system. The objectives of this study were to identify sources of genetic resistance by surveying a diverse set of maize germplasm and to identify any correlated traits. Sixty-four diverse maize lines were planted in the field in three replications in 2006 and 2007 and infested with western corn rootworm (WCR). Root damage was rated on a scale of 0=no damage to 3=complete loss after digging and washing. Root regrowth and root branching were also evaluated. Tests for homogeneity of variance indicated that the 2006 and 2007 data were significantly different so they were analyzed separately. Analysis of variance was performed using proc GLM in SAS. The one way ANOVA from 2006 identified line as a significant source of variability (p-value=0.0137, R²=0.44) in root damage. ANOVA of the root damage data in 2007 revealed that the inbred line and line*Rep effects were a significant sources of damage variation, each with a p-value of <0.0001. Duncan's mean separation test identified significant differences in root damage ratings among the lines. Additionally, this data was analyzed using proc CORR in SAS to determine the correlation between damage rating and days to tassel, days to pollen, number of juvenile leaves, number of transition leaves, and number of adult leaves. A significant negative correlation between the number of juvenile leaves and damage was identified in both years suggesting that lines which undergo later phase transition received less rootworm damage.

P172

Agronomic and basic science utilities of the intermated NC89 x K55 RIL (INKRIL) population

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

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The IBMRIL population has not only served as the first viable bridge between the genetic and physical realms of the maize genome, but has also been widely and successfully used for discovery and functional analysis of QTL. In two cases, we have localized alpha=0.1 bootstrap confidence intervals to single BACs. Separately, we have shown that small physically defined regions of the genome pleiotropically affect multiple phenotypes, providing new functional hypotheses for these QTL. The critical steps in constructing this bridge were the four generations of intermating of the lines prior to recombinant inbreeding. Here we introduce the INKRIL population, which was intermated four times and consists of 420 lines for which inbreeding is nearly complete. The 420 lines have been assayed for linkage and genetic purity at four independently assorting regions in the genome. The per line levels of recombination are equivalent to the IBMRILs. The purity assays have revealed no evidence of residual heterozygosity in the inbred parents and no evidence of pollen contamination. North Carolina 89 appears to have been derived from Mexican central plateau germplasm, based on its hairy sheath, non-stiff stalk and shallow root system. It has yellow-orange kernels. Kansas 55 is derived from the drought tolerant Pride of Saline, has hairless leaf blades, a stiff stalk, deep rooting, white kernels, and has excellent combining ability. More than 65% of 384 SSLP markers assayed have shown a polymorphism for this wide cross and transgressive segregation of traits not obviously different between the parents is widespread. We discuss our effort to positionally clone macrohairless1 using this resource, highlight several genomics utilities and traits for QTL studies, and present a strategic plan for public distribution.

P173

Allelic variation in genes involved in gibberellic acid activity contribute to brace root variability in a diverse germplasm set of *Zea mays*

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Previous QTL mapping studies identified chromosome regions harboring the gibberellic acid mutants, *dwarf3* (*d3*) and *Dwarf8* (D8) as contributing to brace root variation in the intermated B73 x Mo17 recombinant inbred mapping population. Means comparisons between wild-type, and *dwarf3* (GA deficient mutant), and *Dwarf8* (a GA insensitive mutant) revealed a functional role for each underlying gene in brace root formation. The goal of our current study was to use association mapping to examine the effects of naturally occurring allelic variation at the *d3* and *D8* loci on brace root variation in a diverse set of germplasm. A set of 92 diverse maize lines were grown in the field in 2 replications for three years. Each year the number of nodes with brace roots was recorded on at least 5 plants. Least squared means (LSmeans) were calculated using proc mixed in SAS using line as a fixed effect and replication and year as random effects. LSMeans were used for association analysis in Tassel, version 2.0.1, using a mixed linear model. The population structure data and the identity-by-descent probabilities, described previously, were used to account for multiple levels of relatedness observed in the germplasm set. Significant associations (*Pdwarf3* gene, while sites 2905, 2907, 2909, 2916 and 2918 were found to be significant (*Pdwarf3* gene. The R-squared for marker effects ranged from 0.008 to 0.06. Linkage disequilibrium analysis between the significant polymorphisms identified complete gametic phase disequilibrium ($D=1$, $n=41$) between the 677 and 699 sites in the *Dwarf8* gene and 2905, 2907, and 2909 sites in the *dwarf3* gene. These results suggest a potential confounding of QTL effects and subsequent QTL validation may require further genetic and physiological analyses.

P174

Analysis of the root defense metabolome of maize in the presence and absence of western corn rootworm larvae

(submitted by Martin Bohn <mbohn@uiuc.edu>)

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From the 1940s until today more than 12,000 maize accessions, comprising inbreds, synthetics, and open pollinated varieties, as well as maize relatives, like teosinte and tripsacum, have been screened for their level of resistance to western corn rootworm (*Diabrotica virgifera* LeConte, WCR) larvae feeding. After initial evaluations for a wide array of traits associated with different resistance mechanism active against WCR, less than 1% of this germplasm was selected for initiating recurrent selection programs. In general, the selected genotypes were characterized by large root systems and superior secondary root development after root damage caused by WCR larvae. However, no non-transgenic maize cultivars with high level of resistance under moderate to high insect pressure have yet been released. To overcome this problem, we are in the process of evaluating the defense response of maize to WCR feeding in a coherent framework of available methods comprising microarrays and metabolite analysis. Maize cultivar CRW3C6 is moderately resistant to WCR. At the V3 stage plants were subjected to the following treatments: untreated control (Treatment 1), mechanical wounding (Treatment 2), and infestation with WCR larvae (Treatment 3). Root tissue was collected in liquid nitrogen 12h after treatment. Standard extraction protocols were used and metabolic profiles were obtained employing GS-MS. We used univariate and multivariate approaches for data analysis. Across all treatments and extraction protocols more than 700 different biochemical compounds were detected. About 85% of these metabolites were found only in one out of the three treatments. A set of 95 metabolites was detected in all three treatments and 20 of these metabolites displayed significant concentration differences between treatments. These results provide the first step towards the use of metabolic profiles as biomarkers for improving host plant resistance in maize against WCR and may lead to new opportunities to enhance maize breeding.

P175

Analysis of trait variation in intermated and non-intermated recombinant inbred line populations of *Zea mays*

(submitted by Raja Khanal <rkhanal@uoguelph.ca>)

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One explanation for hybrid vigor is that favorable alleles of genes that contribute to hybrid traits are in repulsion configurations and parental inbred lines have closely linked genes that differentially influence traits. To test this hypothesis we developed two recombinant inbred line (RIL) populations from a pair of elite maize inbred lines. One RIL population was generated by selfing F2 plants for five generations, and another intermated RIL (IRIL) population was generated by intermating F2 plants for two generations prior to selfing. We measured five whole plant traits (leaf number, plant height, days to silking, days to pollen shedding and stay green) across 280 lines from both populations grown in two locations with two replications per location. Traits vary more within the IRIL than the RIL population, suggesting inbred lines have closely linked genes that differentially influence whole plant traits. Interestingly, the trait variation explained by population was often higher than the variation explained by genotype and trait means also significantly differed between populations. Our results indicate that intermating influences both population trait means and variances.

P176

Association and linkage mapping of nitrogen metabolism enzyme activities in maize

(submitted by Nengyi Zhang <nz45@cornell.edu>)

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Nitrogen (N) is an essential and often limiting nutrient for maize production. The use of N fertilizers has been dramatically increased during the last few decades. Understanding the genetic basis of nitrogen use efficiency (NUE) will help develop varieties with improved NUE, which could have both economic and environmental benefits. In this study, we used a robotized platform to measure multiple enzyme activities to study the genetics of enzymes involved in the nitrogen metabolism in maize. We initially tested the activity of 12 enzymes across eight inbred lines grown in high and low nitrogen and found a significant genetic effect for 11 of them. Some enzymes also showed genotype N interactions. Eight enzymes, nitrate reductase, ferredoxin-glutamate synthase, NAD-glutamate dehydrogenase, alanine aminotransferase, glutamine synthetase, shikimate dehydrogenase, citrate synthase, and ADP-glucose pyrophosphorylase, were chosen and determined in a panel of 100 diverse inbred lines for association mapping, in the IBM94 population for traditional QTL mapping, and in three groups of diversity hybrids (26 diverse lines crossing with three different parents) for study of heterosis. A significant association was identified between glutamine synthetase activity and an INDEL in one of the corresponding structural genes. With QTL mapping on the IBM94 population, both cis- and trans-aQTLs were identified for some of the enzymes. Results of heterosis for enzyme activities will also be briefly discussed.

P177

Association mapping and genome-wide breeding value estimation for marker assisted selection of parental and elite inbred lines

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Association Mapping (AM) is a population-wide linkage disequilibrium (LD) based mapping approach that uses "unrelated" germplasm for detection of marker-QTL correlations. In a breeding context, AM may be feasible for utilizing germplasm that has extensive phenotypic data; however, estimating the potential of breeding material has many confounding factors caused by population dynamics. With adjustment for population structure and kinship, AM can be used for QTL discovery for Marker Assisted Selection (MAS). MAS with several markers shows greatest benefit when it can be used to increase gains faster and more cheaply than using phenotypic selection alone. The nature of quantitative traits suggests that MAS with only a few markers will be limited by the low numbers of major effect QTL. AM will be used in this study to select QTL of all effect levels; however, methods for error reduction limit AM's utility to discover small effect QTL for MAS. Alternatively, genomic selection (GS) is a type of MAS that uses markers without prior screening or assignment of significance. GS through genome-wide predicted breeding values (GEBV) can be achieved with genome-wide marker coverage, and GEBV has been shown to increase effectiveness of parental selection of inbred crop species. GEBV can be performed for multiple traits simultaneously through addition of large and small QTL effects for all breeding program relevant traits. AM and GEBV will be conducted in a population of 376 advanced breeding-stage (F8-F10) inbred lines in the Cornell Soft Wheat (*Triticum aestivum*) Breeding Program. This study will test the efficacy of these tools in the context of breeding program and will provide critical information for the application of these tools for inbred development of all crop species.

P178

Association mapping for plant architecture in a sorghum population with introgressed dwarfing genes

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The Sorghum Conversion Program was established by the USDA in the 1960s to introgress dwarfing and photoperiod-insensitivity genes into exotic sorghum, with the goal of developing new varieties suitable for mechanized harvest at temperate latitudes. The Program's great success is reflected in the fact that almost every new sorghum release since then contains a Sorghum Conversion (SC) line in its pedigree. However, the genes underlying this conversion process remain largely unknown. Since virtually all SC lines contain introgressed segments from a single, dwarf donor line, BTx406, specific BTx406-derived alleles associate strongly with plant height. We began by performing association mapping on the only cloned dwarfing gene in sorghum, Dw3, using a panel of 378 sorghum inbreds that includes 230 SC lines. The "conversion region" associated with the Dw3 locus spans several megabases, as defined by the relative haplotype homozygosity of lines with and without the dwarfing mutation at Dw3. However, phenotypic associations with individual polymorphisms within this conversion region are highly variable, predominantly because the dwarf haplotype shares many common polymorphisms with non-dwarfs. This problem can be alleviated by using short haplotypes derived from pairs of adjacent markers. We are now focusing on an uncloned QTL for plant height and flowering time on sorghum chromosome 9, which is being fine-mapped using association methodology.

P179

Characterizing differential responses to *C. heterostrophus* in maize near isogenic lines differing for disease resistance QTL

(submitted by Araby Belcher <arbelche@ncsu.edu>)

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We generated a set of near-isogenic lines (NILs) in a B73 background into which 11 previously-identified southern leaf blight (SLB- causal agent *Cochliobolus heterostrophus*) resistance QTL were introgressed. In replicated field trials most of these QTL conferred significant levels of SLB resistance. The ultimate aim of this study is to characterize the interactions between these NILs and *C. heterostrophus*. In particular we wish to compare NILs with different levels of resistance and determine whether there are observable differences on a microscopic and physiological level. We have shown that adult resistance in the field and juvenile resistance in the growth chamber are highly correlated for all but a few lines, with levels of resistance being somewhat dependant on inoculum pressure. Therefore findings made with juvenile plants will likely be relevant to adult plants growing in the field. Using fluorescence and conventional staining, we are working to differentiate the interactions between *C. heterostrophus* and the susceptible backcross parent (B73), the 2 resistant parent NILs (NC330 and NC292), a major-gene resistant line (B73rhm), and select NILs from our population. In preliminary experiments, using a GFP-transformed *C. heterostrophus* strain, some interesting trends were observed: while growth of the fungus was tightly coupled with growth of the necrotic host area in the B73 susceptible line, fungal growth varied greatly (but not predictably) compared to host cell death in the resistant lines. As expected, the overall rate and extent of disease progression was consistently lower in resistant lines. Initial penetration appeared similar between lines. Experiments using conventional staining techniques are now being undertaken. Results will be presented.

