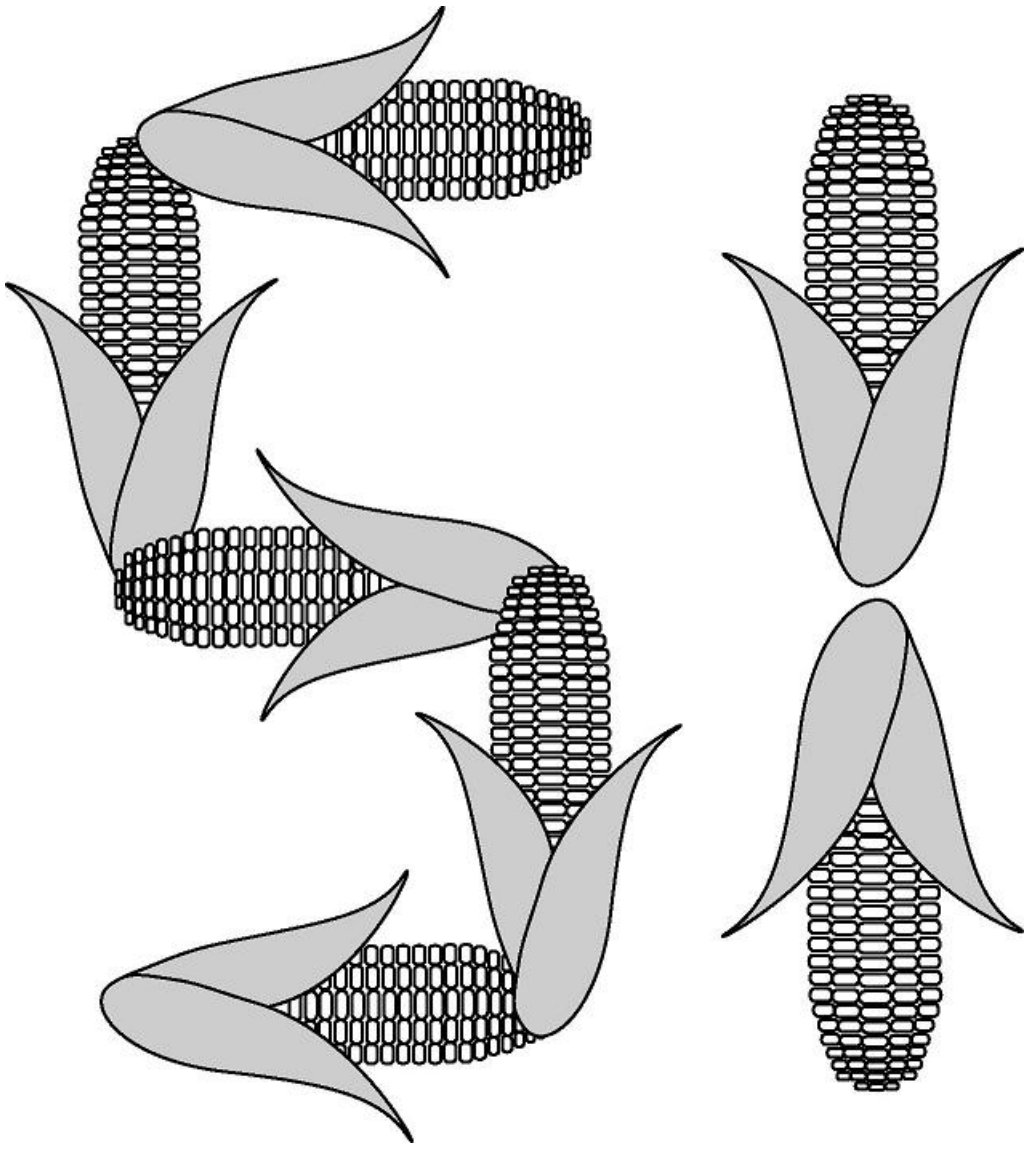

51st Annual Maize Genetics Conference

Program and Abstracts

March 12 – March 15, 2009

Pheasant Run
St. Charles, Illinois

This conference received financial support from:

National Science Foundation
Monsanto
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Dow Agrosciences
BASF Corporation Agricultural Products
National Corn Growers Association and State Partners

We thank these contributors for their generosity!

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Cover art by Yujun Han, University of Georgia

General Information

Registration

Thursday: 3:00 to 6:00 PM: There will be a table in Solarium (near main lobby).
9:00 PM to Midnight: There will be a table in the Mega Center.
Friday: During poster sessions and 9:00 PM to Midnight: There will be a table in the Mega Center.

Meals

All meals will be served buffet style in the Mega Center; serving hours as listed in the Program. Coffee, tea and soft drinks are available at no charge during the beverage breaks.

Talks and Posters

All Talks and the Workshop on Maize Genome Sequencing will be presented in the St. Charles Ballroom.

Posters will be presented in the Mega Center, adjacent to where we will have meals. Posters should be hung Thursday starting at 3 PM and stay up until Sunday morning, but must be removed by 9 AM on Sunday. During the poster sessions each day, presenters are asked to stand by odd numbered posters from 1:30 PM to 3 PM each day and even numbered posters from 3 PM to 4:30 PM each day.

Hospitality

After the evening sessions on Thursday and Friday there will be informal socializing and poster gazing in the Mega Center. Refreshments will be provided each night until 1 AM. On Saturday evening there will be informal socializing in the Mega Center, with music and dancing.

After 1 AM, a double suite on the 16th floor of the Tower (1611, 1613) is available for continued socializing. This is a "private party room" and alcoholic beverages may be brought in; however, you must stay in this room if you are carrying drinks and dispose of trash and bottles in the party room.

Steering Committee

Please share your suggestions and comments about the meeting with the 2009 Steering Committee:

Steve Moose, Chair (smoose@uiuc.edu)
Mike Muszynski, Co-Chair (mgmuszyn@iastate.edu)
Mei Guo (mei.guo@pioneer.com)
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Giuseppe Gavazzi (giuseppe.gavazzi@unimi.it)
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Marty Sachs, local organizer, ex officio (msachs@uiuc.edu)
Georgia Davis, treasurer, ex officio (davisge@missouri.edu)
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Carson Andorf, abstract coordinator, ex officio (Carson.Andorf@ars.usda.gov)

Acknowledgements

Many thanks go to Carson Andorf and Mary Schaeffer for their tremendous efforts in organizing and assembling the conference program and Mike McMullen for the design of the poster. Thanks go to Yujun Han for the winning 51st Maize Meeting logo design. 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 Margy Moore and the Pheasant Run staff for their help in organizing this conference. Thanks go to Karen Cone for her past stewardship in managing the finances for this meeting and Mei Guo, Mike Muszynski, Steve Moose and Georgia Davis 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.

Schedule of Events

Thursday, March 12

3:00 PM – 6:00 PM	**REGISTRATION / POSTER HANGING**
6:00 PM – 7:00 PM	**DINNER**
7:00 PM – 7:15 PM	**ANNOUNCEMENTS**
7:15 PM – 9:00 PM	**SESSION 1 – PLENARY TALKS** Chair: Stephen Moose
7:15 PM	**Curt Hannah, University of Florida** *Genes for ADP-glucose pyrophosphorylase (AGPase); their future and their past.*
8:05 PM	**Joe Ecker, The Salk Institute for Biological Studies** *Arabidomics: Next is now: Sequencing of genomes, epigenomes, transcriptomes, and beyond*
9:00 PM – 1:00 AM	**INFORMAL POSTER VIEWING / REGISTRATION**
1:00 AM – 7:00 AM	**HOSPITALITY**

Friday, March 13

7:00 AM – 8:00 AM	**BREAKFAST**
8:00 AM – 8:15 AM	**ANNOUNCEMENTS**
8:15 AM – 10:15 AM	**SESSION 2 – GENOME DYNAMICS I** Chair: Jane Dorweiler
8:15 AM	**Erik Vollbrecht, Iowa State University** *Properties of transposition and uses in regional mutagenesis of the Dissociation (Ds) transposable element*
8:35 AM	**Jun Huang, Rutgers University** *Paired transposons: natural chromosomal engineers*
8:55 AM	**Chunguang Du, Montclair State University** *Content, distribution, and timing of insertion of Helitron transposable elements in the maize genome*
9:15 AM	**James Estill, University of Georgia** *Distilling transposable element exemplars from host genomes builds a framework for understanding TE diversity in the grasses*
9:35 AM	**Fangpu Han, University of Missouri-Columbia** *Centromere inactivation and reactivation reveal both genetic and epigenetic components for centromere specification*
9:55 AM	**Gernot Presting, University of Hawaii** *Evolution of maize centromeres as revealed by their physical maps*
10:15 AM	**BREAK**
10:45 AM – 12:25 PM	**SESSION 3 – GENOME DYNAMICS II** Chair: Karen Koch
10:45 AM	**Kelly Dawe, University of Georgia** *Recombination within centromere cores*
11:05 AM	**Wojtek Pawlowski, Cornell University** *Chromosome dynamics in live maize meiocytes.*
11:25 AM	**Yujun Han, University of Georgia** *TATE --- A web based pipeline for rapidly retrieving and characterizing gene and transposable element families from genomic sequences*
11:45 AM	**Fusheng Wei, Arizona Genomics Institute** *The Physical and Genetic Framework of the Maize Genome*
12:05 PM	**Taner Sen, Iowa State University** *The MaizeGDB Genome Browser*
12:30 PM – 1:30 PM	**LUNCH**
1:30 PM – 5:00 PM	**POSTER SESSION 1**

Presenters should be at odd numbered posters from 1:30 PM to 3:00 PM.
Presenters should be at even numbered posters from 3:00 PM to 4:30 PM.
Beverages will be available from 3:30 PM to 5:00 PM.

5:00 PM – 6:15 PM	**SESSION 4 – GENOME SEQUENCES: WHAT'S NEW** Chair: Mike Muszynski
5:00 PM	**Doreen Ware, Cold Spring Harbor Laboratory** *Sequence and analysis of the maize B73 genome*
5:20 PM	**Dan Rokhsar, UC Berkeley** *Update on the Mo17 genome sequencing project*
5:40 PM	**Octavio Martinez, Cinvestav Campus Guanajuato** *The characterization of the Palomero Toluqueno genome*
6:00 PM	**Community Discussion and Updates**
6:30 PM – 7:45 PM	**DINNER**
8:00 PM – 9:30 PM	**SESSION 5 – COMMUNITY FORUM ON GENE ANNOTATION: WHAT'S NEXT** Chair: Mike Muszynski
8:00 PM	**Pam Johnson, National Corn Growers Association** *Research & the Recession: How Obama and the Stimulus Package Impact the Future of Agricultural Research*
8:20 PM	**Volker Brendel, Iowa State University** *Community annotation of the maize transcriptome @ MaizeGDB*
8:40 PM	Panel Discussion with Carolyn Lawrence, Jeff Bennetzen, Yan Fu, Jim Uphaus, Volker Brendel, Gernot Presting
9:30 PM – 1:00 AM	**INFORMAL POSTER VIEWING & HOSPITALITY**
1:00 AM – 7:00 AM	**HOSPITALITY**

Saturday, March 14

7:00 AM – 8:15 AM	**BREAKFAST**
8:15 AM – 10:15 AM	**SESSION 6 – BIOCHEMICAL GENETICS / CELL BIOLOGY** Chair: Guiseppe Gavazzi
8:15 AM	**Shawn Christensen, Texas A&M University** *A maize 13-lipoxygenase regulates green leafy volatile and jasmonic acid biosynthesis and is required for resistance to herbivores.*
8:35 AM	**Alice Barkan, University of Oregon** *Site-specific binding of a pentatricopeptide repeat protein defines and stabilizes 5' and 3' mRNA termini in maize chloroplasts: implications for plastid mRNA biogenesis mechanisms and PPR family functions*
8:55 AM	**Romain Fouquet, University of Florida** *The rough endosperm3 locus encodes a predicted splicing factor required for plant development and regulation of cell proliferation.*
9:15 AM	**Jean-Louis Prioul, Universite Paris Sud-CNRS** *Interrelationships between Pyruvate-Pi-dikinase (PPDK) and Opaque-2 in the control of endosperm C/N ratio*
9:35 AM	**Alan Myers, Iowa State University** *Association of enzymes from amino acid- and carbohydrate biosynthetic pathways in multisubunit complexes: A model for regulation of carbon allocation in maize grain*
9:55 AM	**Taijoon Chung, University of Wisconsin** *Autophagy in maize is regulated by development and nutrient availability*
10:15 AM – 10:45 AM	**BREAK W/ BEVERAGES**
10:45 AM – 12:25 PM	**SESSION 7 – DEVELOPMENTAL GENETICS** Chair: Erik Vollbrecht
10:45 AM	**Amelie Gaudin, University of Guelph** *Effect of the Major Domestication Locus, Tb1, on Maize Root Architecture Using an Aeroponic System*
11:05 AM	**Inna Golubovskaya, University of California** *Maize Meiosis and Collection of meiotic mutants*
11:25 AM	**David Jackson, Cold Spring Harbor Laboratory** *Grassy tillers1 encodes a homeodomain protein that controls tiller number and lateral branch length in response to light signals*
11:45 AM	**Beth Thompson, Univeristy of California - Berkeley** *Beyond the ABCs: MADS-box regulation of maize floral development*
12:05 PM	**Brandi Sigmon, Iowa State University** *Evidence of selection at the ramosa1 locus during maize domestication*
12:30 PM – 1:30 PM	**LUNCH**

1:30 PM – 5:00 PM **POSTER SESSION 2**

Presenters should be at odd numbered posters from 1:30 PM to 3:00 PM.
Presenters should be at even numbered posters from 3:00 PM to 4:30 PM.
Beverages will be available from 3:30 PM to 5:00 PM.

6:00 PM – 7:00 PM **DINNER**

7:15 PM – 9:00 PM **SESSION 8 – PLENARY TALKS**

Chair: Stephen Moose

7:15 PM **Luca Comai, UC Davis**
Stay pure or hybridize: pleasures and torments of genomic promiscuity

8:05 PM **Marja Timmermans, Cold Spring Harbor Laboratory**
Adaxial-abaxial patterning of the maize leaf by mobile small RNAs

9:30 PM – 2:00 AM **INFORMAL POSTER VIEWING & DANCE**

2:00 AM – 7:00 AM **HOSPITALITY**

Sunday, March 15

7:00 AM – 8:30 AM	**BREAKFAST**
8:30 AM – 8:40 AM	**ANNOUNCEMENTS**
8:40 AM – 11:30 AM	**SESSION 9 – FUNCTIONAL GENOMICS** Chair: Mei Guo
8:40 AM	**Stefan Scholten, University of Hamburg** *Epigenetic resetting of a gene imprinted in maize embryos*
9:00 AM	**Ruth Swanson-Wagner, Iowa State University** *Regulation of Gene Expression and Parent-of-Origin Effects in Hybrids*
9:20 AM	**Tom Brutnell, Cornell University** *Transcriptome Analysis of Maize Leaf Development Using Illumina Sequencing Techniques*
9:40 AM	**Han Zhao, University of Illinois at Urbana-Champaign** *Genomic responses to a century of phenotypic selection in maize*
10:00 AM	**Owen Hoekenga,** **Robert W. Holley Center for Agriculture and Health** *Iron biofortification of maize grain*
10:20 AM	**Michael Gore, Cornell University** *A First Generation Haplotype Map of the Maize Genome*
10:40 AM	**Yan Fu, Iowa State University** *Whole-genome detection of structural variation and genotyping in maize using high-definition array-based Comparative Genomic Hybridization (aCGH)*
11:00 AM	**Yunbi Xu, International Maize and Wheat Improvement Center** *SNP-chip based genomewide scan for germplasm evaluation and marker-trait association integrated with selective genotyping and pooled DNA analysis*
11:30 AM	**ADJOURNMENT**

Posters

Biochemical Genetics

P1	**David Henderson** <davidh@cals.arizona.edu>	*A New Assay for High Density Microarrays: Targeted, extraction-free measurement of RNA from fresh or FFPE samples*
P2	**Tina Kaiser** <tmkaiser@dow.com>	*A New Herbicide Tolerance Trait to Improve Weed Control in Glyphosate Tolerant Corn*
P3	**Kasey Hames** <kahcg2@mizzou.edu>	*Aflatoxin accumulation in maize starch mutants*
P4	**Robyn Johnston** <johnston@cshl.edu>	*Altered auxin dynamics in the abph1 embryo SAM contribute to aberrant phyllotaxy*
P5	**Alan Myers** <ammyers@iastate.edu>	*Association of enzymes from amino acid- and carbohydrate biosynthetic pathways in multisubunit complexes: A model for regulation of carbon allocation in maize grain*
P6	**Gregorio Hueros** <gregorio.hueros@uah.es>	*Atypical response regulators expressed in the maize endosperm link canonical two component systems to transfer cell differentiation and function*
P7	**Mandeep Sharma** <mxs781@psu.edu>	*Biosynthesis of Multiple Flavonoid Compounds in Maize through the Action of a Flavonoid 3'-Hydroxylase*
P8	**Mingshu Huang** <muh147@psu.edu>	*Camouflage patterning in maize leaves results from a defect in porphobilinogen deaminase*
P9	**Hong Li** <hongli@nature.berkeley.edu>	*Characterization of epigenetic states through heritable changes in MuDR element*
P10	**Gertraud Spielbauer** <gspielbauer@ufl.edu>	*Dosage-dependent genes affecting seed composition or weight.*
P11	**Iffa Gaffoor** <sig2@psu.edu>	*Heterologous expression of a sorghum Myb transcription factor in maize*
P12	**Robert Baker** <rfb11@psu.edu>	*Id1 regulates the expression of the tdy1 carbohydrate accumulation phenotype*
P13	**Ivan Baxter** <ibaxter@purdue.edu>	*Ionomic Profiling of NAM RIL Populations*
P14	**Kalindi LaTorre** <kdl1291@uncw.edu>	*Multiple Stress Dose Response Comparison of Two Genotypes*
P15	**Robert Elshire** <rje22@cornell.edu>	*Next Generation Sequencing with Restriction-enzyme Reduced Representation Libraries*
P16	**Brian Smith-White** <smtwhite@ncbi.nlm.nih.gov>	*Plant Genomic Resources at National Center for Biotechnology Information*
P17	**David Henderson** <davidh@cals.arizona.edu>	*Production of Self-Assembling Autofluorescent Protein (SASAP) Microarrays and Their Application to Phytochrome Research*
P18	**David Johnston Monje** <djohns05@uoguelph.ca>	*Profiling conservation and changes in resident bacterial and fungal endophytes in maize seeds during domestication and diversification in the Americas*
P19	**Andrew Burt** <aburt@uoguelph.ca>	*QTL Mapping a High-Lutein Phenotype in Yellow Dent Inbred Lines*
P20	**Yongrui Wu** <yongrui@waksman.rutgers.edu>	*Redundant Function of Gamma and Beta Zeins in Stabilization of Protein Body Formation*
P21	**John Gray** <jgray5@utnet.utoledo.edu>	*Regulation of Lignin Metabolism in Maize*

P22	**Joerg Degenhardt** <joerg.degenhardt@pharmazie.uni-halle.de>	*Restoring the communication between maize roots and insect-killing nematodes improves control of the maize pest Diabrotica virgifera virgifera*
P23	**Susan latshaw** <latshaw@ufl.edu>	*Sequence indexing of Mu transposons in the UniformMu*
P24	**Thomas L. Slewinski** <tls315@psu.edu>	*Sucrose transporter1 functions in phloem loading in maize leaves*
P25	**Antony Chettoor** <cmants@iastate.edu>	*TRANSCRIPTIONAL REGULATION OF VP1 EXPRESSION IN MAIZE*
P26	**Michael Tessaro** <mtessaro@uoguelph.ca>	*The Design, Engineering, and Application of Whole-Cell Biosensors for Quantifying Maize Glutamine and Soil Nitrate*
P27	**Sylvia de Sousa** <smsousa@ufl.edu>	*The sdh1 mutant alters kernel sugar composition and transcript profiles in addition to its small-kernel phenotype.*
P28	**Andrea Dolezal** <aldoleza@ncsu.edu>	*Transcriptional profiling of Aspergillus flavus infected maize kernels.*
P29	**Thomas L. Slewinski** <tls315@psu.edu>	*psychedelic, a new locus controlling carbohydrate partitioning in maize leaves*

Bioinformatics

P30	**Genevieve DeClerck** <gad14@cornell.edu>	*Gramene: A Resource for Comparative Grass Genomics*
P31	**Jennifer Mingus** <jcmingus@dow.com>	*Development of Bioinformatics Tools to Process Next Generation Sequence Data*
P32	**Carson Andorf** <carson.andorf@gmail.com>	*MaizeGDB: Web Interface and New Features*
P33	**Lisa Harper** <ligule@nature.berkeley.edu>	*How to use MaizeGDB*
P34	**Lisa Harper** <ligule@nature.berkeley.edu>	*New and Improved Phenotypes in MaizeGDB*
P35	**Carolyn Lawrence** <triffid@iastate.edu>	*POPcorn - a PrOject Portal for corn*
P36	**Ethalinda Cannon** <ekcannon@iastate.edu>	*PLEXdb: Plant And Pathogen Expression Database And Web Tools For Comparative And Functional Genomic Analysis*
P37	**Hong Lu** <luhong@iastate.edu>	*Model Genome Interrogator: a PLEXdb module that leverages sequenced genomes for motif discovery via meta-promoter extraction and analysis*
P38	**John Gray** <jgray5@utnet.utoledo.edu>	*Investigation of the role of the ZmNAC1 transcription factor in Zea mays*
P39	**John Gray** <jgray5@utnet.utoledo.edu>	*Investigation of the role of the ZmMYB64 transcription factor in Zea mays*
P40	**John Gray** <jgray5@utnet.utoledo.edu>	*Phylogenomic Analysis of the Trihelix Transcription Factor Family in Grasses*
P41	**Lifang Zhang** <zhangl@cshl.edu>	*MicroRNA analysis in the Maize and Sorghum genomes*
P42	**Christopher Bottoms** <bottomsc@missouri.edu>	*A visualization and query tool for introgression libraries*
P43	**Candice Gardner** <candice.gardner@ars.usda.gov>	*GRIN-Global: An International Project to Develop a Global Plant Genebank and Information Management System*

P44 **Shiran Pasternak** <shiran@cshl.edu> — *MaizeSequence.org Reloaded*

P45 **Toni Kazic** <toni@athe.rnet.missouri.edu> — *Towards a Model of Lesion Formation*

P46 **Jason Green** <jmg00d@mizzou.edu> — *Utilizing Maize-Related Ontologies for Phenotype Text Search*

Cell Biology

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P50 **Katharina Haentzschel** <haentzsc@uni-hohenheim.de> — *Establishment of a methodology for using doubled haploids of maize in a breeding program for Sub-Saharan Africa*

P51 **Zhanyuan Zhang** <zhangzh@missouri.edu> — *Fostering Plant Science Research at MU Plant Transformation Core Facility*

P52 **Bryan Penning** <bpenning@purdue.edu> — *Genetic resources for functional genomics of maize cell wall biology*

P53 **Christine Chase** <ctdc@ifas.ufl.edu> — *Metabolic features of pollen development in male-fertile and S male-sterile maize*

P54 **John Laurie** <jlaurie@email.arizona.edu> — *Molecular Genetic Analysis of Early Endosperm Development by Microinjection of Maize Embryo Sacs*

P55 **Louis Meyer** <ljmr29@mizzou.edu> — *Pollen abortion in CMS-C is associated with reduction of F0 ATP synthase proteins*

P56 **Mo Jia** <mo_jia@baylor.edu> — *Possible Role of Translational Regulation in Determining Opaque Endosperm Protein Quality*

P57 **Rebecca Mroczek-Williamson** <bmroczek@uafortsmith.edu> — *Teaching with Maize: Use of the Yeast One-Hybrid System to Analyze Ab10 Neocentromere Activity in an Undergraduate Setting*

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P59 **Han Zhang** <hzhang@plantbio.uga.edu> — *Unraveling the function of CENH3 and CENPC in kinetochore assembly by ectopic tethering*

Cytogenetics

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P61 **Rachel Wang** <rachelcjw@berkeley.edu> — *Chromatin architecture changes during meiotic pairing revealed by ultrahigh resolution structured illumination (SI) microscopy*

P62 **Debbie Figueroa** <figueroa@bio.fsu.edu> — *Constructing A Cytogenetic Map Of Maize In Oat Addition Lines Using Sorghum BACs As FISH Probes*

P63 **Rick Masonbrink** <remkv6@mizzou.edu> — *Copy Number Increase of Engineered Minichromosomes Derived from the B Chromosome*

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Examination of Mitochondrial DNA Insertion Sites in a Diverse Set of Maize Lines

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Maize chromosome specific painting

P72 **William F. Sheridan** <bill.sheridan@und.edu>
Sorting out dosage effects on maize morphogenesis: additive effects versus gene interactions between nonhomologous chromosome segments

P73 **Donald Auger** <donald.auger@sdstate.edu>
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Developmental Genetics

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P101	**Viktoriya Coneva** <vconeva@uoguelph.ca>	*Metabolic analyses of extremely late-flowering indeterminate1 maize mutants reveal metabolic traits that may be associated with the floral transition in maize*
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P110	**Allison Phillips** <arphilli@stanford.edu>	*The Maize stunter1 Maternal Effect Mutation Affects Male and Female Gametophytes*

P111 **Kristen Leach** <kalp55@mizzou.edu>
The effect of planting density on shoot-born root initiation, and correlated agronomic traits

P112 **Chuck George** <gchuck@nature.berkeley.edu>
The maize tasselsheath4 gene regulates meristem boundaries by repressing ramosa2

P113 **Arco Brunner** <arcobrunner@access.uzh.ch>
The non-reductive mutant 1, a first step towards engineered apomixis in Maize

P114 **Indrajit Kumar** <ikumar2@illinois.edu>
The phytochrome interacting bHLH proteins affect photomorphogenic development in Maize

P115 **Patrice G. Dubois** <pgd7@cornell.edu>
The shade avoidance syndrome in maize: Genetic and hormonal control of seedling elongation.

P116 **Anthony Studer** <studer@wisc.edu>
The use of mixed models to fine map cis regulatory elements located upstream of teosinte branched 1 (tb1)

P117 **Prem Chourey** <pschourey@ifas.ufl.edu>
Two redundant pathways of Trp-dependent biosynthesis of auxin and their possible relationship to sugar metabolism in developing seeds of maize.

P118 **Daniel Hill** <harkius@uwyo.edu>
ZmRAB2A1 partially co-localizes with ER and shows distributed expression through the cytoplasm

P119 **Cima Nowbakht** <cxn211@psu.edu>
sparse inflorescence1, barren inflorescence1 and barren stalk1 promote cell elongation in maize inflorescence development

Epigenetics

P120 **Dustin Mayfield** <drm758@truman.edu>
Effects of rmr1 on Pl1-Blotched

P121 **Thelma Madzima** <tmadzima@bio.fsu.edu>
Genetic and phenotype characterization of maize transgene-reactivated mutants.

P122 **Bernard Mikula** <Berniemaggie@embarqmail.com>
How temperature and light can regulate heritable changes in gene expression

P123 **M. Annie McGill** <mamcgill@wisc.edu>
RNAi-mediated Silencing of a Maize Histone H1 Linker Protein Confers Increased Transformation Efficiency in Maize.

P124 **Kelly Nissen** <kenissen@berkeley.edu>
Temporal shifts in the heritability of Mutator transposons activity in Zea mays

P125 **Yi Jia** <jiayi@iastate.edu>
Unexpected Retrotransposon Expression Patterns in the Shoot Apical Meristems of the mop1 (RNA-dependent Polymerase 2) Mutant

P126 **PoHao Wang** <puw116@psu.edu>
Unstable factor for orange1 (Ufo1) induced histone modification of pericarp color1 gene in maize

Genome Structure / Synteny

P127 **Irina Makarevitch** <imakarevitch01@hamline.edu>
Effect of chromosome number variations on global gene expression

P128 **Qinghua Wang** <qinghua@waksman.rutgers.edu>
Evolution of bz haplotypes in maize and its relatives

P129 **Jian-Hong Xu** <jianhong@waksman.rutgers.edu>
Evolution of Cereal Storage Proteins

P130 **Amber Brown** <brown@bio.fsu.edu>
Mapping of Telomere Length Regulating Factors

P131 **John Matera** <jtm5m3@mizzou.edu>
Mitochondrial Genome Changes Associated with Reversions of S-Type Cytoplasmic Male Sterility

P132 **Michelle Denton** <med55@cornell.edu>
Phylogenetic Examination of Zea and Tripsacum

P133 **Limei Du** <limei@waksman.rutgers.edu> — *Recombination across a gene-rich genetic interval in heterozygous maize haplotypes differing in Helitron and retrotransposon content*

P134 **Edwin Van der Vossen** <evo@keygene.com> — *Whole Genome Profiling: a new method for sequence based whole genome physical mapping*

Genomics

P135 **Shiguo Zhou** <szhou@wisc.edu> — *An Optical Map of the Maize Genome*

P136 **Yunbi Xu** <y.xu@cgiar.org> — *Characterization of a worldwide collection of maize lines through large-scale SNP genotyping*

P137 **Neeru Gandotra** <fno.neeru@yale.edu> — *Comparative Analysis Of C3 And C4 Leaf Development In Rice, Sorghum And Maize*

P138 **kai ying** <yingk@iastate.edu> — *Comparative Genome Hybridization Of Maize Inbreds And Their Wild Ancestor, Teosinte*

P139 **Wei Chen** <wchen3@dow.com> — *Development of Chromosome-specific in silico SNPs in Maize*

P140 **Yonglian Zheng** <yonglianzheng@gmail.com> — *Differential expression of miRNAs in response to salt stress in maize roots*

P141 **Sivanandan Chudalayandi** <chudals@missouri.edu> — *Enhancer Trapping in Maize*

P142 **Qin Yao** <maize@uga.edu> — *Fine-scale analysis of heteroallelic expression in contiguous chromosomal regions in Arabidopsis and maize*

P143 **Matthew Evans** <mmsevans@stanford.edu> — *Functional Genomics of Maize Gametophytes*

P144 **Christophe Liseron-Monfils** <cliseron@uoguelph.ca> — *Gene expression profiling and promoter motif detection in seedling maize roots and root hairs*

P145 **Li Li** <lilsunny@iastate.edu> — *Gene silencing during the domestication of maize*

P146 **Jeff Glaubitz** <glaubitz@wisc.edu> — *Genetic Architecture of Maize and Teosinte*

P147 **Tina Wambach** <twambach@uoguelph.ca> — *Genomic survey of gene expression diversity in Zea mays roots in response to water stress*

P148 **Wojtek Pawlowski** <wp45@cornell.edu> — *High-resolution genome-wide mapping of recombination events in maize*

P149 **Martin Ganal** <ganal@traitgenetics.de> — *Highly polymorphic and haplotype-based SNP markers for maize*

P150 **Wesley Barber** <barber4@illinois.edu> — *Hybrid specific changes in small RNA populations from maize seedling shoot apex*

P151 **Philip Becraft** <becraft@iastate.edu> — *Identifying essential genes for maize kernel development*

P152 **Srinivasa Chaluvadi** <src@uga.edu> — *Influence of host genetics on the rhizosphere microbial community of switchgrass and maize*

P153 **Qihui Zhu** <qzhu@uga.edu> — *Isolation and characterization of resistance gene homologues (RGHs) from switchgrass germplasm*

P154 **Jafar Mammadov** <jamammadov@dow.com> — *Large-scale SNP Discovery in Maize Using Genome Complexity Reduction Approach*

P155 **Jun Pyo Kim** <jkpz2@mizzou.edu> — *Length of Selection around Candidate Genes for Artificial Selection during Domestication and Crop Improvement in Maize*

P156 **Uta Paszkowski** <uta.paszkowski@unil.ch> — *Molecular genetics of arbuscular mycorrhizal symbiosis in rice*

P157 **Andrey Lutsyk** <lytsukandrey@ua.fm> — *Molecular-genetic polymorphism of loci, associated with maize high-temperature tolerance*

P158 **Julie Meyer** <juliem@iastate.edu> — *Physical mapping of the Rf8 restoration of fertility locus in T-cytoplasm maize*

P159 **Stacy DeBlasio** <deblasio@cshl.edu> — *Project update on fluorescent protein tagged maize lines for cell biology and functional genomics*

P160 **Xianran Li** <lixr@ksu.edu> — *QTL-directed candidate-gene NAM analysis to identify natural variations related with maize leaf traits*

P161 **Les Hoffman** <les.hoffman@epibio.com> — *Rapid Plant Seed DNA Extraction and Improved PCR of Plant Tissues Including Seeds and Leaves*

P162 **George Slischuk** <crotallusviperA@ukr.net> — *Restorer of fertility-associated loci maize polymorphism*

P163 **Gaganpreet Sidhu** <gks27@cornell.edu> — *Sequence Diversity and Evolution of Recombination Genes in Maize*

P164 **Maggie Woodhouse** <branwen@berkeley.edu> — *Some classes of stress response genes appear to be more mobile within both Brassicales and the grasses*

P165 **John Gray** <jgray5@utnet.utoledo.edu> — *The Grass Transcription Factor (TF) ORFeome Project and Guidelines for Naming Grass TFs*

P166 **Farag Ibraheem** <fii100@uiuc.edu> — *Transcriptional regulation of nitrogen metabolism in maize*

P167 **Jayanand Boddu** <jboddu@uiuc.edu> — *Transcriptome analyses of developing ear-shoot in selected genotypes of maize*

P168 **Alice Barkan** <abarkan@uoregon.edu> — *Use of Illumina-HTS to identify Mu-insertions underlying mutant phenotypes in the "Photosynthetic Mutant Library"*

P169 **Jon Duvick** <jduvick@iastate.edu> — *What's new at PlantGDB for maize researchers?*

Quantitative Traits / Breeding

P170 **Jennifer Hamilton** <JHamilton4@dow.com> — *A Novel Approach for High Throughput Mapping of Transgenic Insertion Sites*

P171 **Kristen Leach** <kalp55@mizzou.> — *A brown midrib mutant, bm2, decreases Western Corn Rootworm feeding on maize roots*

P172 **Kristen Leach** <kalp55@mizzou.edu> — *A maize population, CRW3 cycle six, is resistant to Western Corn Rootworm at the V3 to V5 stage*

P173 **Chris Grainger** <cgrainge@uoguelph.ca> — *Allele Mining Via Phenotypic Selection in High Carotinoid Derived Maize Inbred Lines*

P174 **Gregory Mahone** <gmahone@illinois.edu> — *Analysis of Inflorescence Architecture Traits in Context of Creating a Commercially-Viable Maize Population*

P175 **Raja Khanal** <rkhanal@uoguelph.ca> — *Analysis of hybrid trait variation in intermated and non-intermated recombinant inbred line populations of Zea mays L.*

P176 **Judith Kolkman** <jmk87@cornell.edu> — *Association Mapping Analysis of Resistance to Northern Leaf Blight in Maize*

P177 **Sara Larsson** <sjl65@cornell.edu> — *Association of Dwarf8 Polymorphisms with Variation in Flowering Time in a Diverse Population of Maize (Zea mays L.)*

P178	**Yunbi Xu** <y.xu@cgiar.org>	*Bulked segregant analysis using the high throughput maize GoldenGate SNP genotyping assay reveals multiple genomic regions associated with kernel hardness and tryptophan content in quality protein maize*
P179	**David Hessel** <dhessel@iastate.edu>	*COGENFITO: a composite genotype finder tool that allows optimization of breeding schemes according to principles of genomic selection*
P180	**Anastasia Bodnar** <anastasia@geneticmaize.com>	*Characterizing seed storage proteins in teosinte and tripsacum*
P181	**Randy Wisser** <rjw@udel.edu>	*Characterizing the selection response by selection and association mapping*
P182	**Laura Shannon** <lshannon@wisc.edu>	*Combined QTL analysis of three maize-teosinte backcross populations*
P183	**Kerry Mayfield** <kerry-mayfield@tamu.edu>	*Confirmation of QTL for Reducing Preharvest Aflatoxin Accumulations in Hybrid Topcrosses*
P184	**Sherry Flint-Garcia** <Sherry.Flint-Garcia@ars.usda.gov>	*Development and Marker Characterization of Maize-Teosinte Introgression Libraries*
P185	**Jianbing Yan** <j.yan@cgiar.org>	*Development of informative markers through association mapping in maize to improve drought tolerance in cereals*
P186	**David Kendra** <david.kendra@ars.usda.gov>	*Disease Lesion Mimics of Maize as a potential source of resistance to Fusarium ear rot*
P187	**Santiago Mideros** <sxm2@cornell.edu>	*Dissecting Resistance to Aspergillus flavus in Maize*
P188	**Jason Peiffer** <jap333@cornell.edu>	*EVALUATION OF STALK STRENGTH QTL BY NESTED ASSOCIATION MAPPING*
P189	**Candice Hansey** <cnhansey@wisc.edu>	*Effect of Plant Morphology and Planting Methodology on Biomass Production and Compositional Characteristics in Maize*
P190	**HIDEKI OISHI** <oishih@jfsass.or.jp>	*Effects of lfy1 gene on some characteristics of an inbred line and hybrids*
P191	**J. Erik Mylroie** <jem135@msstate.edu>	*Evaluation of a Gene Specific Marker in a Population of F2:3 Families for Resistance to Aflatoxin Accumulation in Maize*
P192	**Sofia Silva** <ssilva@illinois.edu>	*Evaluation of starch concentration, grain yield, and response to nitrogen levels in ILP90xB73 derived maize hybrids*
P193	**Kristen Leach** <kalp55@mizzou.edu>	*Fitness comparisons of Bt-resistant and Bt-susceptible western corn rootworm colonies*
P194	**Andrew Doust** <andrew.doust@okstate.edu>	*Foxtail Millet: Developing a Model for Vegetative Branching in Grasses*
P195	**Jose Santa-Cruz** <jhsantac@ncsu.edu>	*GENETIC CHARACTERIZATION OF A QUANTITATIVE TRAIT LOCUS CONFERRING RESISTANCE TO SOURTHERN LEAF BLIGHT OF MAIZE*
P196	**Richard Johnson** <grjohnso@illinois.edu>	*GENOME-WIDE SCREENING FOR CIS AND TRANS REGULATION OF ALLELE-SPECIFIC GENE EXPRESSION IN MAIZE*
P197	**Alexander Thiemann** <Alexander.Thiemann@botanik.uni-hamburg.de>	*Gene expression analysis of european maize inbred lines for the prediction and characterization of heterosis*
P198	**Kristin Chandler** <kchandl@purdue.edu>	*Genetic Analyses and Selection for Carotenoids using Single Kernel Genotyping with Allele Specific Markers*
P199	**Ann Stapleton** <stapletona@uncw.edu>	*Genetic Architecture of Multiple-Stress Responses*

P200	**Seth Murray** <sethmurray@tamu.edu>	*Genetic Diversity within Sweet Sorghum and Association Mapping of Brix and Height*
P201	**Jason Cook** <jpcook4@wisc.edu>	*Genetic Mapping of QTLs Associated with Planat Regeneration in Maize*
P202	**Yunbi Xu** <y.xu@cgiar.org>	*Genetic mapping of head smut and mosaic virus disease resistance and drought tolerance through high-throughput SNP genotyping of a large number of maize introgression lines*
P203	**Sofia Silva** <ssilva@illinois.edu>	*Genetic, Genomic, and Breeding Approaches to Improve Yield and Starch in Maize*
P204	**Hong Yao** <yaoho@missouri.edu>	*Genome Dosage Effects on Heterosis in Triploid Hybrids of Maize*
P205	**Jinliang Yang** <yangjl@iastate.edu>	*Identification of QTLs Regulating Heterosis for Seedling Dry Weight and Cob Weight*
P206	**Cameron Horine** <clhzw6@mizzou.edu>	*Identification of chromosome regions controlling shoot-borne root diameter.*
P207	**Yuhe Liu** <yuheliu1@illinois.edu>	*Integrative analyses of genetic variation in metabolic pathways associated with nitrogen utilization in maize*
P208	**Abby Michenfelder** <asm3734@uncw.edu>	*Iron concentrations within kernels vary between inbred lines and in response to low nitrogen and drought conditions*
P209	**Elhan Ersoz** <ee57@cornell.edu>	*Joint Analyses of Test-cross Hybrids with Inbred-lines Increase Power of Association Under Unified Mixed Model Framework*
P210	**Yunbi Xu** <y.xu@cgiar.org>	*Large-scale screening for drought tolerance at vegetative stage as revealed by biomass measured under water-stressed and well-watered environments, compared with other selection criteria*
P211	**Eunsoo Choe** <echoe1@uiuc.edu>	*Marker assisted breeding for desirable thinner pericarp thickness and favorable ear traits in fresh market waxy corn germplasm*
P212	**Catherine Kandianis** <cbkandianis@gmail.com>	*Modification of Maize Carotenoid Composition through Degradation by ZmCCD1*
P213	**Satya Chintamanani** <satya@purdue.edu>	*Mutant-assisted exploration of natural variation underlying R gene-mediated immunity in maize*
P214	**Jacqueline Benson** <jmb565@cornell.edu>	*Nested Association Mapping of Gray Leaf Spot Resistance in Maize*
P215	**Jianming Yu** <jyu@ksu.edu>	*Nonmetric Multidimensional Scaling Corrects for Population Structure in Association Mapping with Different Sample Types*
P216	**Nengyi Zhang** <nz45@cornell.edu>	*QTL analysis of nitrogen and carbon metabolites using the maize nested association mapping panel*
P217	**Nick Lepak** <nkl3@cornell.edu>	*QTL by Observation of a Field Technician: NAM QTL for Armyworm and Banvel Herbicide Susceptibility*
P218	**Yusheng Wu** <yshmh2@yahoo.com>	*QTL mapping for amylose content in maize (Zea mays L.) using SSR markers*
P219	**Fabiano Pita** <fpita@dow.com>	*Sample size for QTL effect detection – a simulation study*
P220	**Kelly Robbins** <KRRobbins@dow.com>	*Spatial Corrections of single replication field trials using autoregressive and two-dimensional spline models.*
P221	**Peter Balint-Kurti** <peter_balintkurti@ncsu.edu>	*The Genetic Analysis of Quantitative and Multiple Disease Resistance in Maize*
P222	**Genevieve DeClerck** <gad14@cornell.edu>	*The Gramene Genetic Diversity module: A resource for comparative genome analysis in plants*
P223	**Feng Tian** <ft55@cornell.edu>	*The genetic architecture of maize leaf*

P224 **Kelly Robbins** <KRRobbins@dow.com> — *To use percent checks or not: the impacts of trait transformation on genetic parameters and the prediction of future performance.*

Transposable Elements

P225 **Yubin Li** <yubin@waksman.rutgers.edu> — *TED, an Unusual Mutator Like Element in Maize*

P226 **Kevin Ahern** <ka38@cornell.edu> — *A Sequence-Indexed Collection of Ds Transposable Elements in Maize: Platforms for Regional Mutagenesis*

P227 **Yubin Li** <yubin@waksman.rutgers.edu> — *A set of maize lines with marked Ds* transposons for localized mutagenesis*

P228 **Chuanhe Yu** <ych@iastate.edu> — *Ac/Ds elements induce genome rearrangement in transgenic maize and rice*

P229 **Zijun Xu** <zixu@mtu.edu> — *Evolutionary Origin of Retrotransposons Inserted in Rice Promoters and Genes and the Effect of Retrotransposon Insertions in Promoters on Gene Regulation*

P230 **Matthew Oetjens** <oetjensm@gmail.com> — *Helitrons: Their Impact on Maize Genome Evolution and Diversity*

P231 **Christian Restrepo** <civic88@ufl.edu> — *Mu Mapping in Maize and Teosinte Inbreds*

P232 **Donald McCarty** <DRM@UFL.edu> — *New mutants available through MaizeGDB: Stable, sequence-validated Mu inserts in specific UniformMu lines*

P233 **Sanzhen Liu** <liu3zhen@iastate.edu> — *Nonrandom Insertion Patterns of Mutator Transposons in Maize Genes and Chromosomes as Revealed by 454 Pyrosequencing*

P234 **Ann Armenia** <armeniaa@msu.edu> — *Sequence Acquisition by Mutator Elements in Maize*

P235 **Damon Lisch** <dlisch@berkeley.edu> — *Tame transposons and wild genes: Blurring the lines between hosts and parasites*

P236 **Yongqin Wang** <wang.1387@osu.edu> — *Transposon Regulation of A1 Gene Expression*

P237 **Lixing Yang** <lxyang@uga.edu> — *Uncovering Helitrons in the maize genome*

Outreach

P238 **Allison Phillips** <arphilli@stanford.edu> — *A New Outreach Program Trains Undergraduate Students to Mentor and Teach Plant Biology to High School Students*

P239 **Ann Stapleton** <stapletona@uncw.edu> — *Coming of Age of the iPlant Collaborative*

P240 **Michelle Denton** <med55@cornell.edu> — *The genome in a garden: maize mutants and public outreach (1932-2007)*

P241 **Mary Schaeffer** <schaefferm@missouri.edu> — *The maize gene review – a new feature of the Maize Genetics Cooperation Newsletter*

P242 **Anne Sylvester** <annesyl@uwyo.edu> — *Tribal College Outreach III: Transitioning to a University*

Employment Opportunities

P243 **Pioneer Hi-Bred** *Career Opportunities at Pioneer Hi-Bred International*
< http://www.pioneer.com>

P244 **Dow AgroSciences** *Careers at Dow AgroSciences*
<http://www.dowagro.com>

Plenary Talk Abstracts

Plenary 1

Genes for ADP-glucose pyrophosphorylase (AGPase); their future and their past.

(presented by Curt Hannah <Hannah@mail.ifas.ufl.edu>)
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AGPase is a rate limiting enzyme in starch biosynthesis and thus an excellent candidate for engineering. Our approach involves three steps. (1) Variants are isolated and characterized in an E. coli AGPase mutant. (2) Resulting AGPase is purified and characterized for kinetic and allosteric properties and heat stability. (3) Interesting variants are placed into experimental maize lines and commercial hybrids.

Our experimental challenge is developing efficient mutant screens and rational design protocols. Relevant to this is a model that the two AGPase subunits play different roles in catalysis and allostery.

Underpinning this model is the fact that small subunits exhibit stronger sequence conservation relative to the large subunits. We found that while the small subunit is under greater selection pressure, the two subunits are equally susceptible to missense mutations abolishing/altering AGPase activity. Greater selection pressure on angiosperm small subunit stems from the fact that it must function in more tissues and must remain compatible with more pairing partners, relative to the large subunit.

Several strategies have proven useful for mutant selection. We isolated glycogen overproducers by growing E. coli with mutant maize genes at variable temperatures and on variable carbon levels. Intragenic suppressors of heat labile mutants have also been isolated. We designed subunits by focusing on protein motifs from other AGPases that produce superior characteristics. And we have used evolutionary considerations to identify useful amino acids.

Two mutant AGPases have superior characteristics in planta. Maize, wheat and rice plants containing the large subunit Sh2-HS33Rev6 exhibit yield increases up to 68%, 38% and 23% respectively. Surprisingly, yield increase is due to enhanced seed number. Plant weight is also increased. In maize, yield increase occurs only when temperatures during grain fill are above 32 C (92 F). It is presently unclear whether this variant functions only in the endosperm to enhance seed number. A small subunit variant containing sequences from the maize endosperm and the potato tuber enzyme and conditioning an autonomously activated enzyme causes a doubling of seed yield. Yield increase is due to increased seed number.