P180

Developing a haplotype map (HapMap): a resource for complex trait dissection in maize

(submitted by Michael Gore <mag87@cornell.edu>)

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Next-generation sequencing of HpaII methylation-filtered (MF) genomic libraries has allowed us to begin identifying and cataloging SNP variation that occurs in the unmethylated "gene space" of 27 diverse maize inbred lines. These 27 inbred lines have high levels of genetic diversity, low levels of linkage disequilibrium, and are the parents of a 5,000 recombinant inbred line nested-association mapping (NAM) population. The NAM population is an assemblage of 26 populations that are genetically unified through a reference design and provides near gene-level mapping resolution of quantitative trait loci. In NAM, only the parental lines need to be genotyped-by-sequencing at high resolution; then lower resolution markers track the chromosomal segments in their offspring. An estimated 1 million SNP markers, however, are required to define most of the haplotype block structure in the 27 maize lines. Sequencing of HpaII MF libraries was initiated on a 454 GS-FLX instrument, which led to the identification of 10,000s of putative SNPs between B73 and Mo17. We have now transitioned to sequencing HpaII MF libraries on an Illumina 1 G Genome Analyzer, because of its greater throughput and lower cost/Mb. HpaII MF libraries were constructed for each of the 27 maize lines and sequenced multiple times on the Illumina platform. Preliminary results from our bioinformatics analyses of the Illumina sequence data are presented.

P181

Developing a seed DNA-based genotyping system for marker-assisted selection and revisiting the hetero-fertilization phenomenon using molecular markers in maize

(submitted by Yunbi Xu <y.xu@cgiar.org>)

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Leaf collection from the field, labeling and tracking back to the source plants after genotyping are rate limiting steps in leaf DNA-based genotyping. We developed an optimized genotyping method using endosperm DNA sampled from single maize seeds, which can be used to replace leaf DNA-based genotyping. With this method, part of the endosperm was excised from soaked seeds and then ground and DNA extracted in 96-tube plates. The quality of DNA was functionally comparable to that of leaf DNA. Extraction from 30 mg of endosperm yields 3-10 microg DNA, which is sufficient for 200-400 agarose-gel PCR-based markers, with the potential for several million chip-based SNP marker analyses. By comparing endosperm and corresponding leaf DNA of an F2 population, genotyping errors caused by pericarp contamination were found to average 3.8%, depending on the SSR markers used. Endosperm sampling did not affect germination rates under controlled conditions, although under field conditions the germination rate, seedling establishment, and normalized different vegetative index were significantly lower than that of non-sampled controls for some genotypes. However, careful field management can compensate for these effects on germination and seedling establishment. Seed DNA-based genotyping lowered costs by 24.6% compared to leaf DNA-based genotyping due to reduced field plantings and labor costs. It can be used to select desirable genotypes before planting, providing an opportunity for dramatic improvements in the efficiency of breeding systems through optimized combinations of marker-assisted selection and phenotypic selection within and between generations. The genetic and environmental effects of hetero-fertilization are being investigated at the molecular level by comparative analysis of seed (endosperm) DNA versus the corresponding leaf (embryo) DNA using a large number of F2 and three-way crosses growing under different environments. The genetic contribution from maternal gametes was revealed for the first time as an additional contributor to the phenomenon of hetero-fertilization.

P182

Diploid apomixis during haploid induction in maize

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Every year among maternal haploids, produced by using a haploid-inducing line, we reveal maternal diploid plants or, in other words, the result of apomictic reproduction. Attempts to maintain this ability by the means of individual selection were unsuccessful. Probably, this is connected with the facultative status of apomixis which is a very common opinion. Nevertheless, during the last years a genotype, population AP, with a relatively high frequency of diploid apomixis was found - about 1%. However, we can not exclude some specific influence of the haploid inducer in the cases of diploid apomixis.

To avoid the influence of the inducer, the population was crossed with the Mangelsdorf's tester, plus the R1-nj gene (colored embryo and endosperm). Previously it was determined that the tester did not possess a haploid-inducing ability.

After harvesting, kernels with colorless embryos and colored endosperms were selected like possible apomicts. Plants, which will be obtained from these kernels, will be selfed with a purpose to check the expression of the other marker genes of the Mangelsdorf's tester. Absence of any marker-gene expression will be an additional proof of the apomictic way of reproduction in the selected kernels.

For further work, we plan to apply a recurrent selection for apomictic development in the AP population.

P183

Discovery of QTLs for root architecture in maize using a Gasp Flint x B73 introgression library

(submitted by Roberto Tuberosa <roberto.tuberosa@unibo.it>)

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The genetic control of root architecture in maize was studied using an introgression library (IL) developed from B73 x Gasp Flint. This IL includes 72 lines developed following five marker-assisted backcrosses using B73 and Gasp as recurrent and donor parent, respectively. Each IL line retains ca. 30-40 cM of Gasp genome and an estimated 70% of Gasp genome appears represented within the collection. Based on trait differences between the donor and the recipient genotypes, the IL collection will serve as a permanent source of nearly isogenic material for QTL analysis and cloning for morpho-physiological traits such as root and plant architectures, flowering time, yield, etc. IL lines were screened using a simple paper-roll method to search for QTLs controlling root traits at the seminal stage of development (e.g. length and dry weight of the primary and seminal roots, number of seminal roots). The same set of ILs were also grown in pots to study root traits at a later stage of development. Particular striking differences were observed between the two parental lines and among the IL lines for the number of seminal roots developing from the scutellar node. B73 produced an average of 2.8 seminal roots per plant while Gasp did not show any seminal root. Within the IL, we found a few IL lines showing a Gasp-like phenotype, implying that the QTLs controlling this trait are localized on the introgressions carried by these lines. A summary of the data on QTLs for root architecture will be presented. The ILs were crossed with Mo17 and the 72 testcrosses were grown under well-watered and water-stressed conditions in a replicated field trial carried out in 2007 in order to evaluate the effects of root architecture on field performance. The analysis of the field data is presently underway.

P184

Dual Testcross QTL Analysis: a solution to the current rate-limiting steps of positionally cloning QTL in maize

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

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Pinning a phenotypic effect to a nucleotide sequence of a natural allele has obvious value for trait manipulation through both breeding and transgenesis. Thus, as an end, positional cloning of a QTL is well-justified even by arduous means. Fortunately, positionally cloning maize QTL has become increasingly viable with the introduction of intermated RILs, which offer improved primary resolution as well as possibilities to streamline the finemapping process. After QTL positions with confidence intervals are established for a population, lines with recombination events within the bounds of the QTL confidence interval can be identified. Delineating the boundaries of these events has become routine, leaving only the challenge of establishing which QTL allele is carried by each of these lines. Since the identity of the QTL allele carried can't be discerned from flanking marker data, it is often inferred from a regression model. However, definitive evidence is required to support conclusive finemapping, so progeny testing is paramount. The traditional approach has been to breed and analyze NILs, which requires several years. Here we introduce Dual Testcross QTL Analysis (DTQA), a marker-based breeding method and its associated design and analysis tools, which together allow progeny testing to be completed within one year of initial QTL identification. This immediate interrogation of the informative recombination events that are known to exist in the original analysis population drastically accelerates the QTL cloning process by 1) informing the design of the screen for additional recombinants, and 2) streamlining the analysis of the newly identified recombinants.

P185

Fine mapping of maize flowering time QTL on chromosome 1

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In plants, flowering time is the key trait responsible for adaptation to new environments and for evolutionary population substructuring. Using the Nested Association Mapping (NAM) approach, we have analyzed flowering time in maize. Detailed information about the approach and analytical methods used to identify QTL associated with two flowering time traits (days-to-silking and days-to-anthesis) are discussed elsewhere. Briefly, flowering time data were collected from 5000 recombinant inbred lines (RILs) from 25 families that were genotyped and phenotyped for genetic and QTL mapping. One of the largest effect QTLs detected for both flowering time traits maps to a region on chromosome 1 that contains 3 FPC contigs: ctg21, ctg22 and ctg23. This region corresponds to a 2.2 cM interval on the genetic map. The QTL is segregating for a significant additive positive effect within 22 of the 25 families - due to common parent B73 containing the rare allele in addition to what appears to be an allelic series across the entire population. In order to fine map the position of the QTL, genes identified in this region of the physical map are being scored in 77 recombinant inbred lines, which are recombinants for this 2.2 cM region. There are 60 BACs in this interval, 31 of which do not contain any non-transposable element like genes. Of the remaining BACs, 9 of them contain 84 viable gene models, 63 of which did not have any similarity to sequences from protein databases. Of the remaining 21 gene models, 17 of them were amenable to sequencing and genotyping assays. A homolog of *Arabidopsis* flowering time gene *Flowering Locus-D (FLD)* resides within this interval, however, recombinant analyses indicated that it does not contain the functional polymorphism. Progress to date on identification of the functional locus and polymorphisms underlying this QTL will be presented.

P186

Genetic diversity among maize inbreds adapted to hill regions of India revealed by functional SSR markers

(submitted by Jai Dev <jdhp@rediffmail.com>)

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Knowledge about the genetic diversity among maize inbreds is important for hybrid maize breeding. In the present study, the molecular genetic diversity among 82 maize inbreds, procured from three maize research stations situated in the North-Western Himalayan region of India, namely, Almora, Ranichauri and Bajaura, was investigated using gene derived simple sequence repeat (SSR) markers. A total of 70 gene-derived SSRs, distributed uniformly throughout the maize genome, were adopted from the website of Maize Genetic and Genome database (<http://www.maizegdb.org>). These 70 SSRs produced a total of 158 alleles among the inbreds, of which 123 (77.8%) were polymorphic. The number of alleles detected per marker ranged from 1 to 7 with an average of 2.25 alleles per marker. Cluster analysis using unweighted pair group method with arithmetic average (UPGMA) revealed two major groups among the inbreds. The inbreds developed/adapted by/at Bajaura and Rainchauri centers clearly delineated into separate clusters indicating their distinct genealogies. These results also indicated the narrow genetic base of the germplasm being used by different centres. Nevertheless, exploitation of the inbreds from different clusters for obtaining heterotic combinations is possible.

P187

Genetic modulation of harvest index for improved biofuel feedstocks

(submitted by Steve Moose <smoose@uiuc.edu>)

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One of the primary physiological responses to selection for higher grain yields is an increase in harvest index, the proportion of total plant biomass that is harvested as grain. However, recent interest in developing biofuels from agricultural feedstocks has turned attention to alternative maize ideotypes with reduced harvest index that may be optimized for biofuel production. Our recent efforts to characterize nitrogen use efficiency in a broad range of maize germplasm have also characterized genetic variation in harvest index and identified additional phenotypes relevant to enhancing biofuel production. Using hybrids derived from the IBM recombinant inbred line mapping resource, we have identified QTL controlling stover biomass, harvest index, and the response of these traits to N fertilizer. We find that in contrast to grain yield, stover biomass is not highly-responsive to N, raising the possibility of greatly reducing N fertilizer requirements for maize crops grown primarily for stover biomass. We have also extended previous observations that maize hybrids with greatly reduced seed set (and hence harvest index) accumulate high levels of sugar in their stalks. Due to the photoperiod sensitivity contributed by the tropical parent, hybrids derived from crosses between tropical x temperate inbreds show greatly delayed flowering and increased anthesis-silking interval, resulting in enhanced biomass and the accumulation of high stalk sugar concentrations instead of grain. Finally, recent work in Arabidopsis has demonstrated miR172 plays a role in promoting flowering in long days. We show that transgenic overexpression of Glossy15, a known miR172 target, produces a subset of phenotypes associated with photoperiod sensitivity, including enhanced biomass and sugar accumulation. Collectively, these observations suggest a number of paths forward for optimizing maize hybrids for the emerging biofuels industry.

P188

Genetic variation between husk leaves and tillering in forage maize

(submitted by Hee Chung Ji <cornhc@rda.go.kr>)

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Husk leaves that usually tightly enclose field/sweet corn affect leaf sheaths resulted in increase of photosynthetic yield production. The experiment showed that relationship between tiller and husk leaf in field condition and the length of husk leaf of the first-ear has high correlation with that of second-ear($r=0.85^{**}$). The number of tiller also showed high correlation with the length of husk leaves($r=0.68^{**}$). Some possessed long husk leaves, prolificacy as well as tiller character.

Prolificacy maize also appeared tillering character as like grass tiller(gt), corn grass(cg) and perennialism(pe) mutant so on.

At the first considering condition, maize with long husk leaves(lhls) sometimes have unfilled, small ear, irregular ear-row, long shank characters.

Breeding for long husk leaves or tillering maize with ears is more advantage for quality in silage production rather than grain yield increasing because stem is somewhat soft than general Maize.

P189

Genetics of field dry down rate and test weight in early maturing elite by elite maize hybrids

(submitted by Junyun Yang <junyun.yang@ndsu.edu>)

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Test weight and dry down rate are economically important traits of maize (*Zea mays* L.), particularly for varieties grown in production areas with short-growing seasons. The objectives of this research are to better understand the genetic base controlling the expression of test weight and dry down rate and to identify elite inbred lines and hybrids with high test weight and fast rate of dry down. Three North Carolina II mating designs made of elite North Dakota State University (NDSU) and industry lines were used. Hybrids were grown across ND and MN locations in experiments arranged in 12 x 12 partially balanced lattice designs with two replications per location in 2007. Data were collected for days from planting to flowering (DF), ear moisture at four 7-day intervals starting 45 days after pollination (D1, D2, D3, and D4), area under the dry down curve (AUDDC), test weight (TWT), and grain yield. Positive correlation was detected between DF and D1~D4 ($r = 0.75 \sim 0.82$), and between DF and AUDDC ($r = 0.84$), indicating that earlier materials tend to have lower initial moisture content and contribute to lower AUDDC. A negative correlation was detected between AUDDC and TWT ($r = -0.56$), implying that field drying rate has important impact on test weight. Combining ability analyses suggested both additive and non-additive gene action are important for dry down and test weight, but additive effects are more prevalent. Experimental lines ND05-73, ND06-85, and ND06-211 are promising sources of fast drying hybrids while ND06-144 and ND06-50 showed potential for grain yield and TWT. Second year testing is planned for 2008. Crosses between top lines and double-haploid derived lines will be produced in order to obtain mapping populations for both traits in order to confirm results obtained via traditional quantitative genetics.

P190

Genotypic and phenotypic analysis of B73 X Mo17 (IBM) populations after 4 And 10 generations of intermating

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Random mating within a population creates more opportunities for recombination between linked loci. Consequently, the order of loci on the genetic map can be better resolved, and the resolution of loci associated with phenotypes of interest, e.g. QTL, can be increased. The intermated B73xMo17 (IBM) population is a widely used resource for genetic mapping in maize. It was developed by intermating the F2 for 4 generations before deriving recombinant inbred lines (RILs). To further increase the resolution of the genetic map, a second population of double haploid lines (DHLs), IBM-10, was created after six additional cycles of intermating at Iowa State University. The additional 6 cycles of random mating resulted in a higher frequency of recombinants in IBM-10 than IBM, but their effect on phenotypic estimates of population parameters have not been evaluated. To assess the phenotypic consequences of the additional cycles of random mating, IBM RILs and IBM-10 DHLs were evaluated for 9 traits in 2 environments. The populations were assessed for differences in means, variances, and phenotypic correlation coefficients among the traits. A statistically significant increase in genotypic variance was detected only for kernel oil concentration. Changes in phenotypic correlation coefficients among traits were not detected. Population means were significantly larger for IBM-10 than for IBM for growing degree days, plant height, and kernel oil concentration.

P191

High precision growth and high-content phenotyping of complete corn plants

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Quantitative multi-parameter measurements of phenotype development for large numbers of plants is in many cases the key issue to identify advantageous plant traits. This may be to be used in gene identification, selection for further breeding or to test stability and performance of newly developed lines.

For this purpose LemnaTec provides the fully integrated high-throughput technology platform Scanalyzer 3D Conveyor. This HTS platform includes conveyor belts for plant supply to the 3D imaging station or for full greenhouse management, imaging stations for visible and NIR images (water content), chlorophyll and GFP fluorescence imaging of the full plants and thermal imaging. Additionally automatic programmed watering and weighting stations for water use efficiency or drought stress experiments are available. The growth of the plants on moving belts in the greenhouse allow both a statistically sound randomisation and a growth near to field density (moving field concept).

Quantitative non-destructive assessment of plant phenotype parameters (e. g. leaf area, leaf length, colour, internode length) extracted at each point of measurement and phenotypic developments in time like growth rates, stay green and dynamic reversible reaction on drought or other stressors (leaf orientation and rolling) are automatically quantified. The new LemnaLauncher platform provides a strongly extended set of shape and morphological parameters for each plant or even parts of the plant. Together with the new LemnaMiner for fast and convenient data-mining a whole set of new options to look closer in detail on plant growth is available now.

Newly developed imaging concepts for Roots in columns and big containers adding spatially resolved NIR root water extraction efficiency to visible root assessments open new horizons in advanced root phenotyping.

Imaging units designed for corn may be used additionally for a quantitative assessment of a wide range of other plants including wheat, barley, rice and e. g. Arabidopsis.

P192

How do disease QTLs affect the development of northern leaf blight in maize?

(submitted by Ellie Walsh <ekw7@cornell.edu>)

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Our general objective is to understand how disease resistance QTLs affect fungal pathogenesis in maize. Studies on host-pathogen interactions in a range of pathosystems have revealed an array of mechanisms by which plants reduce the efficiency of pathogenesis. With the aim of relating specific loci with effects on specific stages of fungal development, we have mapped QTL for resistance to northern leaf blight (NLB), caused by *Exserohilum turcicum*, using chromosomal segment substitution lines (CSSLs) derived from B73/Tx303. We identified QTLs for NLB resistance using macroscopic disease components, including incubation period (IP), lesion expansion, and diseased leaf area (DLA). Near isogenic lines (NILs) capturing specific NLB-QTLs were generated and are being characterized for detailed microscopic components, targeting different stages of pathogenesis. Preliminary results suggest that the B73 allele at bin 1.02 does not reduce the efficiency of fungal infection, but is effective for reducing secondary hyphal growth surrounding the infection site, as well as inhibiting hyphal growth into the xylem. The effect of this disease QTL on destructive hyphal growth is under further investigation.

P193

Identification of QTL and eQTL for nitrogen use efficiency in IBMRIL x IHP1 population

(submitted by Devin Nichols <dmnichol@uiuc.edu>)

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Nitrogen (N) is often a yield-limiting nutrient in the production of maize. The identification of genes involved in nitrogen use efficiency (NUE) and the use of such genes to develop hybrids with greater NUE could have both economic and environmental benefits. The objective of this study was to identify QTL controlling NUE and its component traits. The mapping population used was a set of hybrids developed by crossing lines from the IBMRI population to the Illinois High Protein (IHP1) inbred. This population takes advantage of the high mapping resolution of the IBMRI and the high N uptake phenotype of IHP1. A set of 243 hybrids was grown with and without supplemental N in an N-responsive environment. Measurements of plot yield, stover biomass, stover N concentration, and grain protein, oil, and starch concentration were made and used to calculate NUE and its component traits. Metabolites including leaf nitrate and earshoot amino acid concentrations were also measured. Additionally, mRNA expression levels of several N-responsive genes were measured in the population using quantitative PCR for use in expression QTL analysis. We will report on progress to identify genes associated with identified QTL, particularly those that influence suites of related physiological traits and their response to N.

P194

Identification of genomic regions associated with ear shoot development and tassel architecture in maize

(submitted by Maria Mayor <mlmayor@iastate.edu>)

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Maize inflorescence architecture is defined by a series of reiterative branching events of the shoot apical meristem and/or axillary meristems. Hence, development of tassel and ear may involve some shared and some unique steps on the same pathway. The objectives of this study are to find genomic regions associated with number of ears per plant, tassel morphology traits and to assess their relationship. Inbred lines C103 and C103AP were used to generate 218 Recombinant Inbred Lines (RILs) to evaluate the variation in ear and tassel traits in 3 different environments. C103 is a single-eared inbred prone to abort its ear shoots in stressful environments while C103AP is an isogenic prolific inbred developed by backcrossing C103 with a donor prolific popcorn population (AP). C103 has lower number of ears per plant (epp), a longer tassel (tl) and an increased number of primary branches in the tassel (tbn) when compared to C103AP. The population of RILs used has intermediate values of epp and tl, while it has lower values than the lowest parent (C103AP) for tbn. Genotyping at 889 SSR loci revealed that approximately 80% of the nuclear genomes of C103 and C103AP are identical by descent; hence, chromosomes were dissected into linkage groups. QTLs were located on chromosomes 1, 3, 8 and on the short arm of chromosome 9. Their effect on the phenotype and interactions will be discussed.

P195

Impact of lignin on resistance to *Aspergillus flavus* infection in maize

(submitted by Lindsay Spangler <lms365@psu.edu>)

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Aspergillus flavus is a pathogenic fungus that can cause the accumulation of carcinogenic aflatoxins in maize and other oil-rich seeds such as cotton and peanut. Caffeoyl-CoA-O-methyltransferase (CCoAOMT), an enzyme in the lignin biosynthetic pathway, is more abundant in maize inbreds resistant to *A. flavus* infection and aflatoxin accumulation than in susceptible inbreds. Evaluation of lignin in cobs resistant or susceptible to *A. flavus* was done by studying lignin composition, cob protein antifungal properties, and characterizing the CCoAOMT gene. Lignin was extracted from an *A. flavus* resistant inbred (Mp313E) and a susceptible inbred (SC212m) 21 days after silking (DAS). Acetyl bromide and thioacidolysis extractions determined that there were no differences in total lignin content between the two inbreds. Thioacidolysis monolignol tests showed that resistant inbreds had two times more guaiacyl subunits than SC212m. Syringyl lignin was more prevalent in SC212m than Mp313E. Although both inbreds have the same amount of total lignin, more syringyl lignin in SC212m may make it easier for *A. flavus* to infect the cob. In vitro antifungal activity assays were performed using resistant and susceptible cob proteins extracted 21 DAS and analyzed 25 days after inoculation. The assay found that Mp313E and SC212m cob protein extracts inhibited fungal growth more than a buffer control, but there was no significant difference in fungal inhibition between the two inbreds. More research is needed to determine the overall impact of lignin on *A. flavus* resistance in maize.

P196

Major and minor QTL as well as epistasis contribute to fatty acid composition and oil concentration in high-oil maize

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High-oil maize is a unique genetic resource for genomic investigation in plants. To determine the genetic bases of oil concentration and composition in maize grain, we phenotyped a recombinant inbred population derived from a cross between normal line B73 and high-oil line By804 using gas chromatography, and genotyped the lines with 228 molecular markers. A total of 42 individual QTL, associated with fatty acid composition and oil concentration, were detected in 21 genomic regions. Five major QTL were identified for measured traits, one each of which explained 42.0% of phenotypic variance for 16:0 (palmitic acid), 15.0% for 18:0 (stearic acid), 27.7% for 18:1 (oleic acid), 48.3% for 18:2 (linoleic acid), and 15.7% for oil concentration in the RIL population. Thirty-six loci were involved in 24 gene-pair epistatic interactions across all traits with explained phenotypic variances ranging from 0.4% to 6.1%. Eighteen candidate genes related to lipid metabolism were mapped, and seven localized within or were close to identified individual QTL, explaining 0.7% to 13.2% of the population variance. These results implied that a few major QTL with large additive effects could play an important role in increased fatty acid compositions and oil concentration. A larger number of minor QTL and a certain number of epistatic QTL, both with additive effects, also contributed to fatty acid composition and oil concentration.