Plenary 2

Arabidomics: Next is now: Sequencing of genomes, epigenomes, transcriptomes, and beyond

(presented by Joe Ecker <ecker@salk.edu>)
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The availability of DNA sequencing technologies that rapidly produce vast amounts of sequence information has triggered a paradigm shift in biology, enabling massively parallel surveying of complex nucleic acid populations. The diversity of applications to which these technologies have already been applied demonstrates the immense range of cellular processes and properties that can now be studied at the single-base resolution. These include, but are not limited to, genome sequencing/resequencing, polymorphism and gene discovery, cytosine methylation mapping, alternative splicing identification, small RNA profiling, as well as mapping of the sites of DNA–protein interaction. These deep sequencing technologies offer plant biologists unprecedented opportunities to increase the understanding of the functions and dynamics within individual cells up to populations of plants. Examples of the types of information that can be captured using now-generation DNA sequencing methods as applied to Arabidopsis will be described.

Plenary 3

Stay pure or hybridize: pleasures and torments of genomic promiscuity

(presented by Luca Comai <lcomai@ucdavis.edu>)
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Genome-wide duplication events are thought to have contributed to diversification of plants. We study incipient and recent polyploidy in Arabidopsis to understand the molecular mechanisms leading to successful hybridization and establishment of polyploids. I will describe genome-wide dosage-sensitive responses important in the success of interploidy and interspecific crosses and loci that affect the outcome. Our observations are consistent with the involvement of both chromatin regulation in the hybrid endosperm and maternal contribution to seed development. I will also describe experiments aimed at elucidating non-additive regulation deriving from hybridization. Our results reveal potential similarities between regulatory disruption during postzygotic incompatibilities and non-additive regulation associated with hybridization and heterosis.

Plenary 4

Adaxial-abaxial patterning of the maize leaf by mobile small RNAs

(presented by Marja Timmermans <timmerma@cshl.edu>)
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Establishment of adaxial-abaxial (upper-lower) polarity is essential for the outgrowth and patterning of leaves. We have shown that in maize, adaxial-abaxial leaf polarity is established through a cascade of opposing small regulatory RNAs. The microRNA miR390 localizes to the adaxial/upper side of leaf primordia where it triggers the biogenesis of a distinct class of 21 nt small RNAs, termed ta-siRNAs. These block the accumulation of miR166, which consequently are restricted to the abaxial side of leaves where they prevent the expression of HD-ZIPIII transcription factors that specify adaxial cell fate. Laser capture microdissection coupled to RT-PCR revealed that the precise spatiotemporal accumulation of these polarizing small RNAs in the maize shoot apical meristem is regulated at the level of precursor transcription, as well as at the level of small RNA processing and/or stability. Importantly, comparison of the expression patterns of small RNA precursor transcripts to those of the mature small RNA provides evidence that small regulatory RNAs can be mobile. To further investigate the possibility that adaxial-abaxial patterning involves mobile small RNAs, we determined the localization patterns of key ta-siRNA biogenesis components in Arabidopsis. This revealed that ta-siRNAs accumulate outside their defined domain of biogenesis. Movement of this low abundant small RNA creates a gradient of accumulation across leaves that maintains the polarized accumulation of abaxial determinants. Our observations indicate that leaves are partitioned into adaxial and abaxial domains via a novel patterning mechanism involving small RNAs as mobile, instructive signals.

Short Talk Abstracts

T1

Properties of transposition and uses in regional mutagenesis of the *Dissociation* (*Ds*) transposable element

(submitted by Erik Vollbrecht <vollbrec@iastate.edu>)
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Mutational analysis has been the cornerstone of genetics and an essential tool to link genotypic variation to phenotypic variation. Hence, gene disruption lines are a fundamental and versatile resource for basic and applied research in any organism, including crop species such as maize. With NSF-PGRP funding (http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=0501713) we have developed a community resource of carefully curated, non-transgenic and sequence-indexed *Ds* insertion lines in the inbred W22. Currently, approximately 2000 lines exist, each with a uniquely located, endogenous *Ds* insertion. These insertions are distributed throughout the genome, such that 85% of the genetic map space is within 4 cM or less of a placed Ds. Given the propensity of *Ds* for local transposition, this collection of lines will allow targeted disruption of the vast majority of genes in maize. In producing and analyzing this foundation for a community resource we discovered new properties of *Ac/Ds* transposition. Using an alignment of a large population of insertion site sequences, our analysis supported previous suggestions that *Ds* insertion does not show DNA sequence preference. However, in a several-base window spanning the insertion site and a few flanking bases, *Ds* did show clear preference to insert into DNA with particular structural properties. More than a dozen physico-chemico indicators of locally destabilized DNA correlated significantly with insertion sites. Additionally, the insertion site was the center of a 16 base pair, palindromic pattern of hydrogen-bond presentation in the major groove of DNA. This is compelling evidence that protein-DNA interaction during reinsertion may involve a symmetrical multimer of *Ac*-encoded transposase molecules. All of these structural properties were also evident in collections of *Ds* insertion sites from heterologous systems. Finally, in pilot remobilization experiments of different *Ds* donors into target genes, the frequency of insertion into a target was proportional to the log of genetic distance between target and donor. These parameters could help optimize remobilization experiments, for example in calculating population sizes necessary to recover *Ds* insertion into a target, or in selecting a gene region to target based on structural properties of DNA. All lines, protocols and data from this project are available to the community, including from our project home page (http://www.plantgdb.org/prj/AcDsTagging/).

T2

Paired transposons: natural chromosomal engineers

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Several observations indicate that compatible ends of separate, yet closely linked, transposable elements (TEs) can interact in alternative transposition reactions. First, pairs of TEs cause chromosome breaks with frequencies inversely related to the intertransposon distance. Second, some combinations of two TEs produce complex rearrangements that often include DNA adjacent to one or both elements. In pairs of TEs in direct orientation (DO), alternative reactions involving the external ends of the two TEs should lead to the transposition of a macrotransposon consisting of both elements plus the intervening chromosomal segment. Such macrotransposons have been hypothesized previously based on deletions, but no macrotransposon insertions have been recovered. To detect macrotransposition, we have analyzed heritable chromosomal rearrangements produced by a chromosome-breaking pair of *Ac* and *Ds* elements situated 6.5 kb apart in DO in a part of the maize genome dispensable for viability. We show here that the postulated macrotransposon can excise and reinsert elsewhere in the genome. In addition, this transposon pair produces other complex rearrangements, including deletions, inversions, and reshuffling of the intertransposon segment. Thus, closely linked TE pairs, a common transposition outcome in some superfamilies, are adept at restructuring chromosomes and may have been instrumental in reshaping plant genomes. We are currently defining the basic properties of the macrotransposition process, and establishing whether a large (>100kb) chromosome fragment can macrotranspose using appropriate combination of TE pairs in DO.

T3

Content, distribution, and timing of insertion of *Helitron* transposable elements in the maize genome

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Helitron transposons were discovered computationally in the genomes of *Arabidopsis thaliana*, *Oryza sativa* and *Caenorhabditis elegans*. However, their abundance is highly variable even among closely related species. *Helitrons* comprise 1-5% of the genome in fruit flies, 0-3% in mammals, >2% in *Arabidopsis*, and 0.03-1.02% in different rice species.

Only a handful of maize *Helitrons* discovered manually have been reported so far in the literature. To overcome this limitation, we developed and implemented in PERL a heuristic searching algorithm for identifying *Helitrons*. Our HelitronFinder program will (i) take FASTA format DNA sequence as input and identify hairpin loop patterns, and (ii) exploit the consensus 5' and 3' end sequences of known *Helitrons* to identify new putative ends (Du et al. 2008). Four out of five predicted *Helitrons* were confirmed to be polymorphic in different maize inbreds. An additional 140 new *Helitrons* were identified by HelitronFinder in the non-redundant GenBank maize sequence database.

The maize sequencing project led by Washington U. in St. Louis has generated thousands of BACs and the whole maize genome sequence is nearly finished. We used the HelitronFinder program to search 2.4 Gb of maize genome sequences consisting of 16,205 maize BACs in the Genome Sequencing Center. We identified over 2,000 non-autonomous *Helitron* copies in the B73 genome. The sizes of the predicted *Helitrons* are 50 kb, 126 bp, 7342 bp, and 11.9 kb for maximum, minimum, median, and average, respectively. The non-autonomous *Helitrons* make up about 1.39% of the sequenced maize genome (33.4/2400 Mb). We have also analyzed the content, distribution, and timing of insertion of *Helitrons* in order to gain a better understanding of the organization of the highly polymorphic present- day maize genome.

T4

Distilling transposable element exemplars from host genomes builds a framework for understanding TE diversity in the grasses

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The study of the distribution and abundance of transposable elements (TEs) in the grasses requires a robust system for circumscribing families within and among host genomes. We have implemented a suite of novel graph theory-based methods that allow for the combination of multiple evidences to generate family level taxonomies for TEs. An outcome of this new approach is the observation that the relative abundance of LTR retrotransposon families in host genomes follows a power law distribution. This gives support to our method of family assignment as well as places the study of TE abundance in the general framework of unified theories of biodiversity. Comparative studies across host genomes can thus directly compare these abundance distributions as well as make use of ecological metrics of diversity. Finally, our approach also identifies extant members of TE families that may serve as representative 'exemplars' of the full diversity of TEs in a host genome. These representative sequences may be used as nonredundant databases of TEs. More importantly, these exemplars will serve as a scaffold for building a full phylogenetic tree of LTR retrotransposons that will allow us to place individual insertion events in the full history of retrotransposon activity in the grasses.

T5

Centromere inactivation and reactivation reveal both genetic and epigenetic components for centromere specification

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A high frequency of stable dicentric chromosomes was found from a centromere tug of war that linked together a large and a small B centromere by inactivation of the small centromere. The inactive centromere can be transmitted from one generation to another by being attached to the larger active centromere. The chromosome produced has a foldback structure so that it can recombine with itself. Intrachromosomal recombination promotes reactivation of the inactive centromere. This reactive process is specific to anaphase of meiosis I. The reactivated centromere binds antibodies against CENH3 and CENPC, molecular correlates of centromere activity, in late anaphase I. This recombination can produce new dicentric chromosomes containing two active large and two inactive small centromeres as reciprocal products of exchange. In the latter, one of the small inactive centromeres can recover function in some cases and transmit to next generation. Homologous chromosome pairing between two copies of the large active and small inactive centromere containing chromosome reduces the frequency of intrachromosomal recombination and thus decreases the reactivation of inactive centromeres. However, recombination between homologues can also produce small-small centromere chromosomes that have been observed to reactivate. These findings indicate an epigenetic component to centromere specification that is reversible.

T6

Evolution of maize centromeres as revealed by their physical maps

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Centromeric retrotransposons of maize (CRM) and the centromeric satellite CentC are the major components of corn centromeres. CR elements have been detected in a wide variety of plants, but their role in centromere evolution remains unclear. Four subfamilies of CRM and their orthologs in rice were recently described. The oldest of these, CRM4, has not been active for some time, while the youngest and most active (CRM1) contains by far the largest number of full-length elements. The high retrotransposition rate of CRM1 elements was recently shown to correlate with the generation of recombinant subgroups. While CRM4 is mostly localized to heterochromatin, CRM1, CRM2 and CRM3 are found in or near the functional centromere as defined by chromatin immunoprecipitation with anti-CenH3 antibody. Physical maps for two corn centromeres, supported by fiber FISH and metaphase FISH, illustrate surprising differences in the distribution of these elements and provide a framework to study their turnover on an evolutionary time scale.

T7

Recombination within centromere cores

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Centromeres are constant features of all eukaryotic genomes, yet they are the most dynamic at the genomic level. Here we describe a novel CENH3 ChIP-marker display method (based on CRM2) that maps kinetochore footprints over high-resolution recombination maps. Each of the ten centromeres was genetically mapped using a set of 274 CRM markers, 65% of which interact with CENH3. Multiple, sequenced, polymorphic markers span each centromere. Careful segregation mapping in the IBM population revealed at least three within-centromere gene conversion events. The complete physical map of centromere 2 allowed us to provide a first measure of linkage disequilibrium within a centromere. Taken together the data show that genetic exchange is common in centromeres, although crossing over rarely occurs.

T8

Chromosome dynamics in live maize meiocytes.

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Meiotic prophase is a period of dramatic spatial repositioning of chromosomes within the nucleus. Maize has been a model system for studying these processes for over 80 years. However, most of our knowledge on chromosome dynamics in prophase I comes from examining fixed meiocytes. Although these observations have been successful in reconstructing the general progression of meiotic prophase, elucidating many aspects of chromosome dynamics is not possible from fixed cells. As a result, the classical reconstruction of meiotic prophase paints a picture of rather slow and orderly chromosome movements. Live imaging of meiosis has remained out of reach for most higher eukaryotes because of the difficulty in culturing isolated meiocytes during meiotic prophase. To overcome this obstacle, we developed a novel system to image in real-time meiocytes in intact maize anthers, which, in contrast to isolated meiocytes, can be cultured. Observations are conducted using multiphoton excitation microscopy, capable of penetrating deep inside the anther locules. We found that chromosomes in zygotene and pachytene show extremely dynamic and complex motility patterns. In zygotene, we can identify several distinct types of movements: (i) very rapid, short-distance oscillations of small chromosome regions, (ii) slower-paced movements of entire chromosomes, and (iii) rotations of the entire chromatin mass within the nucleus. Presence of these different movement types suggests that several independent mechanisms simultaneously direct chromosome motility. In pachytene, the movements are slower than those in zygotene. At this stage we often observe sweeping motions of entire chromosome arms. We hypothesize that chromosome movements in zygotene enable probing for homology between different chromosome segments. The pachytene movements pachytene may facilitate resolving chromosome interlocks. Our preliminary data show that pam1, a maize mutant exhibiting defective chromosome pairing and delayed progression through meiotic prophase, is deficient in chromosome movements, which provides supports to these hypotheses.

T9

TATE --- A web based pipeline for rapidly retrieving and characterizing gene and transposable element families from genomic sequences

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Gene families comprise a large proportion of plant genomes. The ever-growing genomic sequence database provides a good opportunity to study gene family evolution and function. However, most gene family identification programs are restricted to searching protein databases, where data is often lagging behind the genomic sequence data. Here we report a new user friendly web based pipeline, named TATE, which uses either a DNA or amino acid "seed" query to automatically identify and retrieve gene family homologs from a genomic database, characterize gene structure and do phylogenetic analysis. Due to its high speed, TATE is also able to characterize high copy number gene families including transposable elements. TATE was originally developed for an undergraduate classroom and has recently been adapted to the research laboratory where it has been utilized to characterize DNA TEs in the maize genome. In addition, TATE has been evaluated using well annotated datasets, including the ascorbate peroxidase gene family of rice, maize and sorghum, and several TE families in rice. In all cases TATE rapidly recapitulated the known homologs and predicted new ones. We also demonstrated that TATE vastly outperformed similar pipelines and has functionality that is not offered elsewhere. We are working with iPlant to integrate TATE into an annotation pipeline that can be used by advanced high school students and undergraduates worldwide.

T10

The Physical and Genetic Framework of the Maize Genome

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Corn (Maize) is a major crop in the U.S., a main source of global food supply, and an important model system for basic biological research. Knowledge gained from maize can also be used to improve its relatives such as sorghum, wheat, rice, switchgrass and Miscanthus. The Maize Genome Sequencing Project provided a maize reference genome sequence with finished high quality sequence in low copy regions. During the course of the project we continued to update the maize integrated genetic and physical map with sequencing information and public genetic markers. We also built two pipelines, the MTP pipeline for clone selection and gap filling, and the AGP pipeline to build maize pseudochromosomes. At present, the physical map covers 2120 Mb (93%) of the 2300-Mb genome. The updated integrated genetic and physical map has 442 contigs, of which 392 can be anchored to the genetic map, totaling 2073 Mb (97.8% of the 2120 Mb physical map), a significant improvement from 86.1% anchored at the beginning of the sequencing project. Of the 2073 Mb anchored contigs, 1222.9 Mb (57.7% of the physical map) can be ordered and orientated, 387.4 Mb (18.3%) can be ordered, but not orientated, and 462.8 Mb (21.8%) have only approximate genomic positions and are not ordered and orientated. Another 47 Mb (2.2% of the map) have no genome context. In total approximated 850.2 Mb of maize sequence (42.3% of the physical map) still needs to be precisely ordered and orientated. To assess coverage of the maize genome sequence contained within the physical map, we analyzed the 46,873 maize full-length cDNA sequences recently deposited in GenBank and found that 96% could be mapped to the genome. We are now utilizing the maize optical map to assist in ordering and orientating contigs and to determine gap sizes between contigs. Additionally we are utilizing synteny information from the rice and sorghum RefSeqs to help with ordering and orientating contigs. No unpublished ISU IBM Map7 markers were used in this analysis.

T11

The MaizeGDB Genome Browser

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MaizeGDB (http://www.maizegdb.org) is the community database for maize genetics and genomics. As part of a larger effort to develop MaizeGDB into a more sequence-centric resource, we recently implemented a genome browser. The GBrowse platform was chosen for this endeavor based on results of a survey we conducted to learn about community members' desired functionalities and impressions of existing genome visualization software. Data from MaizeSequence.org, PlantGDB, and MaizeGDB are the basis for the initial release. Here I will present the features of the MaizeGDB Genome Browser and describe how the browser is connected with MaizeGDB data as well as off-site resources. I also will demonstrate a number of the browser's unique features and functionalities: e.g., the Locus Lookup tool approximates a given locus' genomic coordinates based on its genetic map position. Along with other tailor-made tools and views of the maize genome unique to the MaizeGDB Genome Browser, this sort of feature provides maize researchers with an accurate picture of the genome, thus enabling researchers to spend less time on the computer and more time testing hypotheses at the bench.

T12

Sequence and analysis of the maize B73 genome

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The Maize Genome Sequencing Consortium was launched with a three-year grant to produce the sequence of the maize (B73) genome. We recently completed the sequencing of 16,600 BAC clones that correspond to a minimal tiling path for the genome. These assembled clones represent a near complete genome sequence of maize with unique regions brought to finished quality. This sequence, accessible via GenBank and, of most relevance to cereal geneticists, via a genome browser (maizesequence.org), provides a more refined view of the maize genome. In this presentation, we will discuss the efforts being conducted during the third year of the project to improve and annotate the maize genome sequence as well as some detailed analysis of a 22Mb gene-rich region of the genome. General findings will be discussed.

T13

Update on the Mo17 genome sequencing project

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As part of the larger tri-agency Maize Genome Project we are sequencing the Mo17 inbred line using 454 pyrosequencing methods. The aim is to recover as much of the Mo17 genome as possible, by de novo and/or assisted methods, and to characterize sequence and structural variation between Mo17 and B73.

This talk will summarize the early analysis of an initial 25 gigabases of shotgun sequence, including technical biases of the dataset and characterization of the SNPs and other variations that have been detected to date. We will also describe current annotation progress and plans for sorghum, update on other grass genomes, and the Phytozome comparative genomics portal for plants.

T14

The characterization of the Palomero Toluqueno genome

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Considering that single nucleotide polymorphisms (SNPs) that distinguish 2 maize inbred lines are on average as significant as those distinguishing humans form chimpanzees, reasonable predictions anticipate that the genetic divergence between inbred lines and native landraces should be far more important. As a consequence, genome sequencing efforts that concentrate on inbred line germplasm might not be sufficient to fully characterize the genome organization and functional diversity of maize. Over the past year we have pursue our characterization of the Palomero genome by applying updated tools used for gene prediction and annotation, improving our validation procedures, and expanding our structural analysis of ultraconserved genomic regions. After removing contigs composed of 100% organellar DNA, our final assembly of the Palomero gene space contains 381,417 unique sequences representing 325.9 Mb. The overall analysis of repeat content indicates that 48 to 58% of nucleotides align with sequences from the TIGR Cereal Repeat database, suggesting that the repeat content of Palomero is significantly lower than previous estimates obtained for B73. A combination of high-speed Blast2Gene homology comparison and GS20-454 transcriptional validation has improved our gene prediction and validation strategy, confirming that the sequence of 646 genomic regions has null or low nucleotide variability in B73, 17 distinct Mexican landraces and 8 different teosintes. Genes contained within these conserved regions are in the process of being functionally analyzed to determine their possible involvement in maize domestication.

T15

Research & the Recession: How Obama and the Stimulus Package Impact the Future of Agricultural Research

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The National Corn Growers Association (NCGA) is a grassroots driven trade group advocating for the 300,000 corn producers in the United States. With approximately 30,000 members, NCGA covers topics ranging from conservation, stewardship, trade, public policy, biotechnology, renewable fuels and research. Currently we have 48 state checkoff and grower groups active within NCGA driving our priorities and keeping us focused on the challenges and opportunities facing U.S. producers.

NCGA spearheaded the formation of the National Plant Genome Initiative (NPGI) funded at $40 million dollars per year. We have since grown that line item to over $100 million a year and have yielded the high quality corn genome sequence as a result. NCGA has remained very active in the development of the program, participating in numerous stakeholder meetings to ensure that the focus of the NPGI remains on innovation in economically important plants. With the release of the next 5-year plan (2009-2013), we are excited about the possibilities for the maize research community to meet many of the objectives set forth by the NPGI.

Additionally, NCGA has long advocated for the development of the National Institute of Food and Agriculture (NIFA) to supplement USDA and NSF research agencies. With this institution authorized under the 2008 Farm Bill, we continue to support the development of NIFA such that it will have the capacity and funding to attract the best scientists to conduct cutting-edge research, education and extension, and to train future agricultural scientists to meet the world's food, feed and burgeoning biofuel needs.

Of course, with the current state of the economy, the future success of agricultural research will be highly dependant on the availability of adequate funding. NCGA will continue to fight for higher levels of research funding, and needs the help of both public and private sector scientists to make us successful.

T16

Community Annotation of the Maize Transcriptome @ MaizeGDB

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A B73 maize genome sequence is pretty much finished. Next generation sequencing technology promises many more genomes to follow soon. To turn the sequence information into knowledge we must carefully and comprehensively annotate these genomes, as well as relationships between genomes (e.g., synteny, homology). Even the limited task of identifying the protein coding genes and their transcripts is currently beyond the grasp of computational approaches alone. A small group of dedicated annotators is also easily outmatched by the volume of data available and being generated. Thus, a combination of computational approaches, dedicated annotators, and the community at large is required. We present already developed infrastructure (computational workflows, genome browser, web-based annotation tools) and a vision for maize genome annotation that promises high quality, expert and community ownership, and teaching opportunities.

T17

A maize 13-lipoxygenase regulates green leafy volatile and jasmonic acid biosynthesis and is required for resistance to herbivores.

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Plants respond to insect attack by using a variety of defense mechanisms. Among the most important molecular signals that activate defense responses are oxygenated lipids (oxylipins) including jasmonic acid (JA) and green leafy volatiles (GLVs) produced by the lipoxygenase (LOX) pathway. GLVs have a dual function in that they directly deter herbivores from feeding and warn neighboring plants to take preemptive measures against potential threats. JA is likewise a potent molecular signal involved in herbivore defense. While the importance of these pathways is recognized, the genes responsible for their detailed molecular and biochemical regulation is still obscure. Here we report on the cloning, localization, mutation, and characterization of a maize 13-LOX isoform. We further show that this LOX isoform is responsible for GLV production and plays an additional role in JA biosynthesis. Moreover, we provide biological evidence that the disruption of this 13-LOX-mediated metabolism leads to decreased herbivore resistance in maize.

T18

Site-specific binding of a pentatricopeptide repeat protein defines and stabilizes 5' and 3' mRNA termini in maize chloroplasts: implications for plastid mRNA biogenesis mechanisms and PPR family functions

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Chloroplast mRNA populations are characterized by complex sets of overlapping transcripts derived by processing from polycistronic precursors. The mechanisms and functional significance of these processing events are poorly understood. We describe a pentatricopeptide repeat protein in maize, PPR10, whose binding helps to define mRNA segments derived from two transcription units in chloroplasts. PPR10 interacts in vivo and in vitro with two intergenic RNA regions of similar sequence. Processed 5' and 3' RNA termini derived from these regions overlap by ~25 nucleotides. The PPR10 binding sites map precisely to these overlapping sequences, and RNAs with these termini are specifically missing in ppr10 mutants. Our findings show that PPR10 serves as a barrier to RNA decay from either the 5' or 3' direction and that a bound protein provides an alternative to an RNA hairpin as a barrier to 3' exonucleases. The results imply that protein 'caps' at both 5' and 3' ends can define the termini of chloroplast mRNA segments. These results, together with recent insights into bacterial RNA decay, suggest a unifying model for the biogenesis of complex chloroplast transcript populations and for the determinants of chloroplast mRNA stability. We hypothesize that the majority of the ~100 chloroplast PPR proteins that lack additional domains serve an analogous 5' and/or 3' capping function.