P197

Mapping and genetic dissection of loci conditioning disease resistance in maize

(submitted by Chia-Lin Chung <cc435@cornell.edu>)

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A range of approaches for QTL analysis have been used to identify, characterize and dissect loci conditioning disease resistance (disease QTLs) in maize. By investigating a set of chromosomal segment substitution lines (CSSLs) derived from B73/Tx303, several QTLs for resistance to northern leaf blight (NLB) were mapped. Two QTLs with large effects, associated with the B73 allele at bin 1.02 and the Tx303 allele at bin 1.06, have been further validated in F2 populations. The effects of the two NLB-QTLs on different stages of pathogenesis is being characterized by detailed phenotyping of the derived NILs. The heterogeneous inbred family (HIF) analysis was explored for targeted QTL mapping and NIL development. Starting with F5 and F6 HIFs derived from B73/CML52 and S11/DK888, we used 74 SSR markers covering 38 bins to identify residual heterozygotes, and generated a series of NIL pairs contrasting for chromosomal regions associated with multiple disease resistance. By testing the NILs for resistance to NLB, gray leaf spot (GLS), southern leaf blight (SLB), anthracnose leaf blight (ALB), anthracnose stalk rot (ASR), common rust, common smut, and Stewart's wilt, we identified several disease QTLs. Most were effective for single diseases. The DK888 allele at bin 8.06 was associated with race-specific resistance to NLB. Race specificity and map position of this resistance locus suggest that it is *Htn1*, a major gene that delays lesion development. The CML52 allele at bin 6.05 was associated with resistance to three vascular diseases - NLB, ASR and Stewart's wilt. Genetic dissection of these disease QTLs is underway.

P198

Mapping quantitative trait loci for kernel oil production in Korean High Oil (KHO) corn

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Increasing the amount of kernel oil is one way of adding value to corn. High oil corn is desired if used as feed because it increases feed conversion ratio, thereby reducing the amount of fat supplements needed for livestock feed. High oil corn is also of particular interest today because of its potential to be a source of both ethanol (from seed starch) and biodiesel (from seed oil). This is made possible by the availability of a new high oil corn germplasm that has at least 20% kernel oil content, which we named Korean High Oil (KHO).

The main objective of this research is to search for QTLs (quantitative trait loci) responsible for the increased amount of kernel oil that can be used for breeding. The mapping population consists of 473 F2 plants. DNA from these plants have been extracted and marker polymorphism survey using simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) is currently ongoing. To date, 73 polymorphic SSR markers spanning chromosomes 1-5 were identified out of the 179 SSR markers tested. Meanwhile, 68 out of the 220 SNP markers distributed throughout the ten corn chromosomes were found to be informative. Polymorphic SSR markers were identified by electrophoresis on a 3% agarose gel, while SNP markers were identified using the Sequenom primer extension and mass spectrometry platform. Phenotyping for kernel oil content is ongoing.

P199

Nested association mapping analysis of tassel architecture

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Nested association mapping (NAM), a combination of QTL mapping and association analysis, is an integrated strategy that allows for genome-wide high-resolution mapping in a cost-effective way. The experimental design of NAM consists of 25 core maize diversity lines crossed to B73 and a set of 200 recombinant inbred lines derived from each set. Here we are presenting the results from initial NAM analysis of tassel architecture. Five tassel architecture traits, tassel branch number (TBN), total tassel length (L1), central spike length (L2), central spike spikelet density (CSD), and primary branch spikelet density (PSD), were measured on this resource in multiple replicates in years 2006 and 2007. We are addressing two questions. (1) What is the exact genetic architecture of tassel architecture, i.e., how many QTL govern the various components of tassel architecture? QTL analysis was based on best linear unbiased predictor (BLUP) estimates of family values across replications. Procedure GLM Select in SAS was used to build QTL models using stepwise selection method and significance level to enter and to stay set to 1×10^{-5} . This model identified ~ 40 QTL explaining for ~70% of the genetic variance, suggesting that >40 QTL are involved in the genetic architecture of TBN. QTL with big effects for TBN were detected in bins 2.00, 3.06, and 7.02. (2) What is the resolution of these QTL? The resolution of the QTL from the initial analysis was in general high and varied, with some flanking markers on the same BAC to 20 BACs between them. Next we will be looking at QTL shared between the various components of tassel architecture to find QTL with pleiotropic effects.

P200

Phenotypic analysis of kernel water relations in the Mo17xH99 RIL population

(submitted by Kendra Meade <kameade@iastate.edu>)

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The relationship between dry matter accumulation and water content in the kernel, the kernel water relation, is indicative of the final kernel weight and maximum kernel volume, both of which are closely tied to grain yield. Kernel water relations were described throughout the grain fill period. Four points were selected to describe key times in the grain fill period. Fresh weight and dry weight were measured at 15, 30, 45, and 60 days after pollination, approximately when dry matter starts accumulating, maximum water content is reached, dry matter stops accumulating or physiological maturity, and after physiological maturity is reached or harvest, respectively. The fresh weight and dry weights were used to determine water content and dry matter content and thus the kernel water relations. These traits were measured in 186 recombinant inbred lines from a cross between Mo17xH99. The population was grown in three replications of an alpha lattice at the Agronomy Agricultural Engineering Resource Center (AAERC), Ames, Iowa in 2007. The results of the phenotypic analysis of these traits are presented here.

P201

Phenotypic diversity of flowering-related traits of maize landraces from the core collection preserved in Chinese National Genebank

(submitted by Tianyu Wang <wangtianyu@263.net>)

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Understanding diversity profile of crop germplasm is the basis of effective conservation and efficient utilization of genetic resources. Our study investigates the phenotypic diversity of four flowering-related traits, including tasseling (T), pollen-shedding (S) tassel-pollenshedding interval (TPI) and anthesis-silking interval (ASI). 799 maize landrace accessions from the core collection preserved in Chinese National Genebank were planted according to multiple-environment trial (MET), and the phenotypic observation of 4 flowering-related traits, including T, S, TPI and ASI, were recorded during the reproduction stage of these accessions. Shannon-Weaver diversity index was calculated based on the phenotypic estimates of these 4 flowering-related traits, and ANOVA was conducted to test the significance of differentiation among the phenotypic estimates and Shannon-Weaver diversity indices. The results of ANOVA showed that the differentiation of phenotypic estimates of all the 4 flowering-related traits significant at 1% level among 32 provinces/regions, and except TPI, the differentiation of Shannon-Weaver diversity indices of T, S, and ASI significant at 1% level. Among the 6 maize regions of China, phenotypic estimates of all these 4 flowering-related traits significant at 1% level, and the Shannon-Weaver diversity indices of T, S, and ASI significant at 5% or 1% level. Among the 6 maize regions in China, the Shannon-Weaver diversity indices of T, S, TPI, and ASI of accessions from Southwest Montane Maize Region are 1.85, 1.90, 0.88, and 1.20 separately. There exists high level of phenotypic diversity of T, S, TPI, and ASI among the maize landrace accessions conserved in Chinese National Genebank, and among 6 maize regions in China, the average phenotypic diversity level of the four flowering-related traits of the accessions of Southwest Mountainous Maize Region was significantly higher than those of the other 5 maize regions, which indicates that there exist relatively high protection value and utility potential in the maize germplasm of Southwest Mountainous Maize Region.

P202

Physiological mechanisms underlying grain yield QTLs

(submitted by Andrea Chambers <aarmst03@uoguelph.ca>)

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We are currently utilizing a novel QTL mapping population structure identical-by-descent recombinant-inbred-lines (IBD-limited RILs) to identify grain yield QTLs and subsequently elucidate their underlying physiology. The key features to this approach are that gross phenotypic differences (e.g., plant height and flowering date) are minimized and population size is substantially reduced. Currently we have identified grain yield QTLs and are dissecting the underlying physiology of how these regions influence grain yield. A subset of 80 IBD-limited RILs from the CG60xCG108 mapping population crossed to CG102 were grown in a 3 rep, 3 year (2005-07) physiology trial at Elora, Ontario. Given that grain yield is essentially the ability of the corn plant to accumulate dry matter and then partition it to the ear (i.e., harvest index), we are examining dry matter accumulation during development and harvest index (HI) in this mapping population. All of the genetic variation for dry matter accumulation in this population is due to differences in dry matter accumulation during the grain filling period. Preliminary analyses suggest dry matter partitioning to the ear (HI) is the most sensitive grain yield component process to environmental variation. Grain yield QTLs have been identified and coincide with QTL for underlying physiological mechanisms. We will further examine how the expression of these physiological QTLs changes under different environmental conditions and begin to investigate candidate genes.

P203

Proteomic analysis of maize leaf tissues from inbred lines resistant and susceptible to fall armyworm, *Spodoptera frugiperda* (J.E. Smith)

(submitted by Ramneek Kooner <rzk126@psu.edu>)

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Fall armyworm, *Spodoptera frugiperda* (J.E. Smith), which are serious pests in the southern United States, show retarded growth when they feed on insect-resistant maize inbred, Mp708. This maize genotype is not only resistant to FAW, but to a number of other Lepidopterans. This study is primarily aimed at investigating proteins in the maize whorl which is the main larval feeding site. In this genotype, a unique, extracellular, 33-KDa cysteine protease (Mir1-CP) accumulates in whorl in response to insect feeding. On the other hand, susceptible inbred Tx601 does not accumulate Mir1-CP in response to larval feeding. In this study we are interested in identifying additional insecticidal proteins present in the resistant maize whorl. To achieve this goal we are performing comparative proteomic analysis by employing gel-based proteomic tools like two-dimensional Difference In Gel Electrophoresis (2-D DIGE) coupled with tandem mass spectrometry to identify differentially expressed proteins from whorls of resistant and susceptible varieties. Inbreds were fed with 3rd instar larvae for 24 hours, and samples were collected around the fed area of yellow-green part of leaf. In our preliminary tests, we were able to see differentially expressing proteins between fed resistant and susceptible inbreds. Further investigation is being done to identify these differential proteins and look for additional herbivore defensive proteins.

P204

QTL analysis of the IBM RIL population of growth characteristics and saccharification potential, part of the Integrated Saccharification Technology Toolkit (ISTT)

(submitted by Bryan Penning <bpenning@purdue.edu>)

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The tremendous natural genetic diversity and extensive toolsets of maize provide a rich resource to identify genes for the improvement of biofuel crops. Maize has numerous gene knockout populations derived from transposon technologies and EMS mutagenesis, lines of diverse alleles for gene mining, such as the maize diversity lines, and recombinant inbred line populations such as the Intermated B73-Mo17 recombinant inbred (IBM RI) lines, and a soon to be completed genomic sequence that can be utilized to build and test models to maximize usable lignocellulosic content in biofuel crops. Recombinant inbred lines (RILs) are immortalized, heavily genotyped populations of plants with sections of different parents intermixed at fixed positions. This allows for continuous studies over multiple years and locations on the same genetic material with the ability to map traits to specific chromosomal loci. RILs also provide a system to look at transgressive segregation and non-co linearity of genes between lines. We have begun a pilot study using the IBM RI lines to find locations of physical differences in stalks, and we are expanding into the study of saccharification differences to improve ethanol yield by manipulating the natural diversity that exists within these two agronomically important maize lines. This will form part of an Integrated Saccharification Technology Toolkit (ISTT) to improve bioenergy crops. So far several quantitative trait loci (QTL) have been found in the IBM RI line linked to general growth characteristics (quantity). In addition, assays for total free sugars, total lignin content, total cellulose, sugar molar ratios, acetylation of sugars, and release of glucose and xylose over time by enzymatic digestion of maize inbred lines, switchgrass, and *Miscanthus* are being performed to test saccharification efficiency (quality) for inclusion in the ISTT. Once discovered in maize, this information can be rapidly deployed to improve all C4 grasses used in biofuel production.

P205

QTL analysis of the shade avoidance syndrome in maize

(submitted by Patrice G. Dubois <pgd7@cornell.edu>)

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Over the past few decades, increases in maize yield have been associated to a greater tolerance to high planting density. The selective absorption of the light spectra by the chlorophyll creates a vegetative shade characterized by a reduced ratio of red (R) to far-red (FR) light. The phytochrome photoreceptors perceive this low R:FR and initiate a series of morphological changes referred to as the shade avoidance syndrome (SAS). In maize, an increase in plant height, a reduction of tillering, an elongation of internodes and leaf sheaths and a reduction in root development characterize the SAS. This repartitioning of resources to increase competitiveness is thought to result in lower grain yields, though the mechanism remains poorly understood. To examine the genetic variation that contributes to the SAS, both the intermated B73 x Mo17 (IBM) and a nested association mapping (NAM) populations were used for QTL analysis. Canopy shade was mimicked in growth chambers using an end-of-day-FR treatment. Elongation after 10 days was measured on both treated and control seedlings and the difference was used as an approximation of the SAS. The NAM population was also evaluated in the field, using plants located in the middle of a row as treatment and border plants as control. Detailed results from these measurements and subsequent mapping analyses will be presented. A better understanding of the mechanisms controlling SAS could benefit breeding efforts aiming at increasing grain or biomass production.

P206

QTL analysis targeting suppressors in Mo17

(submitted by Eric Riedeman <riedeman@wisc.edu>)

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The inbred Mo17 suppresses the phenotypic expression of seed and plant color factors, chlorophyll mutations, dominant morphological mutations, and disease lesion mutations. At Wisconsin we have backcrossed R1 and Cg1 into Mo17 and B73. Mo17 suppresses phenotypic expression of both R1 and Cg1 relative to B73. The IBM population was used to map QTL associated with suppression of phenotypic expression of R1 and Cg1. Mo17Cg1, Mo17R1, B73Cg1, and B73R1 were crossed into lines from the IBM population. Ears of the R1 crosses were visually evaluated for kernel color. Cg1 crosses were grown, and each progeny was evaluated visually for its overall degree of Cg1 expression and other related traits. The collected data was subjected to a QTL analysis. Significant QTL for the R1 crosses were found on chromosome 7 for both the B73 and Mo17 backcross lines as well as on chromosome 3 for the B73 backcross line. Cg1 evaluations of crosses with the B73 backcross line produced a significant QTL on chromosome 5. Mo17 Cg1 crosses resulted in a significant QTL on chromosome 1. There was also a second QTL peak for the B73 Cg1 backcross line in close proximity to the Mo17 Cg1 peak on chromosome 1. The B73 QTL on chromosome 1 was opposite in effect to the Mo17 QTL.

P207

QTL mapping for high amylose starch content in maize (*Zea mays* L.)

(submitted by Yusheng Wu <yshmh2@yahoo.com>)

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There have been two recent advances that have increased interest in the use of high amylose starch. The first has been in the development of thermoplastic starch-based biodegradable plastics. Second, high amylose maize is found as a source of resistant starch (RS), which will lower the risk of colon cancer when added in foods. A maize inbred line, GEMS-0067 (Reg. no GP-550, PI 643420) possesses high amylose modifier gene(s) that, together with the recessive amylose extender (*ae*) gene, raises the starch amylose percentage to at least 70%. GEMS-0067 represents the only public source of high amylose content to date in the US. A study was designed to identify the number and location of modifier alleles and gene interactions among them leading to the development of the high amylose phenotype. An F2 population derived from a cross of (H99ae GEMS-0067) was planted in South Dakota in 2005. Starch amylose content was measured by amylose-iodine colorimetry while the genotypes of individual F2 ears were characterized using SSR markers by agarose electrophoresis. The results showed two potential quantitative trait loci (QTL). One, on chromosome 5, was 33 cM from *umc1365* and another, on chromosome 6, was 36 cM from *umc2313*. They explained 15% and 5% of the total variance of amylose content, respectively. Two gene markers, *umc1997* in the gene encoding starch branching enzyme (*sbe*) IIa and *phi033* in the gene encoding sucrose synthase (*sh1*), segregated in accordance with 13:3; 12:4 and 9:7 ratios based upon chi square goodness of fit tests. Yet the group means of those ratios were not significantly different in terms of F tests.

P208

QTL mapping of an epigenetic modifier

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P11-Blotched is an epigenetically regulated allele of the purple plant1 gene, which activates synthesis of purple anthocyanin pigments. The phenotype of P11-Blotched plants is variegated, but pigmentation can be increased in the presence of a modifier called Suppressor of plant blotching1 (*Spb1*). This effect on pigmentation is correlated with higher levels of P11-Blotched mRNA and altered chromatin organization of the P11-Blotched gene. These features indicate that *Spb1* epigenetically modifies P11-Blotched. To ask if *Spb1* maps at the same location as any of the known anthocyanin or chromatin genes in maize, we crossed the *Spb1*, P11-Blotched stock to a less pigmented P11-Blotched line and produced two F2 populations of 211 and 279 F2 individuals. The phenotypes in both populations showed a continuous distribution and transgressive segregation, suggesting a polygenic trait. Individuals were genotyped at 104 SSR loci and anthocyanin content was quantified. Composite interval quantitative trait locus (QTL) analysis of the two populations both identified QTL on chromosomes 5, 6, 7 contributed by both the *Spb1* and non-*Spb1* parents. Epistacy and ANOVA analysis of an additional population of ~1400 F2 individuals indicated that epistatic interactions are extremely important for high anthocyanin levels. The QTL intervals identified in both studies contain candidate genes that might influence P11-Blotched pigmentation. One of these genes, *intensifier1*, was previously shown to be capable of increasing anthocyanin accumulation, although the mechanism of action is uncertain. Other candidates in the QTL intervals include several chromatin genes with likely, but as yet uncharacterized, roles in epigenetic regulation. The chromosome 6 QTL spans a region that contains the *p11* locus; however, no sequence polymorphism between the two parental P11-Blotched alleles has yet been identified. This suggests that the chromosome 6 QTL is due to modified chromatin structure inherited from *Spb1*, rather than DNA polymorphism at *p11*.

P209

Single segment introgression lines based cloning a QTL for plant high in maize

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Two populations of link-up single segment introgression lines (SSILs) were developed with 87-1 and Zong 3 as the recurrent parents, Hengbai 522 as donor, respectively through backcross-marker assisted selection (BC-MAS). Foreground and background of populations were screened by SSR markers in each cycle of backcross. Genetic evaluation showed that 91 and 50 segments from the donor line were introgressed into Zong 3 Z3HBILs and 87-1 87HBILs SSILs, respectively. In population of Z3HBILs, the average length of a single introgressive segment was 33.5 cM, with 97.3% recovery of background. The total segment length was with coverage of 86.2% of chromosomes. In population of 87HBILs, the average length of a single introgressive segment was 61.8 cM, with 95.7% recovery of background. The total segment length was with coverage of 51.2% of chromosomes. Using population of Z3HBILs, a total of 59 QTLs of 9 traits were identified in BaoDing and Zhang Jiakou, Hebei province. Out of these QTLs, only 9 were identified in both BaoDing and Zhang Jiakou, showing that much interaction existed between QTL and environment, and many QTL clusters controlling yield and its component were detected. Lots of interaction among major QTL of each trait were detected, using plant height and ear height as example, we constructed an interaction network among plant height QTLs and ear height QTLs, several QTLs located in crossing point were detected. F2 population were developed using Z3HBILs containing bnlgl647-phi036 segment in chromosome 3, using 6 newly developed SSR markers, fine mapping a plant height QTL in this region. Meanwhile, 6 contiguous were constructed in target region to confirm this QTL, and further we got a candidate gene of this QTL, zm-GAox3. Improved Z3 and 87-1 with wx introgression were developed, using marker-assisted selection (MAS).

P210

Some aspects of haploid induction in maize

(submitted by Valeriy Rotarenco <rotarenco@mail.md>)

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Recently, rather high effective haploid inducers have been created. However, we consider that there is a need for their further improvement. New inducers should possess a higher frequency of haploid induction, improved agronomical traits and good expression of marker genes, which allow haploids to be identified at different stages - dry seeds, seedlings and mature plants. According to these requirements, an initial material for creating new inducers has been made. In the F2 progeny of six hybrids involving different inducers 235 plants have been selected and self-pollinated. To estimate their haploid-inducing ability, each selfed plant has been crossed with two plants of a synthetic population. The frequency of haploids ranged from 0 up to 18% (5.2% on average). Additionally, the frequencies of embryoless seeds both in the population (2.1%) and in the inducers (5.5%) were estimated. It was revealed a high positive correlation between the frequencies of the haploid and embryoless seeds in the population - the coefficient of correlation was 0.76. Also, a high value of the coefficient of correlation was obtained between the frequencies of the embryoless seeds in the population and in the selfed inducers -0.48. The second character which had a positive correlation with the haploid induction was the frequency of endospermless seeds. The occurrence of such seeds had a significant effect on the seed set (normal seeds) both in the population and in the inducers. As a rule, ears with the high frequency of haploids (more than 10%) had a poorer seed set. Also, it was determined that some of the endospermless seeds possessed viable embryos - they germinated in a thermostat. Therefore, the occurrence of embryoless and endospermless seeds might be used as indirect characters in the selection work for haploid induction. We also suppose that the obtained results will be useful for the further study of the haploid-inducing ability.

P211

Study of Vgt1 using joint linkage and association mapping

(submitted by Maria Cinta Romay <cromay@mbg.cesga.es>)

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Flowering time is a critical trait for maize adaptation to diverse environments. Using a Nested Association Mapping panel (NAM), three early flowering Northern flint populations have validated the previously described Vegetative to generative transition 1 (Vgt1), a major QTN controlling flowering time in ctg 354 of chromosome 8 (Salvi, et al. 2007). Moreover, we have found evidence of another QTN, also at 68 cM, residing near the Ap2-like transcription factor Rap2.7. This QTN induces later flowering in some populations of the NAM panel. In addition, close to Vgt1, another QTL (Vgt2) related with flowering time seems to be present (Chardon, et al. 2005). With the aid of recently developed statistical methods to account for population structure and kinship (Yu, et al. 2006), a 282 line association mapping panel was queried to obtain an alternative estimate of the effect of the allelic series in Vgt1 and Vgt2 on flowering time. These results support additional research to further characterize these complex regions.

P212

The Keygene CRoPSTM technology in field crops

(submitted by Hanneke Witsenboer <hwi@keygene.com>)

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Keygene is providing sequencing services using the Roche GS-FLX and 454 sequencing technology. For crop plant species Keygene is employing 454 sequencing technology to significantly enhance discovery of SNPs and SSRs. To provide sufficient redundancy of generated sequences, genome complexity reduction is applied. Keygene has developed CRoPS1, a method derived from the AFLP technology² (Complexity Reduction of Polymorphic sequences), that combines the power of the AFLP technology as a robust genome complexity reduction method with the power of highly parallel sequence analysis of DNA fragments. The complexity reduction can be easily modified dependent on the genome size of the crop under study.

Over the last year several CRoPS runs have been performed in field crops. In these projects CRoPS has been employed as a SNP discovery method by the simultaneously sequencing of 2 samples. The efficiency of CRoPS as a SNP discovery tool in field crops will be illustrated.

1 van Orsouw et al (2007) PLoS ONE 2 (11): e1172

2 Vos et al (1995) Nucleic Acids Res 23: 4407-4414

The CRoPSTM and AFLP technologies are covered by patents and patent applications owned by Keygene N.V. AFLP is a registered trademark of Keygene N.V. Application for trademark registration for CRoPS has been filed by Keygene N.V.

P213

The analysis of quantitative resistance to foliar diseases of maize

(submitted by Peter Balint-Kurti <peter_balintkurti@ncsu.edu>)

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We are interested in the analysis of quantitative resistance - the dominant form of resistance utilized in cultivated maize. Very little is known about the molecular genetic or physiological basis of this type of resistance in maize or any other crop. Working with the foliar diseases gray leaf spot (GLS), southern leaf blight (SLB) and northern leaf blight (NLB), we are identifying and mapping new sources of resistance from diverse germplasm. We are fine-mapping quantitative trait loci (QTL) using the IBM and NAM populations as well as by classical linkage analysis in segregating populations. We introgressed 13 SLB resistance QTL into B73 to create a set of near isogenic lines which we are using for the detailed characterization of specific QTL.