T19

The rough endosperm3 locus encodes a predicted splicing factor required for plant development and regulation of cell proliferation.

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Plant development requires controlled cell proliferation and cell differentiation. The rough endosperm3 (rgh3) mutant is essential for maize seed and seedling development. Our phenotypic characterization of rgh3 suggests that this locus is required to regulate both cell differentiation and cell proliferation. In the endosperm, rgh3 mutants fail to differentiate the Basal Endosperm Transfer Layer (BETL) and develop ectopic aleurone cells. rgh3 seedlings germinate at a low rate and seedling leaves are typically adherent. Mutant rgh3 plants are lethal within 2-4 weeks of planting; however, the Rgh3 locus is not essential for cell viability. Mutant endosperm tissues are far more proliferative than normal endosperm tissues when grown in vitro. These data suggest that the Rgh3 locus has an essential developmental role. We cloned a Mu1 insertion that is tightly-linked to the rgh3 mutant. The Mu1 element disrupts a U2AF35 Related Protein that we named ZmUrp. Human URP has been shown to be a splicing factor in vitro (Tronchere et al, 1997, Nature 388:397-400). We identified a second rgh3 allele from a directed EMS tagging experiment that suggests the loss of ZmURP protein causes the rgh3 phenotype. ZmUrp produces multiple spliced products with only one variant encoding a predicted protein. A GFP fusion with ZmURP is localized to the nucleolus and nuclear speckles when transiently expressed in Arabidopsis protoplast or in tobacco epidermal cells. These data are consistent with ZmURP having a function in RNA splicing and suggest a role for RNA splicing in regulating cell proliferation and cell differentiation.

T20

Interrelationships between Pyruvate-Pi-dikinase (PPDK) and Opaque-2 in the control of endosperm C/N ratio

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A combined transcriptomic, proteomic and metabolic analysis provided an overview of the main changes occurring in gene expression during maize kernel development. It allowed identifying the set of genes expressed at each development stage and the shift occurring from one stage to the other. During the grain filling period, 12-14 DAP to 40 DAP, the larger modification was an increase in the proportion of storage protein transcripts. The contribution of the enzyme transcripts related to metabolism progressively increased but the proportion of those for protein destination category mainly consisting of chaperonins and folding proteins was always the second one behind the metabolism category. The protein synthesis category which comprises mostly ribosomal protein was also important but it declined at the beginning of maturation. Detailed proteomic analysis of metabolism showed a progressive decline of Krebs cycle enzyme relative to glycolysis which is consistent with a starch synthesis in the hypoxic conditions prevailing in the endosperm. The major observed change in the glycolytic enzymes was an upsurge of the pyruvate-Pi-dikinase (PPDK) in the late filing period (21 DAP onwards). PPDK which forms a co-called futile cycle with pyruvate kinase may provide PEP the substrate for aromatic aminoacids and PPi which reverse the ADP-glucose pyrophosphorylase (Agpase) activity, thus reducing the availability of ADPglucose, the substrate for starch synthases. Thus, PPDK functioning in this way may act as a switch in the starch/protein balance. This hypothesis is substantiated by the data on the Opaque-2 gene encoding a transcription factor with pleiotropic effect affecting lysine content and carbohydrate metabolism, thus acting indirectly on starch/amino acid ratio. The direct effect of O2 on PPDK gene expression provides a clue for explaining the competition between C and N metabolisms. This epistatic relationship between cyPPDK and O2 is further supported by quantitative and association genetics.

T21

Association of enzymes from amino acid- and carbohydrate biosynthetic pathways in multisubunit complexes: A model for regulation of carbon allocation in maize grain

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Protein and starch deposition during maize grain filling represents the culmination of the plant's carbon and nitrogen storage allocation in this high yielding crop. Understanding this process is essential for optimal utilization of maize, because it determines functional features including yield, amino acid distribution, protein content, and starch content. This study advanced our understanding of how the division of carbon flow between amino acid- and starch biosynthesis may be regulated. Biochemical analyses revealed that essentially all starch synthase III (SSIII) exists in a complex of ~670 kDa, and nearly all SSIIa is in a high molecular weight form of ~300 kDa. Mutations of dull1 or sugary2, encoding SSIII or SSIIa, respectively, cause major changes in grain properties, supporting the view that these complexes likely are functionally significant in grain filling. Proteomic analyses revealed that the complexes also include starch branching enzymes SBEIIa and SBEIIb, large and small subunits of ADPglucose pyrophosphorylase (AGPase), the sucrose synthase isoform SUS-SH1, and two pyruvate orthophosphate dikinase isoforms (PPDK). Genetic analyses demonstrated assembly interdependence. For example, high molecular weight complexes of AGPase, PPDK, and SUS-SH1 are missing in dull1- mutants lacking SSIII. Thus, all these enzymes exist together in the same complexes. Co-immunoprecipitation revealed that phosphorylation of one or more components is necessary for stable association of PPDK, SBEIIa, SBEIIb, and SSIIa with SSIII. Recently other workers have proposed that PPDK may function to divert carbon from sucrose into amino acids rather than starch, by affecting the equilibrium of the AGPase reaction. Finding that PPDK and AGPase exist together in a single starch synthetic assembly within the amyloplast suggests a mechanism of this regulation. We propose that these enzyme complexes function as a regulatory valve mechanism that controls carbon flow into starch- or amino acid/protein synthesis, thus determining critical functions of maize grain composition.

T22

Autophagy in maize is regulated by development and nutrient availability

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One of the plant responses to nutritional stresses is to recycle its own constituents by autophagy, a eukaryotic pathway for the bulk degradation in which cytoplasmic constituents and organelles are engulfed and transported to the vacuole for breakdown. Proper autophagy requires Atg (AuTophaGy) genes, which were first identified in yeast and have since found to be highly conserved among animals and plants as well. While defective autophagy in animal is associated with human diseases such as Crohn's disease and various cancer, the physiological roles of autophagy in plants are less clear. By reverse genetics, we previously found that Arabidopsis atg mutants fail to produce the vesicles associated with autophagy and show a hypersensitivity to nitrogen and fixed carbon stresses. Here we describe the Atg system in the maize B73 genome, the expression patterns of key genes, alternative splicing of Atg pre-mRNAs, and the post-translational lipidation of the important autophagy marker, ATG8. Various Atg genes are expressed in all organs tested, with the expression of some dramatically induced by aging and nitrogen deficiency. Subsequently, the level of lipidated ATG8 rapidly increases as leaf tissue senesces either by natural aging or by nutrient deficiency. These and other patterns of Atg gene expression are consistent with proposed roles of autophagy in the basal turnover of cellular constituents, nutrient recycling, and cell death. We also generated and are characterizing transgenic maize plants expressing a Yfp-Atg8 reporter, which should be a useful tool to visualize autophagosomes and autophagic body trafficking in maize under various environmental and developmental conditions.

T23

Effect of the Major Domestication Locus, Tb1, on Maize Root Architecture Using an Aeroponic System

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Maize domestication has resulted in highly modified shoot and inflorescence architecture and size although the effects on the root system remain unknown. The architecture of the root system is critical for plant growth as it determines soil exploration and the plant's competitive advantage for water and nutrient acquisition. We hypothesized that the architecture of the maize root system has been altered during domestication to adapt to above-ground modifications and corresponding changes in plant nutrient requirements. We examined the effects of a mutation at the major domestication locus, Teosinte branched 1 (Tb1), on maize root growth and architecture using an aeroponic growth system: in this system, roots are grown suspended in the air and misted with a nutrient solution to permit dynamic measurements. The homozygous tb1-ref mutant causes a dramatic loss of apical dominance allowing enhanced axillary bud outgrowth. We now report that tb1-ref also causes a drastic modification of the architecture of the post-embryonic root system of modern maize when compared to the wild type inbred. tb1-ref plants allocate more of their total biomass to the root system, but have diminished crown root development in favor of enhanced lateral root growth (branching or total length). The observed tb1-ref root phenotype could be unlinked from the high-tillering phenotype, suggesting that the root phenotype is not an indirect consequence of increased tiller number per se. These results are consistent with the hypothesis that domestication has resulted in changes to the maize root system. We also present here the advantages of aeroponics as a method to uncover genetic diversity and plasticity in the root architecture of adult maize.

T24

Maize Meiosis and Collection of meiotic mutants

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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. A suite of specialized meiotic genes are required to provide for proper reductional segregation. Powerful forward genetics screens have lead 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.

Meiosis occurs in cells that have been developmentally targeted to become meiocytes. This process relies on at least one gene, mac1 (Sheridan, et al.1996). After this cell fate determination step, the cell cycle is switched from a mitotic to a meiotic pathway. The ameiotic1 (am1) gene is a master switch needed to establish the meiotic cell cycle. All meiotic processes require am1, including expression of meiosis-specific genes, establishment of the meiotic chromosome structure, meiosis-specific telomere behavior, meiotic recombination, pairing, synapsis, and installation of the meiosis-specific cytoskeleton.

To ensure separation of homologs at the first reductional division, 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 monopolar oriented. The Afd1 gene affects all these events, and is essential for reductional segregation of chromosomes.

Since we have antibodies against the axial element components ASY1/HOP1 and AFD1/REC8, the synaptonemal central element component ZYP1, and the recombination protein RAD51 we have studied their behavior in 16 mutants. The majority of the mutants we have identified affect both homologous pairing and synapsis. Several mutants (as1, dsy1, mtm14) have non-homologous synapsis. However, dsy2 and ms*_N2415 are genuine synaptic mutants. These genes affect homologous synapsis and are required for establishment and maintenance of the synaptonemal complex. In addition, mtm99-25, has perturbed heterochromatin morphology and these defects in chromosome architecture are associated with non-homologous pairing and synapsis.

T25

Grassy tillers1 encodes a homeodomain protein that controls tiller number and lateral branch length in response to light signals

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Maize domestication and improvement has included selection for reduced tiller and ear number, resulting in increased yield. Despite the existence of numerous mutants affecting tiller production in maize, few have been functionally characterized. In order to understand the genetic mechanism regulating tiller production, we have begun an investigation of the classical maize mutant grassy tillers1 (*gt1*). *gt1* mutants produce numerous tillers, multiple ears, and elongation of all lateral branches compared to wild type. We identified a new allele of *gt1* from an EMS mutagenesis that we subsequently isolated by a positional cloning approach. *Gt1* encodes a class I homeodomain leucine zipper (HD-ZIP), a clade of transcription factors of mostly unknown function. *Gt1* transcripts are localized to the adaxial region of young leaf primordial in axillary buds, but are absent from the meristem. Since decreased tillering and ear number is a shade avoidance response in maize, we investigated the transcriptional dynamics of *Gt1* in response to far red (FR) light. *Gt1* is strongly up regulated after FR exposure, suggesting that it might mediate the shade-induced repression of tillering. *Gt1* also maps within the interval of a domestication QTL for tiller number and lateral branch length, although we have so far failed to find evidence of selection at this locus. A second tillering mutant, *teosinte branched1* (*Tb1*) has a similar phenotype and expression domain to *Gt1*, and is also regulated by FR light. Interestingly, *Gt1* is strongly down regulated in *tb1* mutants, suggesting that *Tb1* acts upstream of *Gt1* to suppress tillering in response to shade. All together, our data confirm that *Gt1* is an important regulator of lateral bud outgrowth in response to shade, and may contribute to natural variation in this important agronomic trait.

T26

Beyond the ABCs: MADS-box regulation of maize floral development

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Grasses, including maize, have a unique floral structure, called a floret. Grass florets contain stamens and carpels, like their dicot counterparts, but also contain unique lateral organs, including lodicules, palea, and lemma. Many floral regulators have been identified and characterized in the model dicots, Arabidopsis and Antirrhinnum, resulting in the well-known ABC model. Some aspects of floral development are conserved between dicots and the grasses, however other aspects are unique to the grasses, and genes that function specifically in grass development await characterization. We have characterized a maize mutant, bearded-ear (bde), required for multiple aspects of floral development, including floral meristem identity, determinacy and organ identity. We cloned bde using a map-based approach and found that it encodes the MADS-box transcription factor, ZAG3. zag3 is a member of the AGL6 clade of MADS-box genes, which are conserved among diverse angiosperms. However, zag3 is the only ALG6-like gene for which a loss-of-function mutant is available. We examined zag3 expression by RNA in situ hybridization and found that zag3 is expressed in the floral meristem, as well as lodicule and carpel primordia. Florets in bde mutants and often initiate extra carpels, a phenotype reminiscent of mutants in another MADS-box gene, zag1. To investigate the genetic relationship between zag3 and zag1, we constructed bde; zag1 double mutants. bde; zag1 double mutants exhibit a novel ear phenotype not observed in either single mutant, in which florets are replaced with branch or inflorescence-like structures, indicating that zag3 and zag1 function redundantly to promote floral meristem fate. MADS-box proteins form complexes to control expression of target genes and we are testing ZAG1 and ZAG3 for physical interactions. We present a model for ZAG3 and ZAG1 function in the molecular regulation of grass floral development.

T27

Evidence of selection at the ramosa1 locus during maize domestication

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Modern maize was domesticated from Zea mays parviglumis, a teosinte, about 8,000 years ago in Mexico. Genes thought to have been selected upon during the domestication of crops such as maize, are commonly known as domestication loci. The ramosa1 (ra1) gene encodes a putative transcription factor that controls branching architecture in maize inflorescences. ra1 is a hypothesized domestication gene because its nucleotide diversity is reduced in maize inbreds and statistical tests indicate that positive selection occurred at some point since maize diverged from its common ancestor with Tripsacum dactyloides. To further investigate the hypothesis of ra1 as a candidate domestication locus, we investigated the developmental consequences of slight alterations of ra1 activity in maize. The basis of crooked rows in weak ra1 mutants was due to the presence of extra spikelets, which disorders the packing of kernels on the ear. To better pinpoint the timing of selection, we sampled the nucleotide diversity of ra1 in a broad panel of teosintes and unimproved maize landraces for subsequent statistical and phylogenetic analyses. We identified significantly reduced nucleotide and allelic diversity for ra1 in maize landraces, but not teosintes, consistent with the hypothesis that ra1 is a domestication locus. In maize landraces, the noncoding 3' sequence showed almost no genetic diversity, suggesting that a regulatory element located in this region may have been the target of selection.

T28

Epigenetic resetting of a gene imprinted in maize embryos

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Genomic imprinting resulting in the differential expression of maternal and paternal alleles in the fertilization products has evolved independently in placental mammals and flowering plants. In most cases silenced alleles carry DNA methylation. Whereas in mammals these methylation marks of imprinted genes are generally erased and re-established in each generation, imprinting marks persist in endosperms, the sole tissue of reported imprinted gene expression in plants. Here we show that the maternally expressed in embryo 1 (mee1) gene of maize is imprinted in both the embryo and endosperm and parent-of-origin-specific expression correlates with differential allelic methylation. This epigenetic asymmetry is maintained in the endosperm, while the embryonic maternal allele is demethylated on fertilization, and remethylated later in embryogenesis. This first report of imprinting in the plant embryo confirms that, as in mammals, epigenetic mechanisms operate to regulate allelic gene expression in both embryonic and extra-embryonic structures. The embryonic methylation profile demonstrates that plants evolved a resetting mechanism of parental imprinting marks, a necessary prerequisite for parent-of-origin dependent gene expression in consecutive generations. The striking difference between the regulation of imprinting in the embryo and endosperm suggests that imprinting mechanisms may have evolved independently in both fertilization products of flowering plants.

T29

Regulation of Gene Expression and Parent-of-Origin Effects in Hybrids

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The mechanisms underlying heterosis are not well understood. Inbred lines B73 and Mo17 produce heterotic hybrids regardless of which plant is used as male. Interestingly, these reciprocal hybrids exhibit significant differences for multiple phenotypic traits. Microarray comparisons identified thousands of differentially expressed genes between the B73xMo17 and Mo17xB73 hybrids at the seedling stage. An eQTL experiment conducted to better understand the regulation of expression in inbred versus hybrid lines detected over 1,500 unique eQTL associations, most of which are trans-eQTL. Surprisingly, we observed that for many trans-eQTL, heterozygous nuclear genotypes (BM) differentially regulate transcript accumulation in a manner consistent with expression being regulated exclusively by the paternally transmitted allele.

T30

Transcriptome Analysis of Maize Leaf Development Using Illumina Sequencing Techniques

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Maize utilizes C4 photosynthesis to harvest light energy and convert it into simple sugars. This specialized form of photosynthesis is achieved through a partitioning of photosynthetic activities between two cell types (mesophyll and bundle sheath) in the leaf. To develop a comprehensive framework for understanding C4 development in maize, we have cataloged a number of anatomical, physiological and gene expression changes that occur in single seedling leaf that is undergoing the transition from sink to source. This data was used to define a set of 1 cm leaf sections that represent four photosynthetic zones: immature, transition, maturation and mature. Through deep sequencing of transcript pools we have generated over 100 million reads from various leaf segments and are constructing a transcriptome map along this leaf gradient. We will present the preliminary analysis of this data and discus some of the challenges and opportunities for utilizing a systems approach for understanding leaf development.

T31

Genomic responses to a century of phenotypic selection in maize

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Evolutionary changes in mRNA expression have contributed to phenotypic differences among populations. Thus, documenting expression variation in response to artificial selection is likely to reveal genomic targets of directed evolution. The Illinois Protein Strains (IPS) include four related maize populations (Illinois High Protein, Illinois Low Protein, Illinois Reverse High Protein, Illinois Reverse Protein) that have been subjected to 108 cycles of divergent recurrent selection for grain protein concentration. We have surveyed DNA sequence and expression variation in the IPS to identify genes that are likely targets of selection. A genome-wide scan of inbred lines derived from the IPS using 384 SSRs demonstrated expected genetic relationships among the IPS, but did not reveal large blocks of linkage disequilibrium that might indicate selection. Genome-scale RNA expression profiling of leaves and developing seeds from the IPS inbred lines found significant expression variation (FDR<0.05) for nearly 30% of the genes detected in all four IPS. A much smaller subset of commonly detected genes (2%) exhibited expression variation that was highly correlated with grain protein concentration; suggesting these genes are likely targets of artificial selection. Candidate selection targets include the prolamin binding factor, the 19kD and 22kD α-zeins (endosperm storage proteins), an endosperm-specific protease inhibitor, genes involved in nitrogen assimilation within leaves, and genes that participate in the remobilization of nitrogen from leaves to seeds. Allelic variation in DNA sequence and expression for these candidate genes is being further characterized in early and recent cycles of the selection experiment, as well as a population of 500 recombinant inbred lines derived from the cross of IHP and ILP. To date, we have obtained strong evidence that three closely-interacting genes in the key pathway modulating free asparagine levels are targets of artificial selection for plant N accumulation and grain protein concentration. One of these genes is a putative bZIP transcription factor that likely regulates the second gene, asparagine synthetase3. The third gene is an asparaginase, which recycles asparagine to glutamine. Collectively, analysis of the IPS populations defines genetic pathways that contribute to phenotypic differences arising from long-term selection and the evolution of these pathways in response to directed breeding.

T32

Iron biofortification of maize grain

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Mineral nutrient deficiencies are a worldwide problem that is directly correlated with poverty and food insecurity. The most common of these is iron deficiency; more than one-third of the world's population suffers from iron deficiency-induced anemia, 80% of which are in developing countries. The consequences of iron deficiency include increased mortality and morbidity rates, diminished cognitive abilities in children, and reduced labor productivity, which in turn stagnates national development. The developed world has made tremendous success in alleviating nutrient deficiencies through dietary diversification, food product fortification, improved public health care, and supplementation. In developing countries, these strategies are often expensive and difficult to sustain. Poverty is the most common cause for dietary deficiency in developing countries, as consumers' dietary choices are limited as regards the quality, quantity, and diversity of foods consumed. The resource-poor typically consume what they grow and are dependent upon a small number of staple crops for the vast majority of their nutrition. Therefore, genetic improvement of staple crops (biofortification) is the most cost effective and sustainable solution to this global health problem. Here we describe an integrated genetic, physiological and biochemical analysis of iron nutrition in maize grain, to discover the genes and compounds that influence grain iron concentration and bioavailability. Multiple quantitative trait loci (QTL) for each trait have been identified and validated via multi-year and/or multi-location testing. QTL have been isolated in near isogenic lines, which were provided to collaborators in five states for planting in Summer 2008. Efficacy of these QTL in multi-location trials will be discussed, as will progress towards identifying the genetic and environmental factors that determine iron nutritional quality in maize grain.

T33

A First Generation Haplotype Map of the Maize Genome

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We have used next-generation sequencing (NGS) to catalogue DNA variation that occurs within the gene space of 27 diverse maize inbred lines. These 27 inbred lines capture many of the common SNPs in maize and are the parents of the Nested Association Mapping (NAM) population. To exploit the association information in the NAM population, potentially several million SNPs need to be genotyped-by-NGS across the recombinationally active, gene space in the NAM parents. To efficiently access this gene space in the NAM parents, we constructed and sequenced three types of restriction enzyme anchored libraries. A methylation-filtered HpaII library was used to identify SNPs in the unmethylated, genic and low-copy fraction of the highly repetitive maize genome. This library was complemented by skimming the same HpaII recognition sites without regard to methylation, which allowed the identification of SNPs in lines with methylated recognition sites. Finally, a 5-bp enzyme that enriched for a low-copy fraction of the genome was used to complement the HpaII-anchored libraries. Taken together, we have generated 30 Gbp of sequence data across the NAM parents, which provide high coverage for ~100 Mbp of the gene space and lower coverage across a substantial portion of the remainder. We are using these sequences to: (1) score SNPs and small indels across the genome; (2) investigate the evolution of major repetitive element classes; (3) examine the structure of linkage disequilibrium (LD) across the genome and its implications for association mapping; (4) evaluate the relationship between recombination and LD; (5) describe patterns of genetic diversity across the genome; (6) determine the degree of differentiation between tropical and temperate maize; and (7) characterize the genic fraction that is not shared with B73. These results should be an excellent resource for fine mapping projects, association mapping studies, designing arrays, and understanding maize evolution and diversity.

T34

Whole-genome detection of structural variation and genotyping in maize using high-definition array-based Comparative Genomic Hybridization (aCGH)

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To study genome-wide structural variation in maize genome, a high-definition maize whole-genome NimbleGen array containing over two million probes was designed based on the genome data of the maize inbred B73 generated by maize genome sequencing project (http://www.maizesequence.org/overview.html). Total genomic DNA samples of the inbreds B73, Mo17, and two Intermated B73 x Mo17 (IBM) recombinant inbred lines (RILs) were used to perform array-based comparative genomic hybridizations (aCGH) with replicates. Statistical analysis of aCGH data revealed a large amount of structural variation between B73 and Mo17, including copy number variation (CNV) and single feature polymorphism. The distribution of the structural variation across the genome is not random. More than 150,000 non-repetitive probes exhibiting significantly different hybridization signal intensities between B73 and Mo17 (FDR<0.0001 and fold-change>2) were used as aCGH-based informative genetic markers for whole-genome, high-resolution genotyping of two IBM RILs. In addition to showing high consistency with previous PCR-based genotyping results, the aCGH-based genotyping of RILs also identified new crossovers in RILs. These aCGH markers are expected to be useful for map-based cloning of genes and QTLs in maize. We thank the "Maize Genome Sequencing Consortium" for making the sequence available prior to publication.