We are using the disease lesion mimic phenotype conferred by the recombinant resistance gene Rp1D21 as a reporter for the defence response pathway. We have shown that the Rp1D21-conferred lesion mimic phenotype is profoundly affected by its genetic background. Using the IBM population, we have now mapped a strong modifying locus on chromosome 10, which may represent an important gene in the defence response pathway.

Foliar disease is generally rated subjectively by eye on an ordinal or percentage scale, which introduces a significant source of error. We have used digital imaging and image analysis for scoring disease in the field and for identifying QTL. Results will be presented.

P214

The genetic analysis of maize root complexity

(submitted by Martin Bohn <mbohn@uiuc.edu>)

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The development of a healthy root system is an important part of the overall plant development program. Root architecture is strongly linked to plant survival under abiotic and biotic stress conditions. The objective of this study was to investigate the inheritance of the primary and secondary root system complexity in maize (*Zea mays* L.). For a total of 231 recombinant inbred lines (RIL) derived from the IBM (B73 x Mo17) population primary and secondary root systems were produced using replicated experimental designs in growth chamber and field experiments, respectively. For the primary root system digital images were taken at days four and eight after germination and the secondary root systems were recovered at male flowering. For each root system image, the fractal dimension (FD) was computed. For primary root systems significant differences among RILs were found in the FD calculated after four (FD1) and eight (FD2) days. For FD1 22 QTL, for FD2 13, and for FD change over time (Δ FD) 12 QTL were found on all ten maize chromosomes explaining between 25 and 47% of the phenotypic variation. Four unique QTL were identified for the dynamics of root growth between day four and eight. For the FD of secondary root systems 25 QTL were identified explaining 47% of the phenotypic variation. The number of common QTL identified for the complexity of primary and secondary root was small. With this study, we demonstrated that FD differences among RILs of the IBM population have a genetic basis. Some of these QTL regions were associated with root mutants providing logical candidate genes for root complexity. Most QTL were located in regions with no further information about possible candidate genes. The genetic relationship of root complexity to ear and tassel inflorescence architecture will be discussed.

P215

The genetic architecture of multiple disease resistance in maize

(submitted by Peter Balint-Kurti <Peter.Balint-Kurti@ars.usda.gov>)

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Quantitative resistance is the dominant form of resistance utilized in cultivated maize. By synthesizing the previously published literature on the locations of disease resistance quantitative trait loci (dQTL) in both rice and maize, we were able to show that dQTL are non-randomly distributed in the genomes of both species. Furthermore several chromosomal segments associated with dQTL for multiple diseases were identified. The maize association analysis population (Flint-Garcia et al. Plant J. 44:1054-1064), consisting of 300 diverse inbred lines, was assessed for resistance to three foliar fungal diseases, southern leaf blight (SLB), northern leaf blight (NLB) and gray leaf spot (GLS), in multiple environments for each disease. After accounting for both maturity and for population structure, significant pairwise genetic correlations between resistances to these three diseases were detected. The maize IBM population (Lee et al. 2002. Plant Mol. Biol. 48:453-461) was also assessed for SLB, GLS and NLB resistance over multiple environments and again significant genetic correlations were detected for resistance to the three diseases. We detected 5 GLS QTL and 11 SLB QTL in the IBM population. Only in one case did GLS and SLB QTL precisely co-localize with each other. We will discuss the possible genetic architecture of multiple disease resistance in maize. Analysis of NLB QTL in the IBM population will also be reported.

P216

The genetic architecture of southern leaf blight resistance revealed by nested association mapping

(submitted by Kristen Kump <klkump@ncsu.edu>)

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Previous research has demonstrated that resistance to southern leaf blight (SLB) varies quantitatively. Several SLB resistance QTL have been mapped at low resolution (~2 - 15 cM) in various genomic regions in several segregating populations. In order to fine-map the resistance genes and to elucidate the genetic architecture of this quantitative disease resistance, we evaluated the nested association mapping (NAM) population, developed as part of the NSF-funded Maize Diversity Project, for resistance to SLB. The NAM population is comprised of 5000 recombinant inbred lines (RIL) derived from crosses between B73 and 25 diverse inbred parents. Using a nine-point scale to rate severity of disease symptoms, we scored each NAM line twice, at different times, in three environments: Clayton, NC during the summers of 2006 (natural inoculum) and 2007 (artificial inoculum) and the winter of 2007 in Homestead, FL (artificial inoculum). Considerable genetic variation among the lines was observed in each environment, and scores on the same lines in different environments were significantly correlated. Preliminary analysis of data from two environments has indicated that resistance to SLB is highly heritable (83%). Flowering time within the NAM population is also highly variable. We have observed a significant association between flowering time and apparent disease resistance; therefore, days to anthesis was used as a covariate to minimize the effects of phenological differences on disease responses. Each line was genotyped with 1106 B73-specific SNP markers. By integrating the NAM map information in conjunction with our phenotypic data, we will map QTL associated with SLB resistance. Exploiting advantages inherent in the NAM design, notably large population size, abundant marker data, and a broad sampling of maize germplasm, we hope to attain a high resolution, global QTL analysis of SLB resistance in maize.

P217

The genetics of GxE: Identification of environment-specific grain yield QTLs in maize

(submitted by Travis Coleman <tcoleman@uoguelph.ca>)

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In maize, detection and analysis of quantitative trait loci (QTLs) helps elucidate complex genetic and physiological processes controlling grain yield. Information gleaned from mapping grain yield QTLs may be put to a variety of ends such as studying wide-scale functional genomics or assisting in breeding programs. Because of the complex nature of grain yield, maize QTLs often exhibit extensive genotype-by-environment (GxE) interaction, thus complicating both genomics and breeding efforts. To examine GxE interaction, we are using an unique set of Recombinant Inbred Lines (RILs) derived from the related inbred lines CG60 and CG108. Due to their pedigree, 67% of each RIL genome is identically fixed and many gross phenological differences are minimized. The 128 RILs (Iodent heterotic pattern) were crossed to a CG102 tester (Stiff Stalk heterotic pattern) to form hybrids. This study relies on 3 locations, 4 years, and 3 plant population densities to generate GxE interaction. Currently 23 environments (combinations of location, year, and plant density) resolve themselves into 8 distinct environmental groups based on GxE interaction in yield trials. The results demonstrate two loci acting as QTLs between environmental groups but generally suggest that most grain yield QTLs are specific to a particular environmental group.

P218

Traits associated with brace root characters implicate light and hormonal signaling pathways

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Maize brace roots provide resistance to lodging by facilitating terrestrial anchorage, and mine the soil for nutrients and water. The goal of this study is to determine the value of traits related to hormonal and developmental pathways and light perception as predictors of brace root development using multiple regression. Twenty-seven diverse maize lines were evaluated in two replications in the field in 2007 for brace root, developmental, and morphological traits. Both forward selection and backwards elimination was performed using a significance threshold of 0.05 for entry or elimination. The results of the forward and backward regression were then compared to models which minimized the Bayesian information criteria. R² for the models ranged from 0.2235 to 0.6117. The total number of brace roots is controlled by both the number of nodes with brace roots and the number of brace roots at each node. The explanatory variables unique to either the number of nodes with brace roots or the number of brace roots at node one were present in the total number of brace roots model. Average internode length and juvenile and transition leaf number were significant predictors of total brace root number suggesting that gibberellic acid (GA) activity is influential in brace root development. Inclusion of node diameter and ear height in the model implies that light perception via phytochromes also plays a significant role. A model incorporating GA and light perception is provided.

P219

Transferring of grain yield component in an inbred line of corn (*Zea mays* L.)

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It is known that the heterosis expression in corn depends of the heterozygous condition of a portion of the genome. The balanced lethal system (BLS) found in corn, allows the study of the relative contribution to hybrid vigour of different chromosome segments that conferring high grain yield. In order to increase grain yield, these chromosome segments can be transferred to inbred lines with low grain yield. In this way, a chromosome segment with high grain yield found in an inbred line of corn, regulated by a balanced lethal system was transferred to an inbred line with low grain yield, through crossing and three backcrosses, with the objective of increase the grain yield. The analysis of variance and principal components analysis showed a significant increase of grain yield in the inbred lines obtained by backcrossing. The search of chromosome segments with high agronomic value in the genome of plants is important to plant breeding.

P220

Uncovering molecular mechanisms of quantitative disease resistance using nested association mapping

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Northern Leaf Blight (NLB), caused by *Exserohilum turcicum*, is an endemic disease affecting maize production worldwide. The most economical and effective method for control of NLB is through the use of genetic resistance in the maize host. Historically both qualitative and quantitative resistance have been important for resistance to NLB. Quantitative resistance is important for resistance breeding for crop species in general, as this type of resistance tends to be more durable and broad spectrum. With the goal of uncovering molecular mechanisms conditioning quantitative resistance to NLB, we have begun evaluating the nested association mapping (NAM) population for resistance to NLB. A sub-set of the NAM lines were evaluated in NY during the summer of 2007 for two components of disease: incubation period (number of days until the appearance of disease symptoms) and disease severity. Using this preliminary data, quantitative trait loci (QTL) for NLB resistance have been localized to several genomic locations previously associated with NLB resistance but with greater precision than previous studies. A total of 22 QTL were mapped with a LOD score of 3 or more and 14 of these QTL had a LOD greater than 5. Most QTL map to regions previously shown to associate with NLB resistance but novel QTL were also detected. The largest QTL in the populations was mapped to a 5 cM region in bin 8.06, which is known to carry the resistance gene *Htn1*. With these populations we were also able to observe allelic series at 8 of the identified QTL. Future analysis with the NAM populations will facilitate higher resolution of QTL and selection of candidate genes which was previously not possible due to the very large regions defined by QTL for disease resistance.

P221

Use nested association mapping (NAM) to fine map maize chromosome 10 flowering time QTL

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Joint linkage analysis and association mapping is a powerful strategy for dissecting complex quantitative traits. Nested Association Mapping (NAM) integrates this strategy with a unified maize mapping population consisting of 200 RILs from each of 25 populations derived from crossing 25 diverse inbred lines to the common inbred line B73. NAM will directly provide genome-wide complex trait dissection by sequencing only the 26 founders of NAM and genotyping 5000 RILs with a finite marker set. Here, we report the progress of fine mapping one of the biggest effect flowering-time QTL identified in NAM. This QTL was initially mapped to a 2cM interval of chromosome 10 and is shared by several populations. Different non-B73 derived alleles showed positive and negative effects, suggesting a possible allelic series pattern at this QTL. Three methods are currently being used to fine map this QTL: 1) saturating the QTL region with additional markers, 2) sequencing 26 founders and projecting the nucleotide polymorphisms on the progenies, and 3) using heterogeneous inbred families (HIFs) segregating at this QTL.

An interesting discovery is that an Flowering Promoter Factor 1 (FPF1) homolog is located in the boundary of the target region. FPF1 has been proved to control phase transition through gibberellin-dependent pathway in Arabidopsis, tobacco and rice. An unusual long-range linkage disequilibrium (LD) pattern surrounding the FPF1 homolog was observed. Selection tests suggest this region may be under selection.

P222

Using nested association mapping to study aluminum tolerance in maize

(submitted by Allison Krill <amk72@cornell.edu>)

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Aluminum (Al) toxicity is a major constraint to maize productivity on acidic soils throughout the world. Phytotoxic Al becomes soluble at pH < 5.5, inhibiting root growth and function and severely reducing yields. Al exclusion and intracellular tolerance are important mechanisms involved in achieving Al tolerance in maize; however, little is known about the underlying genetics. Several related grasses have a single major QTL conferring aluminum tolerance, but this trend has not been seen in maize. Current phenotyping methods to score maize for aluminum tolerance are well established, but these methods limit the use of large genetic resources. The diverse parental lines of the 26 RIL populations of the nested association mapping (NAM) panel exhibit a wide range of aluminum tolerance, making this a valuable resource to map common QTL for this trait in maize. Results of NAM will be presented, showing the usefulness of this resource for mapping complex traits despite phenotypic constraints and low heritability.