T35

SNP-chip based genomewide scan for germplasm evaluation and marker-trait association integrated with selective genotyping and pooled DNA analysis

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The combination of selective genotyping and pooled DNA analysis can be effectively used to replace the entire population for genetic mapping of QTL with relatively small effects, as well as for linked and interacting QTL (Xu et al. 2008, 5th International Crop Science Congress). Using diverse germplasm, it is theoretically possible that one 384-well plate could be designed to cover almost all major gene/QTL controlled agronomic traits of importance in a crop species. In CIMMYT, over 2000 maize plants/lines have been collected from genetics and breeding programs worldwide, which represent phenotypic extremes for many traits including drought tolerance, disease and pest resistance, and grain quality. These lines have been genotyped using one of the two 1536-SNP chips developed through collaborations with Illumina Inc. and Cornell University, allowing us for the first time to test the feasibility of large scale germplasm evaluation and a one-step simultaneous marker-trait association analysis for a large number of agronomic traits. A pilot study on genetic diversity has been carried out using a subset of the collection consisting of 188 inbred lines screened with 849 informative SNPs (Lu et al. 2009, this conference). The use of pooled DNA analysis for genetic mapping with Illumina's Golden Gate/Bead Station platform has been successfully applied. As a part of the large-scale genotyping carried out in this study, two target traits, kernel hardness and maize steak virus, have been mapped through pooled DNA analysis (Babu et al. 2009, this conference). Selective genotyping has been successfully used for genetic mapping of head smut and mosaic virus disease resistance and drought tolerance using 235 BILs based on the allelic difference between groups of resistant and susceptible lines (Hao et al. 2009, this conference). A subset of the collection, consisting of 550 inbreds, was screened under both irrigated and drought stress conditions with significant variation identified for drought tolerance at both vegetative and reproductive stages (Xu et al. 2009, this conference). Both linkage mapping and linkage disequilibrium-based marker-trait association for drought tolerance are underway and the results will be presented at the conference.

Poster Abstracts

P1

A New Assay for High Density Microarrays: Targeted, extraction-free measurement of RNA from fresh or FFPE samples

(submitted by David Henderson <davidh@cals.arizona.edu>)
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The quantitative Nuclease Protection Assay (qNPA™) offers advantages over traditional microarray methods:
• qNPA utilizes a lysis-only protocol, requiring no extraction, reverse transcription, or amplification;
• any biological sample can be tested, including fixed (FFPE) tissue; unfixed cells, tissues, etc.;
• any region of the target genes can be probed (no 3' or 5' bias).

qNPA is particularly suited for high-throughput applications such as chemical genomics, drug discovery, agrochemical discovery, and other genetic screening. The Center for Chemical Genomics and Translational Research (CGTR) was established to provide collaborative and fee-based qNPA screening services to academic and industry partners. Here we highlight CGTR research programs aimed at 1) the development of a high-density qNPA platform and 2) discovery of agents to induce plant cells to auto-metabolize wall oligosaccharides for more efficient conversion into ethanol. As a targeted microplate-based assay, qNPA is currently designed to measure expression of four to 16 genes per well; we are expanding the scope to 2,000 genes per well. Our biofuels screen includes approximately 200 Arabidopsis genes (representing 19 families) putatively involved in cell wall degradation.

P2

A New Herbicide Tolerance Trait to Improve Weed Control in Glyphosate Tolerant Corn

(submitted by Tina Kaiser <tmkaiser@dow.com>)
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Dow AgroSciences has developed a synthetic gene (AAD1) that effectively increases selectivity of the phenoxyacetate and aryloxyphenoxypropionate ("fop") herbicides for in-crop use in corn by metabolic detoxification. This gene has been introduced into corn (Zea mays L.). In this presentation, we describe AAD1 trait development in corn, including event characterization at the molecular level from generation to generation and plant performance in the field. The AAD1 gene was introgressed into Dow AgroSciences elite inbreds from selected donor events. Hybrids made from converted AAD1 inbreds express the target protein at levels that effectively protect the corn plant from yield loss due to 2,4-D injury. Protection or tolerance was observed from the early seedling stage to the reproductive stage at application rates exceeding 4 times typical herbicide use rates. In contrast, the check hybrids had 10-20% yield loss due to 2,4-D injury. Based on our data, it is clear that this technology significantly increases the tolerance of corn to 2,4-D herbicide. The demonstrated tolerance increases the versatility of 2,4-D by eliminating application timing restrictions, allowing selective use of 2,4-D with glyphosate to provide increased control of broadleaf weeds in glyphosate tolerant corn and the use of "fop" herbicides for grass weed control.

P3

Aflatoxin accumulation in maize starch mutants

(submitted by Kasey Hames <kahcg2@mizzou.edu>)
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Aspergillus flavus L. is a soil-born fungal pathogen capable of infecting a wide range of agriculturally important plants. A. flavus infection of Zea mays L. is a serious problem in the southern United States because the fungus not only reduces the quality of harvestable maize, but it also has the ability to produce the secondary metabolite aflatoxin, a potent carcinogen in humans, poultry and livestock. Aflatoxin accumulation in maize is a quantitative trait impacted by a variety of biotic and abiotic factors. One factor of interest is the availability of readily metabolized carbon sources in infected kernels because maize lines with high percentages of starch have been shown to consistently accumulate levels of aflatoxin above the contamination levels set by the federal government. To investigate how changes in kernel starch composition will affect aflatoxin accumulation levels, fifteen starch mutants with a W64A (high aflatoxin accumulating) background were selected and planted over a two year period in Columbia, Missouri. Ears were inoculated nineteen days after pollination with A. flavus isolate NRLL 3357 and harvested at maturity. After the kernels were dried, shelled and bulked, they were ground into flour. An Enzyme-Linked ImmunoSorbent Assay (ELISA) was performed to determine aflatoxin content. Preliminaryresults indicate that several maize starch mutants have significantly higher aflatoxin accumulation levels than W64A with sugary mutants su1-st and su2 being the highest. This research was supported by MU Mission Enhancement funds.

P4

Altered auxin dynamics in the *abph1* embryo SAM contribute to aberrant phyllotaxy

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The maize plant exhibits a very ordered pattern of growth. Leaves of the main shoot axis are initiated singly in an alternate pattern, generating a distichous phyllotaxy. *aberrant phyllotaxy1* (*abph1*) mutants have an altered phyllotaxy – leaves are initiated in pairs in a decussate pattern, rather than the normal distichous pattern. This has been attributed to the enlarged shoot apical meristems (SAMs) of *abph1* mutants (Jackson and Hake, 1999). *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). There is a body of evidence supporting the involvement of auxin and its polar transport in the regulation of phyllotaxy. Polar auxin transport is facilitated by members of the PIN1 family of auxin transport proteins. Recent data indicate that PIN1 expression and auxin levels are reduced in the SAMs of *abph1* mutants. To investigate this interaction further, we observed expression of a ZmPIN1a-YFP fluorescent fusion protein in normal and *abph1* embryos. We found that ZmPIN1a-YFP expression is reduced in the SAMs of *abph1* embryos during early development. In addition, the spatial pattern of auxin accumulation is altered. We propose that reduced auxin levels and PIN1 expression in *abph1* mutant SAMs delays leaf initiation, contributing to the enlarged SAM and altered phyllotaxy of these mutants.

P5

Association of enzymes from amino acid- and carbohydrate biosynthetic pathways in multisubunit complexes: A model for regulation of carbon allocation in maize grain

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Protein and starch deposition during maize grain filling represents the culmination of the plant's carbon and nitrogen storage allocation in this high yielding crop. Understanding this process is essential for optimal utilization of maize, because it determines functional features including yield, amino acid distribution, protein content, and starch content. This study advanced our understanding of how the division of carbon flow between amino acid- and starch biosynthesis may be regulated. Biochemical analyses revealed that essentially all starch synthase III (SSIII) exists in a complex of ~670 kDa, and nearly all SSIIa is in a high molecular weight form of ~300 kDa. Mutations of dull1 or sugary2, encoding SSIII or SSIIa, respectively, cause major changes in grain properties, supporting the view that these complexes likely are functionally significant in grain filling. Proteomic analyses revealed that the complexes also include starch branching enzymes SBEIIa and SBEIIb, large and small subunits of ADPglucose pyrophosphorylase (AGPase), the sucrose synthase isoform SUS-SH1, and two pyruvate orthophosphate dikinase isoforms (PPDK). Genetic analyses demonstrated assembly interdependence. For example, high molecular weight complexes of AGPase, PPDK, and SUS-SH1 are missing in dull1- mutants lacking SSIII. Thus, all these enzymes exist together in the same complexes. Co-immunoprecipitation revealed that phosphorylation of one or more components is necessary for stable association of PPDK, SBEIIa, SBEIIb, and SSIIa with SSIII. Recently other workers have proposed that PPDK may function to divert carbon from sucrose into amino acids rather than starch, by affecting the equilibrium of the AGPase reaction. Finding that PPDK and AGPase exist together in a single starch synthetic assembly within the amyloplast suggests a mechanism of this regulation. We propose that these enzyme complexes function as a regulatory valve mechanism that controls carbon flow into starch- or amino acid/protein synthesis, thus determining critical functions of maize grain composition.

P6

Atypical response regulators expressed in the maize endosperm link canonical two component systems to transfer cell differentiation and function

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Two component signal transduction systems (TCS) were first described in plants in 1996 and rapidly ascribed to the signaling of a number of external outputs, including phosphate and nitrogen availability, usually through hormonal compounds, mostly citokinins. The full TCS structure in plants involves the perception of an external signal through membrane-bound histidine kinases, and subsequent downward transfer of a phosphate group, first to an intermediate phosphotransfer protein, then to a response regulator (RR). In most cases, the RR is a transcription factor (Type B RR), which after phosphorilation changes its transcriptional activity and induces or represses target genes. Among the targets, type A RR are negative modulators of the signaling by TCS, they act competing for the phosphate group with the type B RR that induced their transcription. In 2006 we reported the identification of a type A RR, ZmTCRR-1, with some unusual features: i) It is expressed at a single tissue, the maize endosperm transfer cells. ii) It is regulated by a myb-related protein that is not a type B RR. iii) The protein accumulates in cells that do not transcribe the gene, suggesting trans-cell signaling. In order to identify additional components of this TCS pathway, we are: A) examining the expression of His-kinases and phosphotransfer proteins in developing kernels, B) investigating the localization of the phosphotransfer proteins within the kernels and testing their interactions with ZmTCRR-1 in yeast two-hybrid assays.

P7

Biosynthesis of Multiple Flavonoid Compounds in Maize through the Action of a Flavonoid 3'-Hydroxylase

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The phenylpropanoid pathway in plants leads to the synthesis of several flavonoid end products that include anthocyanin and phlobaphene pigments in maize. Flavonoid 3'-hydroxylase (F3'H) plays a key role in generating flavonoid pigment diversity by 3'-hydroxylation of B-ring. In maize, mutations in the pr1 locus lead to the accumulation of pelargonidin (red) as oppose to cyanidin (purple) pigments in the aleurone cells. We have isolated a putative maize f3′h (Zmf3′h1) gene. Maize populations segregating for pr1 and Pr1 were developed and a genetic ratio of 3:1 was observed for Pr1:pr1. Genetic mapping of the Zmf3′h1 gene confirms the previously known map position of the pr1 locus on chromosome 5L. Further, genetic complementation experiments using CaMv 35S::F3'H1 gene construct established that the putative protein product is capable of performing 3'-hydroxylation reaction both in vitro and in vivo. Transcripts of Zmf3′h1 were detected in floral as well as vegetative tissues of Pr1 plants. On the other hand, pr1 plants did not show any detectable level of Zmf3′h1 mRNA indicating that the pr1 allele used here had a defect that affects transcription of the gene. The defect was identified as an insertion of dinucleotide repeats in upstream promoter region. Further, we show here that Zmf3′h1 is under the independent transcriptional control of both MYB and MYC types of transcription factors that regulate anthocyanins, phlobaphenes, and C-glycosyl flavones accumulation in maize. The expression of Zmf3′h1 was also required for the biosynthesis of luteoforol, one of the flavan-4-ols that is precursor of the phlobaphenes. In addition, we show that pr1 plays a role in biosynthesis of maysin and 3-deoxyanthocyanidins. Maysin and 3-deoxyanthocyanidins have insecticidal and antimicrobial properties, respectively.

P8

Camouflage patterning in maize leaves results from a defect in porphobilinogen deaminase

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Maize leaves are produced from highly polarized cell divisions that result in clonal cell lineages arrayed along the long axis of the leaf. We utilized this stereotypical division pattern to identify a collection of mutants that form pigmented sectors which violate the clonal cell lineages. The *camouflage1 (cf1)* mutant develops nonclonal, yellow-green sectors in its leaves. We cloned *Cf1* by transposon tagging and determined that it encodes porphobilinogen deaminase (PBGD), an enzyme that functions early in chlorophyll and heme biosynthesis. While the biochemical function of PBGD has been characterized, no viable mutations in this gene have been reported in plants. To investigate the in vivo function of PBGD, we characterized the *cf1* mutant. Histological analyses revealed that *cf1* yellow sectors display the novel phenotype of bundle sheath cell-specific death. Light-shift experiments determined that constant light suppressed *cf1* sector formation, a dark/light transition is required to induce yellow sectors, and that sectors form only during a limited time of leaf development. Biochemical experiments determined that *cf1* mutant leaves have decreased PBGD activity and increased levels of the enzyme substrate in both green and yellow regions. Furthermore, the *cf1* yellow region showed a reduced catalase activity level. A model incorporating photosynthetic cell differentiation and PBGD function is discussed.

P9

Characterization of epigenetic states through heritable changes in MuDR element

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The Mutator family of transposable elements in maize has proved to be an excellent model system for understanding epigenetic silencing in plants. Although the Mutator system can be highly complex, we have derived a line that carries a single regulatory MuDR element and a single reporter element. A rearrangement of that single element resulted in Muk, a regulatory transposon that can heritably epigenetically silence one or many MuDR elements. Muk expresses a long hairpin RNA that is processed into siRNAs that trigger methylation of otherwise active MuDR elements. This methylation, and consequent transcriptional gene silencing persists over multiple generations even after the loss of Muk via genetic segregation. Using this system, it has been possible to define a number of distinct epigenetic states that can change over time, and that can be altered by mutations in maize RNA-dependent RNA polymerase 2 (Mop1) and a nucleosome chaperone (Nap1), treatment with 5-azacytosine, exposure to functional transposase and changes in chromosomal position of the target MuDR element. These factors can have distinct effects on the initiation, maintenance and heritability of silenced states. The phenomenology of transposon silencing suggests that epigenetic states can be far more subtle that simply on or off. Rather, they are a dynamic component of the genome that can change over time and that are influenced by local sequence context.

Recent studies indicate intriguing links among chromatin remodeling, histone methylation, DNA methylation and RNA interference. In plants, de novo methylation of cytosines in all contexts, as well as maintenance of CG and non-CG sites requires distinct factors. Epigenetic silencing is also associated with specific modification of histones, such as the methylation of histone H3 at lysine 9 (H3K9Me). Using our MuDR system, it is possible to correlate specific patterns of DNA methylation or histone modification with a variety of genetically defined epigenetic states in order to better define the meaning of those patterns.

P10

Dosage-dependent genes affecting seed composition or weight.

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Kernel composition is an important target for developing improved grain for food, feeds, and industrial processes. Our goal is to identify maize mutants that have significant effects on the chemical composition or weight of seeds. We are focusing 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. We are screening the UniformMu transposon tagging population using single kernel near-infrared spectroscopy (NIR) and seed weights. NIR spectroscopy is an analytical technique that reports seed composition without destroying the seed. We built a semi-automated grain analyzer that collects NIR spectral data and seed weights from individual seeds. We collected data from >4,000 ears and are screening for putative mutants with dosage- and parent-of-origin effects. We selected 89 families from the first 1,700 ears screened for further genetic analysis. The seed weight or NIR classes were separated to determine if seed phenotypes predict heterozygous individuals. Preliminary results from these heritability tests will be presented. In parallel, we sequenced transposon insertion sites from a subset of the UniformMu lines in our seed composition screen using 454 next generation sequencing technology. These transposon flanking sequence tags (FSTs) identified approximately 6,000 insertion sites when the redundancy of the genome sequence tiling path is taken into account. We demonstrate that insertions co-segregating for seed mutant phenotypes can be identified from the FST library.

P11

Heterologous expression of a sorghum Myb transcription factor in maize

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Maize (Zea mays) is one of the most important cereal crops in the world and disease epidemics can be devastating, threatening our food supply and negatively affecting a wide range of industries. Leaf blight is one of the main foliar fungal diseases of maize. The disease inoculum survives in the field debris and can repeatedly infect the maize plant at any stage during its life cycle. Although disease incidence can be reduced by tilling and rotating with non-cereal crops, identification of resistant germplasm is more promising. Unfortunately, most of the maize germplasm screened to date is susceptible to the disease. Sorghum bicolor, a close relative of maize produces a class of compounds (3-deoxyanthocyanidins) in response to fungal attack. These compounds have antifungal properties and confer resistance to leaf blight and biosynthesis of these compounds is regulated through the flavonoid pathway. Although maize produces numerous flavonoid compounds, it does not produce 3-deoxyanthocyanidins in response to fungal attack. We have identified candidate genes and transformed maize in an effort to engineer the biosynthesis of these antifungal compounds and thereby enhance resistance to leaf blight. We have also identified important cis-regulatory elements in promoter of one of the Myb genes. Herein we characterize the response of transgenic maize lines when infected with the fungus that causes southern corn leaf blight.

P12

Id1 regulates the expression of the tdy1 carbohydrate accumulation phenotype

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The genetic regulation of carbon partitioning between leaves and other parts of the plant, such as roots and reproductive structures, plays a critical role in growth and development, but remains a poorly understood process. The tie-dyed1 (tdy1) mutation of maize accumulates excessive levels of carbohydrates in nonclonal, chlorotic leaf regions, indicating that Tdy1 functions in carbon partitioning. Tdy1 is expressed in phloem tissue and encodes a novel, transmembrane protein localized to the endoplasmic reticulum. Preliminary studies of the indeterminate1 (id1) mutation shows Id1 may also be involved in regulating carbohydrate partitioning in leaves. Relative to wild type, id1 leaves show a moderate increase in both starch and soluble sugars, but at levels considerably lower than seen in tdy1 chlorotic tissues. Id1 encodes a zinc finger transcription factor expressed in maturing leaves and is required to promote flowering under long day conditions (Colasanti et al., 1998; Kozaki et al., 2004). In order to determine whether Id1 and Tdy1 genetically interact to regulate carbohydrate partitioning in leaves, we performed a double mutant analysis. Surprisingly, tdy1; id1 double mutant plants resemble id1 single mutants, indicating that id1 is epistatic to tdy1. Double mutants produce an inflorescence only under short day conditions and apparently lack strongly chlorotic regions, showing an absence of the tdy1 variegation phenotype. However, they are slower to flower than id1 mutants, shorter in stature and slightly paler in coloration. Together, these results suggest id1 largely suppresses the tdy1 mutant phenotype. Microscopy studies support these observations. As seen with IKI staining, starch accumulation in tdy1; id1 double mutant leaves is slightly greater than in id1 leaves, but considerably less than in tdy1 chlorotic tissue. In addition, the overall paler coloration suggests a suppression in the severity of the tdy1 chlorosis phenotype but an enhancement in the chlorotic leaf area. Consistent with this, carbohydrate measurements show that tdy1; id1 leaves display slightly higher starch and sucrose levels than id1, but hexose levels similar to tdy1 chlorotic regions. A model discussing these data will be presented.

P13

Ionomic Profiling of NAM RIL Populations

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Plants have adapted to soil with drastically different ionic compositions. To understand how plants have accomplished this, we will need to uncover the plants' physiological responses to the soil environment and the genes that implement these responses. To accomplish this we have employed high-throughput mineral nutrient and trace element profiling coupled with genetics to determine the connections between a plant's genome, and elemental profile, or 'ionome.' To date we have analyzed the shoot ionome of over 100,000 Arabidopsis plants, over 60,000 yeast samples, and thousands of rice and Maize grains, and the data are curated in a publicly searchable online database (www.ionomicshub.org). We have combined these techniques with QTL analysis and association mapping to identify genes important for elemental accumulation in Arabidopsis. We recently extended this approach to Maize kernels using the nested association mapping population. We have analyzed kernels from the NAM parents and five of the NAM RIL populations. We identified over 130 QTLs for 16 elements, including 19 QTLs that were found in more than one population. A strong QTL for Molybdenum accumulation was identified on Chr. 1 in 4 of the 5 populations and co-localized with a Molybdenum transporter.

P14

Multiple Stress Dose Response Comparison of Two Genotypes

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Combinations of stress factors normally occur in the field, and both crop stress and general environmental ecological data suggest that combination stress effects are not easy to predict from single stress experimental analyses. Stress-combination differences may also explain why correlation of specific climate variables with QTL across environments has been difficult. In this study, the difference in compounded UV/drought stress response between B73 and MO77 maize genotypes was tested. The two genotypes, B73 and the IBM RI line MO77, were divided into test groups: a control group, groups with varying levels of compounded UV and drought, a group with UV but no drought, and a group with drought but no UV. Stress was applied to the plants in a week long trial, and then differences in plant height before and after stress treatments were measured. Total shoot mass and total root biomass were also measured after the stress treatment. An ANOVA test was conducted on the three experimental variables. Response surfaces were fit in order to compare the stress reaction of the two genotypes to one another. This comparison will allow us to select a suitable single drought and UV combination dose for QTL mapping.

P15

Next Generation Sequencing with Restriction-enzyme Reduced Representation Libraries

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Efficient SNP discovery via next generation sequencing in maize requires reduction in the complexity of the libraries to reduce repeated sequencing of non-genic regions. In the work to produce the maize hap-map, we resequenced the 27 founders of the nested association mapping (NAM) population on the Illumina GA using three sets of libraries designed with efficiency in mind. The first library was constructed using HpaII which cuts at an unmethylated 4 -base recognition site. HpaII sites in genes are hypomethylated compared to repetitive sequences. Selection of cut fragments enriches for genic regions in the libraries. The second library was also prepared using HpaII, but on whole genome amplified DNA, which is unmethylated. This library allowed us to fill in areas that were differentially methylated in the 27 lines. The third was prepared using BbvI a type IIS restriction enzyme. This enzyme was chosen based on the frequency of recognition sites in genic vs. non-genic regions identified via an in silico digest of the maize genome. This library provides a different sampling of the genic regions than the HpaII libraries. Numerous Illumina runs with these libraries provide a robust data set from which to call SNPs. An analysis of these libraries, including relative importance of repeat classes, cut site frequency, library quality, depth of coverage and current SNP calling pipeline are presented.

P16

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-scale projects (Entrez Genome Projects), 2) datamining of probes (Entrez Probes), 3) datamining of primer sequences (UniSTS), 4) datamining of gene information (Entrez Gene), 5) viewing genome units (MapViewer) and 6) genome-scale services such as whole-genome annotation (Gnomon). 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 40,000 ESTs.
Examples demonstrating use of the developing capabilities of the genomic resources for plants at NCBI will be presented.

P17

Production of Self-Assembling Autofluorescent Protein (SASAP) Microarrays and Their Application to Phytochrome Research

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This project comprises a series of proof-of-concept experiments primarily using Arabidopsis thaliana as the experimental organism. The project is based on the recent description, which we have implemented in our laboratories, of the production of protein microarrays via a process of in situ biosynthesis. This involves spotting onto microscope slides different recombinant DNA constructs encoding the proteins of interest fused to an epitope tag. Mixed with the DNA, and spotted at the same time, is an antibody directed against the epitope. After spotting and immobilizing the mixed DNA/antibody mixture, the microarrays are covered in transcription/translation mix, which produces epitope-tagged proteins from the information encoded in the DNA molecules, which are then captured by the immobilized anti-epitope antibodies. The resultant protein microarrays are employed for a variety of novel downstream assays, which we have devised to examine protein-protein interactions, protein-DNA interactions, covalent modification of protein elements, and mass-spectrometric characterization of the protein elements. These assays are validated by judicious choice of positive and negative controls, and will be extended to address specific biological questions of interest to the plant research community. Below is presented our progress towards the specific goals of optimizing microarray production, developing the MS-based methods for protein identification, and developing high throughput methods for measurement of protein-DNA, protein-protein, and protein-substrate interactions.

P18

Profiling conservation and changes in resident bacterial and fungal endophytes in maize seeds during domestication and diversification in the Americas

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All organisms appear to have resident microbes (endophytes). In plants, endophytes may have beneficial properties including nitrogen fixation, improved nutrient solubilization, and act as biological control agents. We hypothesized that important ecological relationships may have been lost or gained during the domestication and movement of modern maize. We have been profiling microbial communities transmitted through corn and teosinte seed as well as in Mexican landraces and temperate genotypes. We have thus far identified by 16S TRFLP a total of 78 different bacterial endophytes in seeds of 3 temperate corn genotypes, 5 Mexican landraces, and 4 teosintes. By plating seed homogenate on R2A media, we have cultured a total of 77 bacterial strains, and 8 fungal types from seed. These are being evaluated for their ability to grow on nitrogen free media, antagonize pathogens, solubilize phosphate, produce plant hormones, and stimulate plant growth. We have identified maize kernel-associated endophytes that are conserved across all 13 evolutionary and geographic accessions tested, as well as endophytes that appear to be unique to certain genotypes.

P19

QTL Mapping a High-Lutein Phenotype in Yellow Dent Inbred Lines

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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-ε-cyclase (LYC-E) 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 these 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. This population, a testcross to the lower lutein parent, shows transgressive segregation in lutein accumulation indicating that the trait is multi-genic. The hypothesized mechanism is that lycopene-β-cyclase, as well as lycopene-ε-cyclase, is involved in determining this phenotype.

P20

Redundant Function of Gamma and Beta Zeins in Stabilization of Protein Body Formation

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Maize (Zea mays L.) main storage proteins, known as zeins, consist of a super family, falling into four groups based on their structure (alpha, beta, gamma, and delta zeins). Their genes are coordinately expressed ten days after pollination and the proteins are translocated into the lumen of the rough endoplasmatic reticulum, where they are assembled into protein bodies. Previous studies show that gamma and beta zeins are the protein body initiators and alpha and delta zeins are the protein body fillers. Neither alpha nor delta zeins could accumulate at high levels, when expressed in heterologous hosts, unless they are co-expressed with gamma or beta zeins. Taking advantage of RNA interference (RNAi), we successfully silenced the expression of 27-kDa and16-kDa gamma zeins, and 15-kDa beta zeins. In each RNAi transgenic seed, SDS-PAGE analyses showed that the accumulations of alpha and delta zeins were not affected, indicating the redundant function of gamma and beta zeins in stabilization of protein body formation. Although RNAi of 27-kDa and 16-kDa gamma zeins resulted in nearly 20% lower total zein, kernels failed to produce an opaque phenotype. On the other hand, RNAi of 22-kDa alpha zeins, which reduces total zeins by a similar amount, gives rise to an opaque phenotype, implicating different roles in protein-protein interactions.

P21

Regulation of Lignin Metabolism in Maize

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The production of lignin in cell walls is essential for the strengthening of land plants but presents an impediment to the production of cellulosic ethanol from leaf and stem tissues. Much work has focused on elucidating the enzymatic steps involved in lignin metabolism and more recently there has been interest on elucidating the regulation of these genes. Two MYB factors have been linked to the negative regulation of lignin biosynthetic pathway (ZmMYB31 and ZmMYB42). Here we provide a comparative genomics survey of these and other regulatory factors linked to lignin and phenylpropanoid metabolism in maize and related grasses including rice and sorghum. We further report on the overexpression of both the DNA binding and unique domains of these proteins and their use in the identification of the cis-regulatory elements both in vitro (by SELEX assay) and in vivo (by ChIP-Seq assay).

P22

Restoring the communication between maize roots and insect-killing nematodes improves control of the maize pest Diabrotica virgifera virgifera

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When attacked by herbivorous insects, plants emit volatiles that attract natural enemies of the insects. Here we demonstrate for the first time that such volatile signals can be manipulated to improve crop protection. Roots of many maize varieties emit (E)-β-caryophyllene in response to feeding by the western corn rootworm, Diabrotica virgifera virgifera,, which attracts entomopathogenic nematodes that infect and kill this root pest. However, most North American maize varieties have lost the ability to emit (E)-β-caryophyllene and may therefore receive little protection from the nematode. To restore nematode attraction, a maize line that does not emit (E)-β-caryophyllene was transformed with a (E)-β-caryophyllene synthase gene, resulting in constitutive emissions of this sesquiterpene. In D. v. virgifera-infested field plots in which nematodes were released, transformed plants received significantly less root damage and had 60% fewer adult beetles emerge than control lines. This demonstration that plant volatile emissions can be manipulated to enhance the effectiveness of biological control agents opens the way for novel and ecologically sound strategies to fight crop pests.

P23

Sequence indexing of Mu transposons in the UniformMu

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We have developed new methods that enable efficient sequence-indexing of high-copy transposon insertions in the maize genome based on Next-Generation sequencing technologies. Our approach includes several key features. 1) Unbiased representation of Mu insertions in the genome is ensured by preparation of template from randomly sheared genomic DNA fragments. 2) A series of nested primer-extension and PCR reactions result in very high fidelity single-stranded template containing less than one part per thousand non-specific amplification products. 3) Multiplexing of up to 64 samples per sequencing reaction on the 454-instrument is enabled by incorporation of a 4-base error-detecting barcode sequence. High-throughput mapping of the insertions in the B73 genome is performed by parallel BLASTN implemented on the high-performance computing grid. We have applied this method to indexing UniformMu maize lines arrayed in 24 X 24 2D grids as well as pooled DNA samples of 48 lines. The accuracy of the axis assignments within the grid were verified by PCR analysis of a large sample of insertions. Large scale confirmation of germinal inheritance of insertions was obtained by analyzing and comparing insertions in F2 and F3 derived lines. The demonstrated accuracy of the method has enabled implementation of higher-dimensional (4D) pooling strategies that substantially reduce the costs of library preparation. Indexing of 4D arrays representing 8000 lines is currently in progress.

P24

Sucrose transporter1 functions in phloem loading in maize leaves

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In most plants, sucrose is exported from source leaves to carbon-importing sink tissues to sustain their growth and metabolism. Apoplastic phloem loading species require sucrose transporters (SUTs) to transport sucrose into the phloem. In multiple dicot plants, genetic and biochemical evidence has established that SUT1-type proteins function in phloem loading. However, the role of SUT1 in phloem loading in monocot plants is not clear since the rice (Oryza sativa) and sugarcane (Saccharum hybrid) SUT1 orthologs do not appear to function in phloem loading of sucrose. A SUT1 gene was previously cloned from maize (Zea mays) and shown to have expression and biochemical activity consistent with a hypothesized role in phloem loading. To determine whether SUT1 is essential for phloem loading in maize, a sut1 mutant was isolated and characterized. sut1 mutant plants hyperaccumulate carbohydrates, display leaf chlorosis, reduced rates of photosynthesis, and premature senescence. In addition, sut1 mutants have greatly reduced stature, altered biomass partitioning, delayed flowering and stunted tassel development. Cold-girdling wild-type leaves to block phloem transport phenocopied the sut1 mutants, supporting a role for maize SUT1 in sucrose export. Interestingly, both sut1 mutants and cold-girdled plants displayed the novel phenotype of secreting soluble sugars from the hydathodes on the leaf margins or from wounds on the leaf surface. Furthermore, application of 14C-sucrose to abraded sut1 mutant and wild-type leaves showed that sucrose export was greatly diminished in sut1 mutants compared to wild type. Collectively, these data demonstrate that SUT1 is crucial for efficient phloem loading of sucrose in maize leaves.

P25

TRANSCRIPTIONAL REGULATION OF VP1 EXPRESSION IN MAIZE

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Seed maturation and dormancy are important for stand establishment, yield, seed quality and longevity in crops. Several plant hormones are key regulators of seed maturation and the dormancy-germination transition. During the maturation phase of seed development, abscisic acid (ABA) regulates the expression of genes, including a transcription factor network, which promotes maturation and represses seed germination. The Viviparous1 (vp1) gene, encoding a B3 domain-containing transcription factor, is an important mediator between the seed maturation and germination programs in maize and is a key regulator in the response to ABA. To further understand the regulation of seed maturation, we are examining the transcriptional regulation of Vp1. A 958-base Vp1 promoter fragment that confers proper expression to a GUS reporter was identified. Five oligonucleotides of the promoter region have been identified that specifically bind embryo nuclear proteins in electrophoretic mobility shift assays. Using yeast one-hybrid screens, we have identified S13. S13 binds to the CE1L element of the VP1 promoter and appears to be cereal specific. S13 is targeted to the nucleus and is a Zn2+ binding protein. Functional assays to understand the role of these proteins in the transcriptional regulation of VP1 are currently ongoing.

P26

The Design, Engineering, and Application of Whole-Cell Biosensors for Quantifying Maize Glutamine and Soil Nitrate

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We are interested in engineering new tools for scientists and extension specialists interested in improving nitrogen use efficiency in maize. In maize and other organisms, glutamine is the first amino acid to be synthesized following nitrogen uptake, and it is then used in the biosynthesis of other amino acids. Glutamine also serves as a signalling molecule in maize and other plants, serving to regulate and communicate the nitrogen status of the plant, including nitrogen uptake and remobilization. Glutamine is thus a useful amino acid to quantify in plant tissues. There are several ways in which to measure free glutamine including HPLC and GC-MS, however most of the currently available methods have the disadvantages of being expensive, time consuming, requiring extensive sample preparation and some level of technical expertise. To overcome these disadvantages, we have created a whole-cell glutamine biosensor. This biosensor was generated by transforming a glutamine auxotrophic strain of E. coli with a plasmid that contains a constitutively active Lux operon. In the presence of exogenous glutamine, the biosensor cells multiply and emit photons of light, detectable using a luminometer. We have optimized the biosensor to improve sensitivity, linearity, specificity and robustness of detection with maize tissue extracts. The use of this biosensor has the potential to allow free glutamine to be measured in multiple plant tissue samples quickly and at low cost to facilitate high-throughput studies. In addition to the glutamine biosensor we are engineering a whole-cell biosensor for the detection and quantification of nitrate in soil samples. This biosensor is being created using laboratory directed evolution of promoters that are responsive to nitrate, and a combinatorial design strategy to create biosensors which are responsive to different concentration ranges of nitrate. We will discuss how these biosensors may be used for laboratory and field applications with maize.

P27

The sdh1 mutant alters kernel sugar composition and transcript profiles in addition to its small-kernel phenotype.

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Sorbitol dehydrogenase (SDH) in maize kernels could mediate a centrally-positioned, second-step in metabolism of imported sucrose; that fructose use (fructose + NADH <-> sorbitol + NAD). This is significant because fructose is produced regardless of how sucrose is cleaved (via invertase or sucrose synthase), and sorbitol dehydrogenase provides a potentially advantageous alternative to other paths of fructose use and sensing. To help test hypotheses for the significance of SDH in maize, we identified an sdh1 mutant, as noted earlier, by screening the UniformMu maize population. The sdh1 mutation reduced maximal SDH activity in developing kernels to less than 6% of wild- type levels. The resulting phenotype was a 21% smaller kernel under field conditions (dried-seed weight at maturity, significant to p<.001). In the present work, a 3'-UTR profiling strategy was used to generate gene-specific, sequence-based expression profiles for tissues of wild-type and sdh1 kernels. These tissues included pedicel + transfer region, embryo, embryo proximal region, peripheral endosperm, and pericarp. The 454-transcript profiles identified altered expression of genes for specific metabolic pathways between regions within kernels, and between these zones in wild-type and sdh1 mutants. A decrease was observed in the expression of the genes involved in the putative sorbitol pathway. Metabolic analyses also showed that the sdh1 mutation 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. In addition, sdh1 decreased mannose levels in the kernel, and levels of arabinose and xylose in the pericarp. The starch content was decreased in the kernel, but no difference was observed in the protein content. These results demonstrate multiple effects of sdh1 mutation in the maize kernel and importance of the sorbitol pathaway in metabolic and physical flux of carbon in developing maize kernels.

P28

Transcriptional profiling of *Aspergillus flavus* infected maize kernels.

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Maize kernels are susceptible to infection by the opportunistic pathogen *Aspergillus flavus*. Infection results in a reduction of grain quality and contamination of kernels with the highly carcinogenic mycotoxin, aflatoxin. To gain a better understanding of the host response to infection by the fungus, transcription of approximately 9,000 maize genes were monitored during the host-pathogen interaction. RNA was extracted 4 days after infection of kernels at the developmental stages of blister, milk, dough, and dent, and hybridized to custom made Affymetrix GeneChip DNA microarrays. Analysis of these microarrays revealed a complex transcriptional response by maize to infection. Responses included the up regulation of over 1,000 maize genes with a fold change of two or greater. Included in this response was increased expression of genes coding for proteins characteristic of plant defense such as, but not limited to, Pathogenesis-related (PR) proteins, a xylenase inhibitor, and ethylene biosynthetic enzymes. In addition, other transcripts of interest found differentially expressed in the interaction were those associated with the basal endosperm transfer layer, BETL-4 and the cell wall invertase *miniature seed 1*. Additional experiments are being performed using a beta-glucuronidase- expressing *A. flavus* strain, as well as, histological stains to visualize fungal colonization within the kernel. Combined, these data give insight into maize defense mechanisms used against the opportunistic pathogen *A. flavus* and may lead to the development of control strategies for this disease.

P29

psychedelic, a new locus controlling carbohydrate partitioning in maize leaves

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Carbohydrate transport and allocation are essential to all aspects of plant growth, development and metabolism. Carbohydrate partitioning entails the fixation of carbon in the photosynthetic source tissues of the plant, followed by carbon translocation through the phloem to the non-photosynthetic sink tissues. Although this process has been studied at the physiological and biochemical levels, little is known about the genes that regulate this process. To identify genetic regulators of carbohydrate partitioning, we have identified mutants that are defective in carbon allocation, in particular, in the export of fixed carbon from photosynthetic leaf tissue. Here we describe a new recessive mutant, psychedelic (psc), which displays stable, non-clonal yellow and green sectors within the leaf. Yellow psc sectors hyperaccumulate starch and soluble sugars, but green sectors appear comparable to wild-type tissue. Lateral and intermediate veins (classes of veins where hypodermal sclerenchyma are present) usually delineate sector boundaries, suggesting that there may be a mobile signal involved in sector formation. psc mutants only produce yellow sectors when grown under high-light regimes, but are indistinguishable from wild-type plants when grown under low light. Furthermore, psc mutants display a reduction in plant height, leaf number, tassel size and branching, and kernel number and weight. This altered biomass partitioning is hypothesized to be a downstream effect due to carbohydrate retention in the yellow sectors leading to reduced export from leaves to developing sink tissues. Using molecular markers, we found that the psc mutation is unlinked to all previously characterized carbohydrate hyperaccumulation mutants. Investigation into the functional role of psc in maize will further our understanding of the genetic control of carbohydrate partitioning in plants.

P30

Gramene: A Resource for Comparative Grass Genomics

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Gramene (www.gramene.org) is a curated, open-source, data resource for comparative genome analysis in the plant kingdom, with emphasis on the grasses. Gramene is comprised of database modules that integrate publicly-available information about genomic sequence, genes, proteins, biochemical pathways, maps and markers, QTL, and genetic and phenotypic diversity. Ontologies (controlled vocabularies) are used to associate the different types of data. Maize researchers and breeders can take advantage of known microsynteny between the different species of the grass family by using maps and genomic sequence as a reference point for gene and marker discovery in maize.

Gramene releases data updates and feature improvements on a semi-annual basis. Online tutorials and help documents provide users with an overview of how to conduct a wide variety of operations on the database. Gramene is a collaborative effort between Cold Spring Harbor Laboratory, the Department of Plant Breeding and Genetics at Cornell University and various national and international projects dedicated to cereal genomics and genetics research.

P31

Development of Bioinformatics Tools to Process Next Generation Sequence Data

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High-throughput next generation sequencing such as Illumina and 454 systems produce large number of short reads. For example, one Illumina run produces approximately 50 million reads that are each about 50 base pairs in length. To fully utilize these resources for SNP discovery and validation using chemistries such as Illumina's GoldenGate assay or Taqman assay, the short reads can be extended by aligning them to a reference sequence such as that of maize inbred line B73 and recovering the flanking regions. In this study, a bioinformatics pipeline was developed to facilitate this task. The short sequences were first BLASTed against the NCBI maize high-throughput genomic sequences database. The sequence of the best match bacterial artificial chromosome (BAC) clone was retrieved. Using an internally developed bioinformatics approach, we were able to identify 100 bp of flanking sequence from both sides of the identified SNP. The results demonstrated the usefulness of bioinformatics tools, both publicly available and custom developed, which has tremendously increased the efficiency and accuracy of our sequence extension study and accelerated our SNP discovery and development process.

P32

MaizeGDB: Web Interface and New Features

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MaizeGDB (http://www.maizegdb.org) is the research community's database for maize genetics and genomics. Here I present the latest updates to the MaizeGDB web interface organized into four major areas: new features, upgraded features, newly integrated web pages, and upcoming work. One new feature is a Locus Lookup tool that allows researchers to define a genomic window within the MaizeGDB Genome Browser that should contain a given locus for which only genetic map information is known. Two new tools have been upgraded: the BLAST tool is now integrated with the Genome Browser and the Annotation Manager has been redesigned to allow researchers to more easily add notes to MaizeGDB records. To allow for a more sequence-centric focus, the following MaizeGDB web pages have been integrated with the Genome Browser: Locus, FPC Contig, Bin, BACs, Sequence, EST, and cDNAs. Upcoming work will focus on creating a new overall look and feel, improving speed and usability, enhancing the accessibility of our data and tools, and expanding the search capabilities at MaizeGDB.

P33

How to use MaizeGDB

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The MaizeGDB team is committed to providing easy access to maize data and to be responsive to the requests of the maize community (http://www.maizegdb.org/). The website has many new features this year, and here we describe how to use them. We now offer short movie tutorials (http://www.maizegdb.org/tutorial/index.php) on many aspects of the database. You can request a custom movie, phone call help, or an institutional visit by emailing Lisa Harper at ligule@nature.berkeley.edu or calling at 510-559-5629.

Our major new feature is a GBrowse-based Genome Browser (http://www.maizegdb.org/gbrowse) that provides access to sequence data generously provided by the Maize Genome Sequencing Consortium and PlantGDB well integrated with MaizeGDB's genetic and genomic information. Access to the MAGIs and ZmGI will become available in the near future. Stop by to learn about the MaizeGDB Genome Browser's functionality and associated tools! Examples will be given on how to use the new "Locus Lookup" and the Genome Browser to aid in cloning your favorite genetically mapped gene. Also, we point out new features such as additional definitions of the genetic maps (http://www.maizegdb.org/map.php). As always- we rely on your feedback to improve MaizeGDB to better suit your needs. Please write to us (http://www.maizegdb.org/personnel.php) early and often!!

P34

New and Improved Phenotypes in MaizeGDB

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MaizeGDB is overhauling its phenotypic descriptors and tools to enable researchers to better document outcomes from phenomics projects. As the scale of data generated by projects increases and demand for access to information grows, we recognize an emerging need for the community to (1) gather and document phenotypic data using standards and (2) document phenotypes easily while walking through the field.

At this point, MaizeGDB requires the use of the Phenotypic Controlled Vocabulary (http://www.maizegdb.org/phenotypeterms.php) for data contribution. However, we add to the Controlled Vocabulary as researchers request new descriptors and can use your exact terms as synonyms to improve search functions and to better document what you observe in the field. Please check out the Phenotypic Controlled Vocabulary and help us to better tailor it to meet your needs!

To help researchers document their phenotypes as they walk through the field, a MaizeGDB Stand-alone Phenotype Browser is under development that can be deployed to handheld devices. Because the Phenotype Browser is not yet completed, your guidance on how this system should function is requested! Please stop by.

P35

POPcorn - a PrOject Portal for corn

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Maize researchers cannot easily leverage all available genetic and genomic data because the online locations of all resources are not easy to find and the sequence-indexed resources generated by individual projects must be searched independently. In addition, it is often the case that when a project's funding period ends, the generated data are lost because they are not moved to long-term repositories: these once-funded project sites degrade over time and sometimes disappear entirely. This project will overcome these challenges in collaboration with the community of maize researchers by launching POPcorn (PrOject Portal for corn), a needs-driven resource and data pipeline. POPcorn will make available (1) a centralized Web-accessible resource to search and browse ongoing maize genomics projects, (2) a single, stand-alone tool that makes use of Web services and minimal data warehousing to enable researchers to carry out sequence searches (BLAST) at one location that return matches for all participating projects' related resources, and (3) a set of tools that enable collaborators to migrate their data to MaizeGDB, the long-term model organism database for maize genetic and genomic information, at their projects' conclusion.

P36

PLEXdb: Plant And Pathogen Expression Database And Web Tools For Comparative And Functional Genomic Analysis

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PLEXdb is a plant expression database that supports Affymetrix microarray designs for plants and plant pathogens. PLEXdb provides microarray annotation and visualizations of raw and normalized experiment data across treatments in the form of boxplots, heatmaps, dendrograms, expression graphs, scatterplots and quality control plots. Experiment data deposited in PLEXdb is hand-checked for MIAME/Plant compliance and completeness then processed by normalizing the raw data using RMA and MAS5.0. This process produces tables and graphics from the resulting data. To facilitate the use of PLEXdb at any stage of data analysis, experiments may be kept private, shared worldwide with collaborators and reviewers, or released directly to the public. A suite of analysis and visualization tools permits in-depth exploration including searching for homolgous genes on different microarray platforms and mapping microarray probe sets onto model genomes. New features include: submission of experiment data to GEO on researchers' request; GO, EC, and KEGG annotation of probe sets; the Microarray Platform Translator; the Gene OscilloScope which calculates variation in the expression of a specific gene across multiple experiments; and tools for creating, analyzing, and annotating gene lists. PLEXdb uses standardized vocabularies such as the gene and plant ontologies to improve searching and cross-experiment comparisons. PLEXdb also links to data and tools provided by other relevant databases such as PlantGDB, Gramene, GrainGenes, HarvEST, and UniProt.

P37

Model Genome Interrogator: a PLEXdb module that leverages sequenced genomes for motif discovery via meta-promoter extraction and analysis

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The language that provides instruction to the transcriptional machinery of cells remains poorly understood. Characterization of motif function (word meaning) and relative position (word order, usage, grammar) helps further our understanding of this cis-regulatory language. The two commonly used approaches for identifying putative regulatory motifs capitalize on 1) co-expression during development and/or across treatments, and 2) motif retention in independent lineages as evolution occurs. Model Genome Interrogator (MGI) facilitates a powerful fusion of these approaches. For single or batch queries, MGI uses the sequenced genomes of rice and/or arabidopsis to identify the putative orthologues (metagenes) corresponding to probesets from any of the 16 microarray platforms supported by PLEXdb (www.plexdb.org). For each metagene identified, MGI allows researchers to view annotations and gene models, and to extract sequence data from promoters, exons, introns and UTRs. The following use case scenario illustrates several of many MGI utilities. For a set of maize genes found to be co-expressed across several conditions via a microarray experiment, maize probe identifiers are used in an MGI query to find orthologous genes in rice. The promoters of these metagenes can then be retrieved by the researcher using the MGI web interface, and subsequently subjected to cis-regulatory element searches using standard methods. When a motif is shared among the metapromoters beyond what could occur by chance, coordinate regulation of these genes in both maize and rice can be inferred. If such an hypothesis can be borne out via further testing, it suggests that the regulatory mechanism is sufficiently important to have been conserved through 50 million years of evolution in both independent lineages. Thus, MGI facilitates identification of conserved non-coding sequences (CNS) that are predicted to regulate whole sets of genes, creating a synergism of co-expression and CNS approaches for documenting the language of promoters.