P223

Variation in pollen competitive ability in diverse maize lines

(submitted by John Fowler <fowlerj@science.oregonstate.edu>)

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Although pollen occupies a small fraction of the angiosperm life cycle, it is of interest for both basic and applied scientific reasons. Seed production depends on a functional male gametophyte achieving fertilization following pollination. Pollen also serves as a vector for gene flow between plant populations. Recently, propagation of transgenic plant cultivars has raised concerns about viable pollen dispersal and its role in introducing transgenes into non-transgenic populations. We are interested in understanding the genetic basis for pollen fitness, and are developing methods to predict whether certain plant cultivars or genotypes pose greater particular risks for transgene escape via pollen-mediate gene flow than others. As a first step in investigating natural variation that affects pollen fitness, we are measuring pollen competitive ability (PCA) in a set of maize lines recommended by the Panzea project to maximize diversity. We use a pollen-mixing method employed previously by Sari-Gorla, Ottaviano, Pe and co-workers and implement it in a Latin Square experimental design. With each line tested on at least four days, we found significant day-to-day differences in PCA. Despite this, a range of significant differences in PCA (relative to the reference W22-R line) among maize inbreds was identified, up to almost 4-fold between MS71 and P39. In addition, the two hybrids (B73xMo17 and T232xCM37) tested in our initial round of experiments produced pollen that was more competitive than the inbred parents. These results are consistent with earlier work demonstrating correlation between sporophytic vigor and pollen fitness (reviewed in Ottaviano & Mulcahy 1989). Data from a second round of experiments, incorporating additional inbred lines and hybrids, is being analyzed and will be presented.

P224

A maize genome modification system based on alternative transposition

(submitted by Chuanhe Yu <yhc@iastate.edu>)

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McClintock found that some kinds of maize chromosome rearrangements are associated with transposition of Ac/Ds elements. We have found that alternative transposition involving a pair of directly oriented Ac/Ds transposon termini can induce deletions or inverted duplications; and that transpositions involving a pair of reverse-oriented Ac/Ds transposon termini can induce deletions, inversions or translocations. Here, we tested whether alternative transposition can be used to develop a genome modification tool.

We designed two types of transgenic constructs: one contains a pair of directly oriented Ds termini, and the other contains a pair of reverse-oriented Ds termini. The maize *c1* gene, which specifies colored kernel aleurone, is used as a visible marker for genome rearrangements; the Ds termini are inserted flanking exon 3 of the *c1* gene. To enable genome-wide distribution, we inserted the *c1::Ds* segment within a second transposon (*I/dSpm*), which itself is inserted into a maize *p1* gene for kernel pericarp color. The transgenic stock is crossed by *En/Spm* transposase lines, and transposition of the engineered *I/dSpm* element results in red pericarp sectors. A number of red pericarp kernels were screened by PCR and inverse-PCR to identify those in which the engineered *I/dSpm* element had transposed. To date we have obtained approximately 20 independent lines in which the *I/dSpm* element has inserted into different maize chromosomes. By crossing these plants with a stable Ac transposase line (*p1-vv5145*), we obtained many candidate rearrangement kernels with loss of *c1* function. PCR analysis and sequencing showed that the plants grown from these kernels carry a variety of chromosomal rearrangements, including deletions, inversions or translocations. We anticipate that this maize genome modification system will facilitate maize functional genome analysis and possibly be useful for corn breeding.

P225

A sequence-indexed collection of Ds insertion lines in maize: insights into transposon and genome biology

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The maize transposable elements *Activator* (*Ac*) and *Dissociation* (*Ds*) were the first transposons discovered by Barbara McClintock over 50 years ago. The biology of these elements has been extensively characterized by studies in maize and in heterologous systems, where they have also been exploited as tools for insertional mutagenesis. Such reverse genetics resources are among the maize research community's paramount needs as the first draft of the maize genome sequence nears completion. Through NSF funding (><http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=0501713>), we are developing a sequence-indexed, two-component *Ac/Ds* gene tagging platform for maize. The goal of the project is to uniformly distribute up to 10,000 endogenous *Ds* elements around the maize genome in a similar number of single- or low-copy *Ds* lines, sequence-index the insertions and place them on the genome sequence. For forward and reverse genetics applications, these simple insertion lines are publicly available with or without an independently segregating *Ac*. We present results from the ongoing project, including accessing and using the collection (<http://www.plantgdb.org/prj/AcDsTagging/>) and insights into transposon and genome biology from analyzing the first 1,000+ transpositions at the local, intrachromosomal and interchromosomal level. DNA sequences that flank the *Ds* elements are exceptionally low in repeat content, leading to a high proportion of insertions in annotated genes. Selection for unlinked transpositions recovers randomly distributed insertions. Data from selections for linked transpositions indicate the collection will be useful for local mutagenesis, which may be critical for knocking out both copies of tandem duplicated genes as occurs with high frequency in maize. At sites of *Ds* insertion, DNA sequence is markedly nonrandom in both a palindromic consensus sequence for the eight base pair target site duplication (TSD) and in several nucleotides on either side of the TSD.

P226

A sequence-indexed mPing transposon collection for gene tagging in rice

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Minature inverted-repeat transposable elements, miniature Ping (mPing) have recently been shown to be active in rice. However, the copy number of mPing elements varies greatly between lines. For instance, the cultivar Nipponbare contains > 50 elements whereas 93-11 carries less than 10 elements. A putative autonomous element, Ping maps to chromosome 6 in Nipponbare (Japonica), but is not detectable in 93-11 (Indica). Thus, Ping may have mediated the widespread amplification of mPing elements in this cultivar. DNA blot surveys of 5 US cultivars (Kaybonnet, Orion, Cypress, Lagrue, Labelle) indicated a low copy number of mPing insertions (5-15 copies) and that the Ping element is absent from all lines. To develop a sequence-indexed collection of mPing insertions in widely used US cultivars, we introduced active copies of Ping into the US cultivars by crosses to Nipponbare. F3 generation seedlings resulting from a Kaybonnet x Nipponbare cross were examined for evidence of transposition and putative mPing footprint alleles were detected in some lines. Currently, we are developing a low copy mPing line through backcrosses to the Kaybonnet parent as starting material for a large-scale mutagenesis. We have also developed methodologies to amplify and sequence mPing insertion sites using 454 sequence technology. Our goal is to develop a reverse-genetics resource of non-transgenic lines utilizing US germplasm that will be readily accessible to plant scientists and breeders in the US and abroad.

P227

Epigenetic changes in MuDR elements over time

(submitted by Damon Lisch <dlisch@berkeley.edu>)

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MuDR elements can be heritably silenced by Mu killer (Muk). Once silenced, elements at most positions remain silenced indefinitely. Here we present evidence that silencing, even after the loss of Muk, is a progressive process that deepens over time. Thus, an element that is inactivated by Muk can be reactivated by an active element, but only in the generation immediately after the loss of Muk. In subsequent generations, the silenced element becomes refractive to reactivation. We also show that a silenced element can be hypomethylated by an active element, and even transcriptionally activated in the presence of that element. However, in deeply silenced elements the heritable component of silencing is not lost, so once the active element is segregated away, the previously inactive element becomes silenced again. We suggest that these data can be used to distinguish between epigenetic marks that determine silence from those that determine transgenerational memory. These experiments also suggest that competency to respond to an activator can be encoded epigenetically, and the degree of epigenetically encoded competency can change over time.

P228

Family-specific developmental expression patterns of maize transposable elements

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Transposable elements are mobile DNA segments that are found in nearly all eukaryotic genomes and compose over half of maize DNA. There are several types of transposable elements, including many different families of retrotransposons, DNA transposons, and helitrons. These elements are potentially mutagenic when active, so their mobility is suppressed by overlapping gene silencing pathways. In *Arabidopsis thaliana*, vastly different families of transposable elements have coordinated epigenetic regulation patterns across a developmental timeline. *Arabidopsis* transposable elements become active at the specific time and in the specific tissue where the cellular machinery responsible for gene silencing is down regulated. In contrast, maize transposable elements do not show coordinated developmental activity. Instead, individual maize transposable elements have their own family-specific developmental regulation. This family-specific epigenetic control of transposable elements may be due to the evolutionarily recent transposable element activity in the maize lineage, and this suggests intriguing differences in the regulation of transposable element-induced epialleles in maize and *Arabidopsis*.

P229

Helitron discovery and description across the plant kingdom

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Helitrons are recently discovered eukaryotic transposons that are predicted to transpose by a rolling-circle mechanism. They are present in most plant and animal species investigated, but were previously overlooked partly because they lack terminal repeats and do not create target site duplications. Helitrons are particularly abundant in flowering plants, especially in maize, where they frequently acquire one or more gene fragments. These acquired fragments are often fused into chimeric transcripts after intron processing. Hence, Helitrons appear to employ exon shuffling to create chimeras that may evolve into novel genes. We have developed a structure-based approach to identify novel Helitrons, and have used this strategy to analyze several plant and animal genomes, leading to the discovery of hundreds of new Helitrons. Analysis of these Helitrons has uncovered mechanisms of element evolution, including sequence acquisition, and has provided insights into their transpositional history and secondary effects on evolved genome structure.

P230

LTR retrotransposons in the rice (*Oryza sativa*) genome: recent amplification bursts followed by rapid DNA loss

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LTR retrotransposons make up the major part of plant genomes and their activity is considered as one of the major processes in the evolution of plant genome size and structure, along with polyploidy. Characterizing the amplification and elimination of these sequences is therefore a major goal in understanding the structural dynamics of plant genomes. To quantify these two forces, we analyzed the insertion and subsequent elimination of 41 LTR retrotransposon families in the almost complete (~365Mb) rice genome (*Oryza sativa*, cv. Nipponbare).

Using a new method to estimate the insertion date of both truncated and complete copies, we could precisely describe the amplification and elimination of LTR retrotransposon sequences in the rice genome. We show that most of the copies have inserted within the last 5 My, in a burst-like pattern, and that the amplification process varies both in timing and intensity between families. Copies are subsequently efficiently eliminated from the genome, through both solo LTR formation and the accumulation of deletions.

We estimate the half-life of LTR retrotransposon sequences in the rice genome to be less than 3 My. However, if only the neutral accumulation of small deletions is taken into account, this half-life would be close to 19 My, revealing that this process is not a major force of LTR retrotransposon removal in the rice genome.

Altogether, these results reveal a high turn-over of LTR retrotransposon sequences in the rice genome and provide an explanation for the rapid differentiation of intergenic regions in grasses.

P231

Mu mapping in diverse maize inbreds

(submitted by Charles Hunter <ibe@ufl.edu>)

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Changes in numbers and sites of insertion for transposable elements during the evolution and domestication of maize may tell us much about these processes and about the differences among various maize inbred lines. Mu transposons are found throughout diverse maize germplasms. They have been an invaluable genetic tool, being employed to identify gene function through both forward and reverse genetics. When a Mu element is found close to, or within a gene, changes in that gene's expression typically occur. Each maize inbred line utilized for genetic research lacks the Mu activator element (MuDR), and therefore contains a specific complement of stable Mu elements. By mapping the Mu elements in diverse maize inbreds, we may learn much about the development of these lines. Examination of the differences between which lines retain Mu elements in various genes may also shed light on specific characteristics of the maize inbreds and the Mu-induced gene mutations they carry. Completion of the maize genome in the B73 inbred has allowed us to map the B73 Mu elements bioinformatically by searching the genome using conserved Mu terminal inverted repeat (TIR) sequences. The UniformMu population was generated by introgressing Robertson's Mutator into W22 at the University of Florida, and a database of MuTAIL-PCR generated Mu flanking sequences was developed for this population. By searching this database for sequences flanking B73 Mu elements, we obtained preliminary evidence for the conservation of specific elements among the two inbred lines. About half of the B73 Mu elements also appear in the W22 inbred. Insert validity and locale were verified using PCR primers flanking B73 and W22 Mu elements. The presence or absence of each is also being tested in selected maize inbreds (including Mo17), and in the maize ancestral teosintes (*T. mexicana* and *T. parviglumis*).