P38

Investigation of the role of the ZmNAC1 transcription factor in Zea mays

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The NAC family of transcription factors is defined by a N-terminal helix-turn-helix domain, that is conserved amongst the Petunia NAM, Arabidopsis ATAF1, and Arabidopsis CUC2 proteins. This is a large family of transcription factors widely distributed in plants but not found in other eukaryotes. There are at least 150 NAC family members in rice and at least 214 in maize but functional roles have been assigned only to a handful of these genes. We have chosen to study one of these NAC family members of unknown function and here we report the relationship of ZmNAC1 (www.grassius.org) to NAC proteins in maize and other grass species (sorghum and rice). In order to study the binding specificity of ZmNAC1 we have cloned the DNA binding domain into a Gateway expression vector containing an N-terminal His-tag (pDEST17). We will report on our progress to affinity purify this fusion protein and use it in SELEX experiments to identify it's preferred DNA binding motif. We will also report on the pattern of expression of the ZmNAc1 gene in different plant tissues during maize development.

P39

Investigation of the role of the ZmMYB64 transcription factor in Zea mays

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MYB factors represent a heterogeneous group of proteins that is ubiquitous in eukaryotes, most notably in plants, and which contain 1-4+ MYB repeats (R1, R2 etc). In maize there are estimated to be at least 260 MYB factors with 2 MYB repeats that belong to the R2R3 MYB subfamily. We have examined the repertoire of maize R2R3 MYB factors that are most similar to ZmMYB1 (C1) which is a key regulator of flavonoid metabolism and chosen one ZmMYB64 for further study. Using phlyogenomic comparison we report the relationship of ZmMYB64 to MYB factors in maize and other grass species (sorghum and rice). In order to study the binding specificity ZmMYB64 we have cloned the DNA binding domain into a Gateway expression vector containing an N-terminal His-tag (pDEST17). We will report on our progress to affinity purify this fusion protein and use it in SELEX experiments to identify its preferred DNA binding motif. We will also report on the pattern of expression of the ZmMYB64 gene in different plant tissues during maize development.

P40

Phylogenomic Analysis of the Trihelix Transcription Factor Family in Grasses

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The Trihelix (THX) family of transcription factors in plants exhibits one or two THX DNA-binding motifs that bind to GT cis regulatory elements to regulate transcription. To date, DNA-binding proteins characterized by the THX motif have been described only in plants, and may therefore be involved in plant-specific processes. There is experimental evidence from Arabidopsis and rice that these roles are mainly in fruit and seed development. We have taken advantage of the near complete maize genome to identify at least 20 THX family members in corn and have performed a phylogenomic comparison to those in rice and sorghum. The DNA binding domain of one of these (ZmTHX1 (www.grassius.org)) was cloned as a His-tag fusion protein in pDEST17 for study of its binding specificity. We report on the overexpression of ZMTHX1 in E. coli and purification of the DNA binding domain in its native conformation. We also report on the binding specificity as determined by SELEX and on the tissue specific expression of this transcription factor during maize development.

P41

MicroRNA analysis in the Maize and Sorghum genomes

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MicroRNAs (miRNAs) are small non-coding RNAs that play an important role in many plant biological processes by negatively regulating gene expression through cleavage of target mRNAs or translational inhibition. The targeted functional categories include transcription factor activity, stress response, metabolism, and hormone signaling. To better understand the evolution of this class on non-coding genes, we have used the available draft genomes of sorghum and maize to computationally predict known miRNA families. The results related to conservation and species-specific features of miRNAs families will be presented. We have experimentally validated several members of the 16 most conserved miRNA families in maize and sorghum by RACE. Preliminary data of deep sequencing of small RNAs, from a variety of different developmental stages and stress-treated tissues in sorghum and maize, provide information about their expression dynamics as well as additional support for our computational predictions.

P42

A visualization and query tool for introgression libraries

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Near isogenic lines are powerful resources to analyze phenotypic variation and are important in efforts to map-base clone genes underlying mutants and traits. With many thousands of distinct genotypes, querying introgression libraries for lines of interest is an issue. To make it more tractable, we created a tool to graphically display and query such data. This tool incorporates a web interface for displaying the location and extent of introgressions. For comparative purposes, each marker is associated with the genetic position of a reference map. Users can search for introgressions using marker names or chromosome number and map position. This search will result in a display that gives the names of the lines with an introgression at the given position. Upon selecting one of the lines, color-coded introgressions in all 10 chromosomes of the line are displayed graphically. Then, upon selecting a chromosome, the user is taken to a web page that shows all of the markers on the chromosome along with the introgressions. A feature in development is the option to download query results as text as well as high-resolution publication-quality images corresponding to those displayed on the screen. This tool will be used to display maize teosinte introgression libraries, which comprise over 600 lines with introgressions defined with >500 SNP loci. Using our tool will allow quick access to lines with introgressions of interest. It will furthermore provide the means for producing figures for publications.

P43

GRIN-Global: An International Project to Develop a Global Plant Genebank and Information Management System

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The mission of the GRIN-Global Project is to create a new, scalable version of the Germplasm Resource Information System (GRIN) to provide the world's crop genebanks with a powerful, flexible, easy-to-use plant genetic resource (PGR) information management system. The system will help safeguard PGR and information vital to global food security, and encourage PGR use. Developed jointly by the USDA Agricultural Research Service, Bioversity International and the Global Crop Diversity Trust, GRIN-Global will be deployed in selected plant genebanks worldwide for 2010.

The .NET Framework and Visual Studio development environment were chosen for the project. A core set of web services, enterprise services or other technologies will update data stored locally or on networks, distribute centralized data to off-site systems, and enable third party data sharing. The database and interface(s) will accommodate commercial and open-source programming tools, be database-flexible (MySQL, MS SQL Server, Oracle), and require no licensing fees. The database will be deployable on stand-alone computers or networked systems. Iterative programming strategies will support continuous product evaluation and refinement; advanced prototypes will be extensively beta-tested. Bioversity International will deploy GRIN-Global internationally, working cooperatively to document the new system in Arabic, English, French, Russian and Spanish, translate its interface, and implement it in developing countries. Implementation will be monitored and barriers to adoption identified. The impact of system use will be evaluated by users during and following database implementation.

P44

MaizeSequence.org Reloaded

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MaizeSequence.org is the official website of the Maize Genome Sequencing Project. It is a comprehensive web portal that provides full public access to maize sequences as well as a wide array of derived annotations. Developed in close collaboration with Gramene, the website provides graphical navigation through an Ensembl-based genome browser, contextual and sequence-based querying, and complete snapshots of the data in various formats. The available annotations are for the most part automatically computed through an extensive annotation pipeline developed for the project, although other data sets are imported directly to the site or else dynamically integrated with remote data sources. The website is closely integrated with other online resources. Cross-species annotations such as gene orthologues and syntenic blocks are directly linked to contextual views on Gramene. Users can seamlessly navigate from graphical displays to corresponding views on the new MaizeGDB Genome Browser. Website and data updates are released through a well-defined deployment process. The website has been enhanced to provide greater ease-of-use and reliability. The integration of open APIs and social media such as Twitter enable the user community to collaborate and keep up-to-date with the latest news and information about the project. This work was funded by the NSF/DOE/USDA "Sequencing The Maize Genome" project (NSF #0527192).

P45

Towards a Model of Lesion Formation

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The production of spatially discrete leaf lesions in maize is controlled by a minimum of 25 -- 53 lesion mimic genes and a maximum of probably at least 200 genes. Genetic background, temperature, and light are all known to affect the frequency and rate of lesion production in the mutants. Lesions produced by mutants mimic many features of those produced by pathogens, such as fungi. Lesions can be either chlorotic, necrotic, or a mixture of the two types; in some mutants chlorotic lesions appear to develop into necrotic ones.

The number of mutant genes and the complexity of their phenotypes offer an opportunity to study the mechanisms underlying such complex phenotypes; and concomitantly, to develop computational methods to infer the network of reactions that operate those mechanisms. The development of predictive models that account for the phenotypes is central to both biological understanding and computational techniques. Here I present my initial low-resolution transition state model of lesion formation; an eyeball deconvolution of the lesion mimic phenotypes into their components; and the biological and theoretical implications of this information.

P46

Utilizing Maize-Related Ontologies for Phenotype Text Search

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Considerable time, money, and effort have been put forth toward the tedious development of several ontologies relevant to the maize community, e.g. the Plant Ontology (PO), Phenotype and Trait Ontology (PATO), Gene Ontology (GO). The primary aim of these ontologies is to list, describe, and relate concepts within their respective domains. By producing a unified vocabulary for a domain in this way, misunderstandings resulting from variations and discrepancies in term usage may be eliminated. As such, the primary use of ontologies thus far has been for the annotation of various texts. For example, “a bif2 ear showing barren patches on rachis and missing kernels,” would be linked to (PO:0020136), (PO:0020055), and (PO:0009001) for ear, rachis, and kernel, respectively. In other words, ontologies have mainly been exploited for concept linkage while the entire organizational structure, i.e. the defined relationships between concepts, has been largely unutilized. We have developed a phenotype free-text search mechanism that combines information retrieval techniques with ontological structure, typically directed acyclic graphs (DAGs), to improve the quality of retrieved results. To accomplish this, we developed an approach that automatically annotates a phenotype description repository with a given set of ontologies. A submitted query is first annotated and then expanded utilizing the relationships between ontological concepts. The weighting of an expanded term is derived from its relationship in the ontology to the annotated query concept. The testbed for the system was the set of phenotype descriptions in MaizeGDB (4104 distinct descriptions).

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P47

A BACTERIAL SIGNAL PEPTIDE IS FUNCTIONAL IN PLANTS AND DIRECTS PROTEINS TO THE SECRETORY PATHWAY

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This study summarizes the work done to establish the role of a bacterial signal peptide in the localization B subunit of the heat labile enterotoxin (LT-B) of Escherichia coli in plant cells. Previous work has shown that LT-B can accumulate in the starch granules of transgenic maize kernels. To investigate the targeting properties of the bacterial LT-B protein and its native signal peptide in plant systems, we have generated a series of translational fusions of LT-B with GFP and have studied their expression and subcellular localization in protoplasts and leaves of Arabidopsis thaliana and in cells of maize embryogenic callus and maize endosperm. Results show that LT-B with its native signal peptide (BSP) or with a maize 27kD gamma-zein signal peptide (ZSP) fused to GFP accumulates in the endoplasmic reticulum (ER), ER-derived bodies and starch. It is also demonstrated that the bacterial signal peptide is sufficient to localize GFP to the secretory pathway of the plant cells. The findings suggest that BSP is functional as a signal peptide in plant cells, and that plant-derived LT-B follows a route analogous to its native route in the secretory pathway of bacteria. However, in plants, the protein is not secreted but retained in the ER, ER-derived bodies, and to a lesser degree, in plastids. The results presented here are of great importance for future production of recombinant protein and edible vaccine production in plants, as they highlight the importance of targeting and subcellular localization in functional products.

P48

Analysis of the maize root-hair transcriptome and its responsiveness to inbred and hybrid backgrounds

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Root hairs have been an invaluable system for the study of tip growth, cell-wall dynamics, nutrient uptake, and other biological processes. We have profiled the root hair transcriptome as a means of learning more about their physiology, metabolism, genetics, and general biology. To do so, we employed 454-based, 3'-anchored cDNA sequencing for quantification and identification of the transcript content of root hairs isolated from lab-grown maize seedlings. By anchoring sequences at the 3' UTR, transcript identification becomes not only gene-specific, but also highly allele specific. Roots from maize seedlings were frozen in liquid nitrogen for collection of root-hair cells by 'scraping' the epidermal surface with a spatula. Root hair samples were thus collected from W22 and B73 inbred seedlings, as well as from those of a W22xB37 hybrid. Each of the samples were assigned a unique 4-base key code during library construction to track their respective sequence reads from the total 454-generated sequence data. Transcripts of interest included cell wall-related genes, such as those of the Cellulose Synthase-Like D subfamily, which have been implicated in root hair formation by genetic studies in multiple species. In addition to shedding light on the biology of root hairs, these data also highlight differences between the W22 and B73 inbreds, and the W22xB73 hybrid. Approximately 50% of the 100 most abundantly expressed genes show significantly different levels of expression between the three maize lines. Over- and under-dominance was also evident for about 15% of genes examined thus far. Results demonstrate the feasibility and efficacy of profiling a single, fully-differentiated cell type using a method that captures allele specificity of transcripts.

P49

Characterization and Identification of Protein Body ER-Associated Proteins

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Maize is an important protein source in many developing countries especially in Central America and Africa and a major source of feed in the US. The discovery that certain mutants of maize, especially *opaque2*, have a positive effect on nutritional value led to the development of Quality Protein Maize. However, mutants with the opaque phenotype are susceptible to damage from pests and disease as a result of their soft, starchy kernels. The FL1 gene encodes a novel membrane protein that resides in the ER membrane surrounding the protein body (Holding et al. 2007). This protein participates in protein body formation by facilitating the localization of 22-kD α-zein between the γ-zein-rich periphery and the core of the protein body. Furthermore, it is essential for the vitreous endosperm formation. We hypothesize that other proteins on the surface of protein body ER that are not present in the cisternal ER play a role in the development of the protein body. These proteins may affect the phenotype by altering the cross-linking structure of the zein proteins. We have begun to investigate this by cell fractionation of the maize endosperm on discontinuous sucrose gradients and analyzing the differences in protein composition among the fractions. Once these proteins have been identified, their function will be investigated in wild type and opaque endosperm maize lines. This work was supported by an Undergraduate Research and Scholarly Achievement grant from Baylor University.

P50

Establishment of a methodology for using doubled haploids of maize in a breeding program for Sub-Saharan Africa

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In commercial maize breeding programs doubled haploid (DH) plants have been used extensively. Especially since this technology may significantly reduce the time required for developing new varieties. Several methods have been established for producing doubled haploids in maize often employing inhibitors of cell division like colchicine.

This study is aimed at implementing DH-lines in breeding programs for Sub-Saharan Africa. In this context the method for doubling of chromosomes has to be simple, i.e. doubling in vivo. Furthermore, negative impact on human health and the environment should be avoided. Towards these goals a standardized screening system was established with diploid seedlings (3 days after germination) of the maize inbred line A188. Tips of primary roots were subjected to regimes of different physical treatments or incubated in potential mitotic inhibitors. As a positive control seedlings were treated with colchicine. A qualitative evaluation of the different treatments was performed by estimating mitotic indices of squashes of root tips. Afterwards, the DNA contents of nuclei isolated from treated root tips was measured quantitatively by flow cytometry and compared to untreated controls. Overall, physical treatments of root tips did not result in an elevated level of cells with a doubled chromosome number. However, mitosis was effectively blocked by herbicides like oryzalin, pronamide, and amiprophos methyl (APM) and thus doubling of chromosome numbers in maize root tips was achieved. Attempts failed to further enhance the effect of these compounds by synchronizing cell division of root tip cells.

The results of our experiments showed that oryzalin, pronamide, and APM effectively doubled the chromosome number of root tip cells. Furthermore, it's important to note that these compounds act specifically in plants at micro molar concentrations. Experiments are under way to double chromosome numbers of haploid seedlings in the breeding program for Sub-Saharan Africa.

P51

Fostering Plant Science Research at MU Plant Transformation Core Facility

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Since its establishment in 2000, the University of Missouri (MU) Plant Transformation Core Facility has been providing state-of-the-art research support services in genetic engineering of maize (Zea mays) and soybean (Glycine max). Located inside the new MU central campus greenhouse, the Sears Plant Growth Facility, the Plant Transformation Core Facility is aiming at promoting gene discovery, crop improvement, and funding opportunities for the plant science research community. Our staff is strongly dedicated and committed to providing various types of transformation support services and conducting research in transgenic technology development with a focus on maize and soybean. The facility service categories include both standard and customized transient as well as stable transformation for maize and soybean upon the user's request. The transformation system for maize utilizes Agrobacterium-mediated approaches using immature zygotic embryos as starting materials. The Agrobacterium approach has been our current emphasis starting from year 2003. We employ the Agrobacterium-mediated cot-node transformation system for soybean transformation. We have recently established fast and reproducible sorghum (Sorghum bicolor) transformation system and now are ready for providing sorghum transformation services. Current research activities are geared towards developing high-throughput transformation, improving the quality of transgene integration and sufficient gene regulation to meet the needs of crop improvement and functional genomics. One of our most recent efforts is in developing efficient switchgrass transformation process to meet the need of biofuel crop engineering. Our specific interest in soybean genetic engineering is to regulate several economically important genes conditioning soybean seed polyunsaturated fatty acids and short chain sugars, secondary metabolites, abiotic stress, virus resistance, etc. Some of these studies are conducted as collaborations with on- and off-campus researchers. More details of these activities will be presented at the conference.

P52

Genetic resources for functional genomics of maize cell wall biology

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Grass species represent a major resource of our food, feed, and fiber crops and potential feedstocks for biofuel production. Most of the biomass is contributed by cell walls that are distinct in composition from all other flowering plants. Identifying cell wall-related genes and their functions underpins a fundamental understanding of growth and development in these species. Toward this goal, we are building a knowledge base of the maize genes involved in cell wall biology, their expression profiles, and the phenotypic consequences of mutation. Over 750 maize genes were annotated and assembled into 21 gene families predicted to function in cell wall biogenesis. Comparative genomics of maize, rice, and Arabidopsis sequences reveal differences in gene family structure between grass species and a representative dicotyledonous species. Deep sequencing of the maize unfertilized ovary showed that transcript abundance varies more than 100-fold between members of a single family of cell wall-related genes. When compared to expression for immature ovules of rice and Arabidopsis, different sets of genes were expressed in the grasses compared to Arabidopsis. These differences in gene family structure and expression between Arabidopsis and the grasses underscore the requirement for a grass-specific genetic model for functional analyses. A UniformMu population proved to be an important resource in both forward- and reverse-genetics approaches to identify hundreds of mutants in cell wall genes. A forward screen of field-grown lines by near-infrared (NIR) spectroscopic screen of mature leaves yielded several dozen lines with heritable spectroscopic phenotypes. Pyrolysis-molecular beam-mass spectrometry confirmed that several NIR-mutants had altered carbohydrate-lignin compositions. Novel methods to characterize phenotypes are proven essential to establish specific gene functions for cell wall biogenesis.

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P53

Metabolic features of pollen development in male-fertile and S male-sterile maize

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In the S type of cytoplasmic male sterility (CMS-S), developing maize pollen collapses following the mitotic division that generates bi-cellular pollen from uni-nucleate microspores. The mitochondrial reading frame associated with this phenotype predicts a hydrophobic peptide related to ATP synthase subunit 9. To examine the potential role of mitochondrial bioenergetics in CMS-S pollen collapse, mitochondrial protein and ATP accumulation was examined during normal and CMS-S pollen development. ATP levels did not differ between CMS-S and normal genotypes at the microspore or early bi-cellular pollen stage. The accumulation of mitochondrial respiratory complex I-V subunits was reduced in both CMS-S and normal microspores compared to immature ears and later pollen stages. In contrast, mitochondrial anti-oxidant proteins were abundant in microspores compared to bi-cellular pollen. These observations raise the question of which catabolic pathways support maize microspore development and support a model of CMS-S pollen collapse mediated by mitochondria-produced reactive oxygen species and declining levels of protective/anti-oxidant proteins. Isozyme gels revealed abundant NADH dehydrogenase activity consistent with a non-phosphorylating pathway of electron flow through type II NAD(P)H dehydrogenases and the alternative oxidase of microspore mitochondria. Lactate dehydrogenase and alcohol dehydrogenase accumulation patterns were inconsistent with significant aerobic fermentation at the microspore stage. The relative abundance of MnSOD, higher in microspores than in bi-cellular pollen, supports a model in which MnSOD protects the microspores from collapse. Reverse transcription-PCR based on the four known MnSOD genes of maize revealed microspore-specific transcript variants. Further experiments will involve the expression of these MnSOD forms in bi-cellular pollen, to attempt to rescue CMS-S pollen from collapse.

P54

Molecular Genetic Analysis of Early Endosperm Development by Microinjection of Maize Embryo Sacs

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Cereal endosperm is an important source of food, feed and industrial raw material for several applications including biofuels. With such an important role in society, it is imperative that we understand all aspects of endosperm development, starting from its inception at fertilization. However, due to the small size and relative inaccessibility of most endosperms, early molecular events in endosperm development are largely unknown. Maize endosperm provides a unique opportunity to study cereal endosperm development. This is because the maize central cell is large, follows a normal developmental pattern when cultured in vitro, and can easily be microinjected, allowing introduction of foreign nucleic acids and proteins. These advantages, together with the application of small RNA technologies currently used in animal studies, will enable rapid progress in our understanding of endosperm development and our ability to manipulate it. We are investigating how microinjection can be used to deliver nucleic acids into live, intact maize embryo sacs. During the first three days after pollination, maize endosperm undergoes several rounds of mitosis without cytokinesis, forming a large multi-nucleate cell. Endosperms from embryo sacs isolated during this period show a ~90% survival rate when cultured in vitro. When plasmid DNA is microinjected into the syncytium, ~35% of the endosperms formed express the introduced construct. The outcome of overexpression and RNAi knock-down of genes presumed to be important for endosperm development will be discussed.

P55

Pollen abortion in CMS-C is associated with reduction of F0 ATP synthase proteins

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Cytoplasmic male sterility type C (CMS-C) is atypical compared to other CMS types as no novel chimeric transcript or protein has been found correlating with the phenotype. However, a tassel-specific decrease for both ATP6 and ATP9, which are mitochondrially encoded components of the ATP synthase F0 subunit, was discovered in CMS-C. This decrease in levels was not observed when the restorer of fertility gene (Rf) was present. Other components of the ATP synthase complex analyzed did not appear to be altered in CMS-C, indicating that the cause of pollen abortion in CMS-C is affecting the stability or formation of F0. The CMS-C genome has two copies of the atp9 gene. One is the normal copy of atp9 (atp9-1), which is present in other maize cytotypes but is transcribed ~20 fold less in CMS-C. The other copy of atp9 (atp9-2) is the predominant atp9 transcript in CMS-C and has a chimeric 5'UTR resulting in the use of a different promoter and a larger transcript (4kb instead of 1kb). We propose that the atp9-2 transcript is inefficiently translated. When synthesis demand is high, as in the tapetum layer of an anther, the mitochondria are unable to compensate and ATP9 levels decrease. This results in the formation of fewer F0 subunits and ATP synthase complexes, ultimately causing a reduction in ATP levels and abortion of the developing pollen.

P56

Possible Role of Translational Regulation in Determining Opaque Endosperm Protein Quality

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Maize starchy endosperm mutants have improved protein quality but poor agronomic traits. To better understand the mechanisms that lead to the starchy endosperm phenotype, we performed a proteomic analysis of wild type and opaque2 mutants. Several lysine rich proteins were increased in *opaque2*. Prior studies using Affymetrics analysis found that there were some shared changes in gene expression in several opaque mutants including o1, o2, o5, o9, o11, floury2, defective endosperm B-30 and mucronate. The majority of the mutants had increased ribosomal protein gene expression, which indicated that protein synthesis was likely to be altered in these mutants. This result is consistent with a change in TOR-kinase signaling which is a central signal transduction pathway that regulates cellular growth in response to nutients, particularly amino acids, mitogens, growth factors and hormones. One important aspect of TOR kinase signaling is mediated by translational regulation and this may partially explain the accumulation of specific lysine-rich proteins in *opaque2* mutants. The signaling pathways of TOR kinase in mammalian cells are well studied and many regulatory factors have been identified. Although many aspects of TOR-dependent signaling are conserved between different species, there is still little known about these mechanisms in plants. Expression analysis of the TOR kinase pathway genes and investigation of homologues in plants will increase our understanding of this signaling pathway and its role in endosperm development.

P57

Teaching with Maize: Use of the Yeast One-Hybrid System to Analyze Ab10 Neocentromere Activity in an Undergraduate Setting

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Traditionally undergraduate laboratory courses at non-research universities are organized as weekly exercises, independent of one another, arranged to accommodate a particular set of learning objectives. Consequently these courses rarely provide undergraduates with the opportunity to take part in novel experimentation with the potential for publication. Here we arrange our undergraduate laboratory course to allow students to take part in answering a novel scientific question, and use the experimental process to drive the learning objectives of the course. We propose using the Yeast One-Hybrid system to analyze Ab10 neocentromere activity in maize. Previous experiments have shown that the TR-1 and 180bp repeats of the Ab10 chromosome act as neocentromeres during meiosis (Yu, et al 1997; Hiatt et al 2002). Here we will attempt to identify a protein or proteins that interact with the TR-1 and 180bp repeats of maize by utilizing the Yeast One-Hybrid system. Here we present the expected procedures, learned techniques, and student learning objectives of the project.