P232

Mu transposition alters the transcriptome and proteome of developing anthers

(submitted by David Skibbe <skibbe@stanford.edu>)

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MuDR/Mu are a highly active transposon family moving by either cut only (or cut-and-paste) in strictly somatic tissues or net replicative transposition (absence of excision alleles) in reproductive tissues. Aside from the MuDR-encoded MURA and MURB proteins, other factors required for Mu transposition, particularly those contributing to the developmentally specific behavior, have yet to be identified. To address this question and assess the overall impact of a highly active transposon on the transcriptome and proteome of developing anthers, RNA and protein was extracted at three developmental stages (mitotic, pre-meiotic and meiotic) from Mu-active and spontaneously inactive stocks. From transcriptome profiling on a 44,000 element Agilent 60-mer oligonucleotide array we found that approximately 30,000 unique genes are expressed at each stage, and statistically significant differences representing all GO categories were represented in the comparison to Mu-active and inactive matched stages. Of the ~10% (ca. 3000) differentially regulated transcripts per stage, there was approximately equal representation by the active or inactive individuals. The effect of Mutator activity on the host proteome was determined using 2D-Difference Gel Electrophoresis. Of the approximately 2,500 protein spots visualized, Mu-active and -inactive individuals exhibited 48 statistically significant differentially regulated spots. In contrast to the transcriptome experiments, 47 of the 48 spots were up-regulated in the Mu-active lines. Peptides in 30 spots were sequenced, and the majority encoded genes with metabolic functions. Twenty-one of these 30 were represented on the oligonucleotide array, and twenty were up-regulated at both the RNA and protein levels; transcript modulation was less than the magnitude of protein change. Interestingly, transcriptome profiling experiments comparing Mu-active lines with or without Mukiller identified unique sets of differentially expressed genes, suggesting non-equivalence of the silenced state arising spontaneously and silencing induced by Mukiller. D.S.S. is an NIH Ruth L. Kirschstein Post-Doctoral Fellow.

P233

Regulation of the Ping transposase proteins

(submitted by Charles Nathan Hancock <cnhancock@plantbio.uga.edu>)

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The Pong-like transposable elements (TEs) rely on two separate proteins for mobilization (ORF 1 and TPase). These transposase proteins are of special interest because they also mobilize the abundant Tourist-like miniature inverted repeat TEs (MITEs). We have shown that expressing both the Ping ORF 1 and TPase proteins results in mPing MITE transposition in Arabidopsis (using a GFP reporter). However, most of the mPing transposition occurs early in development as shown by the presence of small GFP spots in the cotyledons, hypocotyls, and root tissues, with large sectors of GFP in the true leaves. Our goal is to determine what mechanisms regulate the activity of the Ping transposase proteins. Protein localization is a common regulatory mechanism for DNA binding proteins. We found that ORF1 contains a likely nuclear localization signal (NLS), while the TPase protein has a predicted nuclear export signal (NES). We confirmed the localization of the Ping proteins by performing crude nuclei purifications. These experiments showed that the ORF1 protein is primarily localized to the nuclei and the TPase protein was mostly cytoplasmic. We have also shown that the Ping ORF 1 and TPase proteins can be co-precipitated, indicating that they form a complex that catalyzes the excision and insertion reactions. Together, these results suggest that the physical separation of these proteins functions to regulate their activity. We are currently testing how altering the NLS and NES, and thus protein localization, effects overall activity. These results should indicate what role this regulatory mechanism plays in Pong-like element transposition.

P234

Retrotransposons associated with rice genes and their evolutionary history

(submitted by Wusirika Ramakrishna <wusirika@mtu.edu>)

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Retrotransposons comprise a significant fraction of the rice genome. In an effort to understand the role of retrotransposons associated with genes in regulating them, we identified one sixth of rice genes as being associated with retrotransposons, with insertions either in the gene itself or within its putative promoter region. Among genes with insertions in the promoter region, the likelihood of the gene actually being expressed was shown to be directly proportional to the distance of the retrotransposon from the translation start site. In addition, retrotransposon insertions in the transcribed region of the gene were found to be positively correlated with the presence of alternative splicing forms. Some of the retrotransposons that are part of full-length cDNA (fl-cDNA) contribute splice sites and give rise to novel exons. Furthermore, evolutionary conservation of the retrotransposon insertions in six genes was investigated in 95 cultivated and wild rice genotypes. Four out of seven retrotransposon insertions appear to predate the ancestral *Oryza* AA genome. Two retrotransposon insertions in gene 1 arose after the divergence of Asian cultivated rice from its wild ancestor. Furthermore, the retrotransposon insertion in gene 3 appears to have occurred in the ancestral lineage leading to temperate japonicas. Conservation of retrotransposon insertions in genes in specific groups, species, and lineages might be related to their specific function.

P235

The evolutionary relationships of high- and low-copy-number LTR-retrotransposons in maize genome sequence data

(submitted by Regina Baucom <gbaucom@uga.edu>)

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LTR-retrotransposons comprise 70-80% of the maize nuclear genome and are commonly arranged as complexes of nested elements in the spaces between genes. The majority of this repetitive DNA is represented by a few retrotransposon families that have amplified to high copy number. There are also many families present at low copy number; previous work has uncovered the presence of approximately 61 LTR-retrotransposon families present at less than 30 copies. While the evolutionary relationships, as well as the average time of transposition of these high-copy-number families has been investigated, very little is known about the properties of the low-copy-number element families, their time of insertion in the maize genome, or their relationship to the families present in high copy number. We used an automated pipeline to query the available maize genome sequence to identify intact LTR-retrotransposon, with special emphasis on finding previously uncharacterized, low-copy-number elements. We then grouped these elements into families using the program RepMiner, and subsequently analyzed the phylogenetic relationships among both low- and high-copy-number LTR-retrotransposons.

P236

Transposon pairs and chromosome rearrangement

(submitted by Jun Huang <junhuang@waksman.rutgers.edu>)

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Some DNA transposon structures cause chromosome breaks. McClintock (1952) discovered chromosome breaks by a transposable element (TE) that turned out to be Double-Ds, two 2-kb Ds elements inserted one inside of the other in inverse orientation (IO) (Doring et al., 1984). Pairs of closely linked, but separate, transposons can also cause chromosome breaks, the frequency of breakage being an inverse function of the intertransposon genetic distance. TE pairs in IO have the 5' end of one transposon and the 3' end of the other in direct orientation (DO), thus capable of undergoing alternative transposition reactions, which lead to dicentric formation and, therefore, chromosome breakage.

However, transposon pairs in DO do not have such a structure, hence an alternative model was proposed to explain the chromosome-breaking properties of Ac and Ds (Ralston et al., 1989). In this project, not only have we found evidence supporting the chromosome breakage model in DO, but also other novel events, for instance, macrotransposon excision and reinsertion events and intertransposon recombination events.

P237

Maize production and poverty alleviation in the Federal Capital Territory Abuja Nigeria

(submitted by Michael Adedotun <agriclinkcooperative@yahoo.com>)

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Poverty in rural and semi-urban areas of Federal Capital Territory is acute and is proving unmanageable even in relatively wealthy urban settlements. The present government has proposed strategies to alleviate poverty in the rural areas of F.C.T. Perhaps, the most important challenge for government is to foster and commercialise small-holder agricultural production of maize to alleviate poverty.

This paper therefore surveys the potential of growing maize in the Federal Capital Territory, the problems encountered by some of the farmers growing maize, such as processing marketing, marketing oppourtinities and transportation and seed plantings and how international countries could help in processing of maize in the F.C.T.

P238

Plant breeding educational videos

(submitted by Shawn Kaeppler <smkaeppl@wisc.edu>)

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We are producing a series of videos on controlled pollination methods in plants, and on plant breeders of various crops. The controlled pollination method videos are targeted to high school and college students with some background in plant biology. They will be useful for students learning about plant breeding methods, and may be of interest to backyard and commercial hybridizers of various crops. The plant breeder videos are intended for a general audience, and are intended to inform the public about plant breeding and to interest and encourage people to consider a career in plant breeding. When completed, these educational videos will be available through the Wisconsin Plant Breeding and Plant Genetics program website (pbpg.wisc.edu). We anticipate that a video on corn pollination and a video describing sweet corn breeding will be available at the time of the meeting.

P239

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

(submitted by Natalie Fredette <nf04d@fsu.edu>)

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The *Maize-10-Maze* project is a public outreach effort of the *Cytogenetic Map of Maize* project (www.cytomaize.org). Our goal was to produce a fun and educational self-guided public tour of the maize genome. The karyotype of maize was used to develop a field-scale version of the maize genome for public exploration. Each of the 10 rows represents a single chromosome. Over 100 selected mutants were grown in this chromosome map garden, arranged according to the natural genetic order of the corresponding gene loci. The criteria for choosing which mutants to include were that the mutant (1) should exhibit a visually striking or cool plant or seed phenotype - such as *Knotted1* or *lazy plant1*, or (2) should be of agronomic importance - such as *brittle endosperm1*, or (3) should be of major scientific or historic importance - such as *teosinte branched1*. Undergraduate students developed weatherproof field placards that describe each mutant, providing information for self-guided public tours of the maize genome. Each placard lists the phenotype, genotype, original reference, mode of inheritance, genetic location, and sample photographs. We hosted two public field days in the summers of 2006 and 2007 in conjunction with Florida A&M University's Forestry and Conservation Education (FACE) Summer Program. Photography and web-based resources from the *Maize-10-Maze* project have been developed and used to support classroom activities at middle school, high school, and college levels. We found that the *Maize-10-Maze* project helped to engage a broad audience in the use of maize mutants for understanding plant growth and development. The project was also useful for discussing the value of public funding for plant genome research.

P240

Tribal College Outreach II: Reaching students through faculty training

(submitted by Anne Sylvester <annesyl@uwyo.edu>)

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Tribal Colleges (TC) have a unique mission among community colleges because they serve as learning as well as cultural centers for local communities. The Little Big Horn College (LBHC), Crow Agency MT, is an established tribal college that has been instrumental in promoting education and cultural preservation for the Crow people. We have worked towards enhancing science education in the area of genetics, molecular and cell biology at LBHC by focusing on teaching to the most practical needs of the students and the Crow tribe. To date, we have conducted workshops for students that focus on classical genetics through study of horse coat color genetics, the genetics of human diseases such as diabetes, and current relevant issues such as transgenic crops. A second workshop held at the University of Wyoming brings students to the university environment. For this workshop, we focus on molecular genetics by teaching about DNA through training in the use of PCR for bacterial water quality testing. A major component of success when working with TC is the importance of cultural exchange and also follow-up with the students and faculty. Given the need to provide more permanent impacts to the teaching environment, we have recently added a third dimension to our outreach by extending the teaching to TC faculty as well as students. The one-on-one interaction between researchers and TC faculty is essential. To accomplish this, we initiated a pilot workshop for TC faculty prior to student workshops. By acting as a liaison between university faculty and TC faculty we hope to provide more permanent connections and impact more students more effectively. Researchers interested in participating in a faculty workshop in Montana are encouraged to contact Anne Sylvester or Dave Jackson to learn more about plans for the coming year.

P241

Evgueni V. Ananiev (1947-2008): A career dedicated to understanding the chromosome

(submitted by Olga Danilevskaya <olga.danilevskaya@pioneer.com>)

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Evgueni V. Ananiev, 60, a Research Scientist at Pioneer Hi-Bred and fellow Cooperator, passed away January 10, 2008 after a courageous battle with cancer. Evgueni's career came full circle from his initial exposure to science as a temporary technician measuring human chromosomes in the cytology lab of the famous Russian geneticist A.A. Prokof'eva –Belgovskaya to his ultimate achievement of the construction of the first linear artificial chromosome in maize. This poster celebrates Evgueni's contributions to understanding chromosome structure and function throughout his career. We highlight Evgueni's accomplishments in Russia including his graduate studies at Moscow State University investigating X-chromosome dosage compensation and position effects, his seminal discovery of transposable elements in *Drosophila* in 1976 as a post-doctoral fellow in the labs of V.A.Gvozdev and G.P. Georgiev and his leadership of a lab in the Institute of General Genetics working on plant genetic engineering. Also highlighted are Evgueni's achievements in the USA in Ron Phillips and Howard Rines labs at the University of Minnesota, where he made fundamental discoveries of the structural elements of maize centromeres and organization of "knobs", and his work at Pioneer Hi-Bred, where he successfully led a team that developed the maize artificial chromosome in 2006; which is considered the highpoint of his career. Evgueni authored 69 publications and his legacy will leave a significant mark in chromosome biology. His passion for science was a leading motivation of his life and was an inspiration for those that knew and worked with him.

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