P58

Understanding the control of division plane orientation in plant cells

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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 division site delineated by the PPB. New cell walls are often incorrectly orientated during asymmetric cell divisions in the leaf epidermis of the *discordia (dcd1, dcd2, and dcd3)* maize mutants. 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. We identified an additional gene, *alternative discordia1 (add1)* in the maize genome, which encodes a protein 96% identical to DCD1. The inbreed line A619 was identified as an *add1* mutant but does not have a noticeable phenotype. *dcd1; add1* RNAi lines and double mutants have abnormal division planes and fail to form PPBs in both symmetrically and asymmetrically dividing cells. An antibody that recognizes DCD1 and ADD1 localizes these proteins to PPBs and, more surprisingly, the cortical division site that remains after PPB breakdown. Considered all together, these experiments suggest that phosphatase activity regulated by DCD1/ADD1 is needed for PPB formation and cortical division site establishment. Additionally, progress on the cloning of *dcd2* and *dcd3* will be presented as well as the initial characterization of *asc1*, a cyclin D mutant identified by the Scanlon lab with abnormal asymmetric cell divisions.

P59

Unraveling the function of CENH3 and CENPC in kinetochore assembly by ectopic tethering

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Most centromeres in complex eukaryotes are characterized by long tandem arrays of satellite repeats, but these repetitive sequences are neither necessary nor sufficient for centromere activity. Recent research suggests that centromeres are defined by their active interaction with kinetochores, conserved protein complexes that assemble on the centromeres for chromosome movement and segregation. CENH3 and CENPC are among the “foundation proteins” of the kinetochores, which are present in all eukaryotes and bind to centromeric DNA throughout a cell cycle. They are thought to play a role in recruiting other components of the kinetochore and assembling a functional centromere-kinetochore complex. Here we describe a strategy for targeting kinetochore proteins in maize, based on tandemly arrayed binding sites for the Lac repressor, LexA activator, and Gal4. We show that large tandem arrays can be generated and transformed into maize by a combined approach of overlap extension PCR and co-bombardment. A LacI-YFP fusion protein binds to the inserted array in vivo. By means of this tagging system, we’re expecting to achieve specific localization of CENH3 and CENPC in order to assemble a functional kinetochore ectopically.

P60

Characterization of mtm99-14, a meiotic mutant with defective synaptonemal complex assembly

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Meiosis is essential for almost all eukaryotes, producing haploid gametes with new genetic variation. Most meiosis-specific events occur during the first meiotic prophase, when meiotic recombination takes place and the homologous chromosomes pair and synapse. During synapsis the axial element cores of the leptotene chromosomes align and a central element assembles between them to form a tripartite structure, the synaptonemal complex (SC). The mtm99-14 mutant exhibits defects in pairing and assembly of the SC filaments. TEM images of the SC spreads of mtm99-14 mutant prophase nuclei revealed abnormal and irregular lateral elements. When examined by immunolocalization of various SC components, the localization pattern of AFD1 and ASY1, both of which are part of the axial element, were as in wild type while ZYP1, a component of the central element, is not loaded properly onto the chromosomes during zygotene, resulting in foci or patches of ZYP1 staining. However, ZYP1 does elongate between homologously as well as non-homologously paired chromosomes in pachytene. These results suggest that mtm99-14 mutant is defective in synapsis due to anomalous formation of the axial element and/or their maturation into the lateral element during zygotene. We present the results of cytological characterization as well as progress in cloning the gene, including evidence for a tightly linked Mu insertion.

P61

Chromatin architecture changes during meiotic pairing 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. At the leptotene to zygotene transition, chromosome architecture undergoes a dramatic structural remodeling, including the elongation of heterochromatin and expansion of euchromatin. Chromosome pairing, repair of meiotic DNA breaks and synapsis are all dependent on successfully passing through this transition. However, the reorganization of leptotene chromosome architecture and the role of chromosome remodeling at this transition in regulating downstream meiotic prophase events are not understood.

Structured illumination (SI) is a method of ultrahigh resolution light microscopy that overcomes the 250 nm limit of resolution of conventional light microscopy, and reaches a resolution less than 100 nm in the x, y and z axes. We used SI to explore the architecture of meiotic chromatin at the leptotene to zygotene transition and during pairing by using antibodies to monitor the distribution of an euchromatin marker (histone H3-lysine 4 dimethylation), a heterochromatin marker (histone H3-lysine 9 dimethylation) and an axial element component, AFD1. We found that euchromatin forms thin and long fibers, expanding further from the axial elements than DAPI-stained chromomeres. In contrast, heterochromatin forms thick and compact fibers, and mainly colocalized with the DAPI stained chromomeres. We are investigating the chromatin architecture of a gene-rich region and its position relative to heterochromatin and chromosome axes by FISH-immunofluorescence. In addition, we are examining the meiotic chromatin structure of am1-praI, a mutant that is arrested at the leptotene-zygotene transition and a cohesion complex mutant, afd1, that is defective in axial element organization. These novel views of meiotic chromosome organization revealed by 3D-SIM will provide insights into how meiotic chromatin architecture changes and affects meiotic processes.

P62

Constructing A Cytogenetic Map Of Maize In Oat Addition Lines Using Sorghum BACs As FISH Probes

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We are developing a pachytene cytogenetic FISH map of the maize (Zea mays L.) genome using maize marker-selected sorghum BACs as described by Koumbaris & Bass (2003, Plant J., 35:647). The two main objectives are to produce a high-density FISH map of chromosome 9 (Amarillo & Bass, 2007, Genetics, 177:1509) and a core marker FISH map of the other maize chromosomes. Thirty-two maize marker-selected sorghum BACs have been cytogenetically mapped onto maize chromosome 9. This map established an overall conservation of marker colinearity with linkage maps at 5 cM resolution while uncovering regions of maize genome hyperexpansion relative to sorghum. Preliminary FISH data for chromosomes 1, 4, and 6 is also presented along with data for FISH mapping duplicated loci in maize using a single BAC. In addition, an RFLP full-length insert sequencing (FLIS) project has produced annotated GenBank sequence accessions for over 150 widely mapped maize RFLPs, which map to more than 300 loci across the maize genome. These results facilitate analysis of the maize and sorghum genomes by using common markers to integrate their physical, linkage, and cytogenetic pachytene chromosome maps. The FISH map, maize-10-maze, and RFLP projects are described at cytomaize.org and mapping records and FISH images are made available at MaizeGDB. As part of a public outreach program, we used the map of maize genes/mutations to guide production of a maize field map garden (see Denton et al., poster for information on maize map gardens).

P63

Copy Number Increase of Engineered Minichromosomes Derived from the B Chromosome

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Engineered minichromosomes provide the potential to accumulate numerous transgenes on an independent chromosome for faithful expression and no linkage drag. Our lab has produced engineered minichromosomes via telomere truncation with the simultaneous addition of reporter genes and site-specific recombination cassettes. Using minichromosomes derived from the supernumerary B chromosome, we are testing the accumulation capacity of minichromosomes. The minichromosomes have lost the B chromosome accumulation mechanism, i. e., nondisjunction at the second pollen mitosis followed by the preferential fertilization of the egg by the B containing sperm, but adding a normal B chromosome to the genotype can restore this function. Using FISH we are selecting for the accumulation of various sizes of transgenic and nontransgenic minichromosomes. The GUS transgene incorporated in the telomere truncation construct is being used to study how minichromosome numbers affect transgene expression. In maize, about 15 B chromosomes can be accumulated without affecting vigor, and with various sizes of B-derived minichromosomes we can investigate the role of chromatin in establishing the biological limits of chromosome number in plant cells. Because these minichromosomes have significantly less chromatin than a normal B chromosome, it might be possible to accumulate even greater numbers of minichromosomes. However, saturating the spindle or metaphase plate could also become a limiting factor. Here we present data on the effects of accumulating minichromosomes in maize.

P64

Crossover interference in Maize

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In most organisms, if one crossover (CO) occurs at a given position on a chromosome, there is a lowered probability that a second one may arise nearby during the same meiosis. This is referred to as CO interference. COs are produced from precursors (DNA double-strand breaks), through two distinct pathways. Class I COs (most frequent), show definite interference, whereas class II COs do not seem to interfere. At least one CO is required per bivalent to ensure proper segregation of homologs during meiosis ("obligatory CO").

Several mathematical models have been proposed to model CO interference. Inferring the model parameters from biological data allows one to measure and characterize interference. Most models (e.g. "gamma" model) used so far are based on stationary renewal processes (SRPs), and do not take into account multiple pathways, nor the obligatory CO. We developed a model called "Forced Initial Crossover" (FIC), which incorporates the obligatory CO within an SRP framework, and the "Precursor-Sharing Pathways" (PSP) model, based on the mechanically-motivated beam-film model. In our PSP model, a common pool of precursors is concurrently processed by two pathways leading to class I and class II COs. We impose that class II COs are non-interfering, and that the obligatory CO belongs to class I.

Using the models FIC and PSP, as well as the standard gamma model as a reference, we analyzed mapping data of late recombination nodules along Maize bivalents (Anderson et al., Genetics 2003). We inferred the proportion of non-interfering class II COs for each chromosome (from 0 to 20%), and the intensity of interference among class I COs. Within any of the models, the proportion of class II COs is strongly positively correlated with the intensity of interference among class I COs. This result suggests a chromosome-by-chromosome regulation of interference, presumably to control CO number.

P65

Diversity of repetitive sequence arrays in maize lines as visualized by fluorescence in-situ hybridization

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One of the resources generated by the Maize Diversity Project is an extensive collection of nested association mapping (NAM) lines for joint QTL-association analysis and high-resolution mapping in maize. These lines were derived from twenty six progenitor lines chosen to maximize trait diversity. We examined the progenitor lines and other inbred lines to determine the hybridization patterns on somatic chromosomes of specific repetitive sequence arrays. Sequences found to be reliably present include the TAG microsatellite (1L), the 5S ribosomal gene cluster (2L), Centromere-4-specific sequence (4C), subtelomere clone 4-12-1 (5S and 8L), and the 45S nuclear organizing region (6S). Significant variability was observed in the position and copy number of knob related sequences (180-bp repeat and TR-1 tandem array) and the copy number of Centromere-C-specific sequences among different maize chromosomes. Variation was also detected for other sites of the TAG microsatellite. Three of the NAM progenitor lines were found to be heterozygous for arrays on one or more chromosome pairs. This work reveals the cytological diversity in maize and could provide insight into mechanisms involved in the evolution of repetitive arrays.

P66

Effect of genomic balance on quantitative traits and gene expression

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Maize is endowed with an unusually rich repertoire of tools to examine the effects of chromosomal dosage. Several studies of global gene expression in aneuploids from a variety of species have been published recently with widely varying conclusions, which appear to depend on the mode of normalization. Our previous studies had indicated that many genes are inversely or directly modulated by a change in chromosomal dosage and that several genes present on the varied region showed dosage compensation. To revisit this issue at multiple ploidy levels in order to examine the specific question of genomic balance on gene expression, 2-D DIGE protein analyses were performed on haploids, haploids plus the long arm of chromosome 1 (1L) versus diploids with one, two and three copies of 1L. Plants were classified via root tip chromosome karyotyping and grown in the greenhouse. The addition of 1L to a haploid has a much more dramatic effect on the phenotype than addition to a diploid. In each comparison, matched protein samples from mature leaf tissue were differentially labeled with Cy3 and Cy5 with an internal spike of the mixture labeled with Cy2. The analyses indicated that when chromosomes are added to the genotype, the amounts of some proteins are unchanged, but the majority was reduced to varying degrees within the inverse limits of the chromosomal dosage in the aneuploid versus the euploid. In other words, the extra chromosome in the haploid caused reductions to varying degrees down to about 50% whereas the trisomic versus the diploid had reductions ranging down to about 67%. Because the majority of proteins are modulated in the same direction, normalization to the average of all proteins, as is often performed, greatly obscures the effect. A few extreme outliers both up and down were also observed at both ploidy levels. The monosomic showed many changes within the direct and inverse limits relative to the diploid. Phenotypic comparisons were also made for chromosome arms 5S and 9S with the extra chromosome in the haploid having a more severe effect than in the diploid indicating the generality of the balance effect on quantitative traits.

P67

Examination of Mitochondrial DNA Insertion Sites in a Diverse Set of Maize Lines

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The transfer of mitochondrial DNA (mtDNA) to the nuclear genome is an ongoing and frequent process in plants. The 570 kb maize NB mitochondrial genome has been divided into 20 overlapping pieces (in cosmids). These cosmids were used as fluorescence in situ hybridization (FISH) probes on maize metaphase root tip chromosomes. Our lab previously found a surprisingly large amount of variation among mtDNA insertion sites in 10 genetically useful maize inbred lines. This study has now been expanded to examine mtDNA insertion sites in a set of lines that represent the natural diversity of maize, including the Nested Association Mapping (NAM) lines. Several lines from a single sweet corn pedigree have also been surveyed. This work will determine the extent of the variation of mtDNA insertion sites among maize lines.

P68

Identification of a Maize Neocentromere in an Oat-Maize Addition Line

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We report a neocentromere event on maize chromosome 3 that occurred due to chromosome breakage. The neocentromere lies on a fragment of the short arm that lacks the primary centromere DNA elements, CentC and CRM. It transmitted in the genomic background of oat via a new centromere (and kinetochore), as shown by immunolocalization of the oat CENH3 protein. Despite normal transmission of the maize fragment in most progeny, neocentromeres appear to vary in size within the same tissue, as shown by fluorescent measurements. A secondary truncation in one line lowered mitotic transmission to 3% and precipitously reduced the size of the chromosome. The results support the view that neocentromere formation is generally associated with major genomic disturbances such as wide species crosses or deletion of an existing centromere. The data further suggest that new centromeres may undergo a period of instability that is corrected over a period of several generations.

P69

Inactivation of an A chromosome centromere

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We have previously reported the inactivation of numerous B chromosome centromeres that occurred in dicentric chromosomes, which resulted in stable minichromosomes. In the process of analyzing the reciprocal translocation T1-5(8041), an extra array of centromeric repeats was found on the chromosome. Maize centromere specific sequences CentC and CRM were used to confirmed the dicentric situation by FISH.
Multicolor FISH was used to confirm the translocation involved chromosomes 1 and 5.

We investigated the localization of CENPC, CENH3 and histone H3 phosphorylated at Ser-10 in this translocation chromosome using immunocytochemistry and FISH. The results indicate that only one centromere assembles the centromere specific proteins. These results extend the number of inactive centromeres described in maize and illustrate that inactivatioin can occur for both A and B chromosome centromeres.

P70

Isolation and Amplification of a Maize Abnormal Chromosome 10 Translocation Line

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In a small percentage of both domesticated maize and teosinte the normal chromosome 10 (N10) has been replaced with abnormal chromosome 10 (Ab10). Ab10 is defined cytogenetically by the addition of a terminal piece of foreign DNA onto the long arm of N10. This piece of DNA includes both euchromatin and a distinctive, large, heterochromatic knob. The additional DNA is known as the Ab10 haplotype, and is responsible for neocentromere activity and preferential segregation (meiotic drive). In the presence of Ab10 all knobs are transformed into neocentromeres and any loci that are heterozygous for knob size are subject to meiotic drive. This ensures the transmission of the larger knobbed chromatid to the progeny at rates of up to 83%. These unique properties of Ab10 have been studied since the 1940s; however, the genes which control these properties remain unknown. To address this problem, we are developing methods to isolate and amplify DNA from individual chromosomes containing the Ab10 haplotype. Using a chromosome 1/Ab10 translocation line (T1-Ab10) we will isolate and collect T1-Ab10 chromosomes via laser capture and flow sorting in parallel. Whole genome amplification kits will then be used to obtain enough DNA from the isolated chromosomes for 454 sequencing. This will allow us to sequence the Ab10 haplotype and uncover the genes which control neocentromere activity and meiotic drive. Additionally, we hope to gain further insight into the evolutionary history of the Ab10 haplotype.

P71

Maize chromosome specific painting

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Whole chromosome paints or chromosome banding patterns are powerful methods for detection of chromosomal aberrations, gene localization, studying chromosomal rearrangements, phylogenetics and chromosomal evolution. Although the maize genome is rich in repetitive elements (up to 70%), they are not suitable for classical banding paints unlike some species. On the contrary some abundant repetitive elements are not specific and are spread along all maize chromosomes; the copy number of others substantially varies among chromosomes of different maize genotypes. For developing whole chromosome painting probes, selection of chromosome specific sequences and depletion of repetitive elements from the probe collection are necessary and were attempted by two approaches: first, by sequence analysis of mapped BAC clones and amplification of their unique regions, which were pooled as a probe (chromosomes 8 and 9). The developed probe collections were used for chromosome painting by fluorescent in situ hybridization. Second, we used laser-capture chromosome microdissection followed by DNA amplification and subtractive DNA hybridization (chromosomes 1 and 10).

P72

Sorting out dosage effects on maize morphogenesis: additive effects versus gene interactions between nonhomologous chromosome segments

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We previously reported that about one-third of 48 B-A-A translocation stocks tested displayed an altered plant phenotype in plants that contain an extra copy of the two A chromosome segments (hyperploid). In most cases the hyperploid plants were significantly reduced in height, and often other traits, when compared to their euploid counterparts. We have suggested that these dosage sensitive effects may be either the additive effects of hyperploidy for the two chromosome segments or a result of gene interaction between them. Here we report the effects on plant development of simple B-A translocations that include most of the same chromosome arms that were involved in the previous study of B-A-A hyperploid effects. Hyperploidy for a simple B-A carrying either chromosome arm 1S, 1L, 5S or 10L exhibited sufficient decrease in plant traits to suggest that additive effects can explain a reduction in plant traits experienced in hyperploid B-A-A stocks carrying segments of these arms. In contrast, hyperploidy for chromosome arm 3L, 4L, 5L, 6L and 7L had sufficiently small or no effects on plant traits to suggest an interaction of genes when regions of two of these arms are present in a B-A-A chromosome causing a reduction in plant traits. Additionally, in light of these recent results, examination of our previously reported B-A-A data suggests that when a region of one of this latter group of chromosome arms is present in a B-A-A chromosome together with a region of 1S, 1L 5S or 10L, it may ameliorate the negative effect of that second region. We will also report the identity of 15 new confirmed B-A-A translocations involving chromosome arms 1S, 1L, 2S, 2L, 3S, 3L, 4S, 4L, 5L, 6L, 7L, 9S, and 10L.

P73

The Effects of Inbred Background upon Maize B Chromosome Behavior

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Maize B chromosomes are supernumerary, having no known genes and almost no effect upon plants that possess them. They have properties that apparently contribute to maintaining Bs within populations. One is the ability of the B to transmit efficiently through meiosis as a monosomic; failure results in B-loss. A second property involves non-disjunction of the B chromosomes at the second pollen mitosis, which results in one sperm possessing two Bs, while the other has none. Related is a third property, preferential fertilization, where the sperm possessing the two Bs preferentially fertilizes the egg. Previous studies have identified B chromosome regions that are important to these properties, but little work has examined how the essential A genome contributes to these behaviors. A translocation between a B and chromosome 9 (TB-9Sd) was introgressed into several inbred lines. The B-A translocation has a color marker, C1, which allows the presence or absence of this chromosome to be monitored. Plants possessing TB-9Sd from each inbred were crossed as males onto several inbred lines. B-loss was highest among crosses where L289 served as the males and least with W23. Among the females, B-loss appeared highest with B73 and lowest with L289. The female effect is remarkable since B-loss was expected to have taken place in the male, prior to fertilization. Non-disjunction rates were lowest with W23 males and highest with L289 and W22. Again, the female parent had an effect; W22 females consistently yielded higher rates of kernels that demonstrated non-disjunction in the pollen. Interestingly, preferential fertilization was not strongly demonstrated in these data. Only B73 x L289 gave a preferential fertilization rate approaching 60%. Indeed, for W22 x W23 the trend was the opposite with 67% preferential fertilization of the central cell, which develops into endosperm.

P74

ragged seedling2 encodes an ARGONAUTE7-like protein and is required for proper leaf patterning

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ragged seedling2 (RGD2) is required for mediolateral leaf development in maize (*Zea mays*). Plants homozygous for the recessive mutation rgd2-R form a range of mutant leaf phenotypes, including half leaves, split leaves and radial leaves. Previously described radial leaf mutants lack either adaxial or abaxial identity; however, rgd2 radial leaves maintain dorsiventral polarity. Positional cloning has shown that rgd2-R mutants contain a *Mutator* transposon in an *argonaute7-like* (*ago7*) locus. Efforts to obtain additional mutant alleles of *rgd2* are underway. AGO7 is required to produce trans-acting small interfering RNAs (ta-siRNAs), which ultimately regulate the transcript level of *auxin response factor3a* (*arf3a*), an abaxial identity gene. Previous analyses showed AGO7 forms a complex with microRNA390 (miR390) to cleave the non-protein coding *tas3a* transcript. Non-mutant maize plants express miR390 in the adaxial surfaces of young leaf primordia and throughout the incipient primordium, but rgd2 mutants show an expanded miR390 expression domain throughout the shoot apical meristem, suggesting RGD2 may be required to properly localize miR390. The *tas3a* cleavage fragment is stabilized by SUPPRESSOR-OF-GENE-SILENCING3 (SGS3) and turned into a double-stranded RNA (dsRNA) by RNA-DEPENDANT RNA POLYMERASE6. The dsRNA undergoes phased cleavage by DICER-LIKE4 to form ta-siARFs, which form a complex with AGO1 to cleave *arf3a* trancripts. The morphology of the rgd2 mutant is macroscopically equivalent to the leafbladeless1 (lbl1) mutant; *lbl1* encodes the maize orthologue of SGS3 and is predicted to function directly downstream of RGD2. Although *rgd2* is expected to be epistatic to *lbl1*, rgd2/lbl1 double mutants exhibit synergistic shootless phenotypes. These genetic data suggest that in addition to its role in ta-siARF biogenesis, LBL1 functions in a separate pathway(s) apart from RGD2. qRT-PCR analyses of *arf3a* in rgd2 shows that *arf3a* levels are increased, and that this increase alone is insufficient to confer an abaxialized leaf phenotype.

P75

Auxin Evo-devo: Genetic and genomic approaches to understanding the role of auxin in shoot development

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Auxin regulates almost every aspect of plant growth and development. A better understanding of the role of auxin is fundamentally important to basic plant biology and crop improvement. Previous research has demonstrated both conservation and diversification of the role of auxin in maize and Arabidopsis. At least six gene families are required for auxin-mediated organogenesis but how these genes are integrated into a single pathway is uncertain. This project will leverage tools and resources generated from prior NSF-funded genome projects to further our understanding of how auxin regulates shoot development, with an emphasis on maize shoot organogenesis. To identify additional genes functioning in auxin-mediated organogenesis, we will use high-throughput forward genetics and a novel method for transient local auxin induction to perform comparative expression profiling in both maize and Arabidopsis. Phylogenetic analysis will guide reverse genetic functional analysis of newly identified genes. Furthermore, phylogenetic characterization of all gene families identified, in combination with comparative expression analyses, will test the conservation and diversification of mechanisms of auxin action in all flowering plants. Determination of the molecular, cellular, biochemical and genetic interactions between components in the pathway will provide the detailed information necessary to begin assembling a network of gene interactions. Statistical and mathematical modeling will integrate the expression profiling and interaction data to construct a gene regulatory network which will provide hypotheses for future experimentation. Together, these inter-disciplinary approaches will expand our basic knowledge of plant development and contribute to ongoing efforts to improve maize and other crops for use as both food and fuel.

P76

BLADEKILLER1 functions in SAM maintenance and leaf blade formation as a putative regulator of cytokinin signaling

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The maize gene bladekiller1 (blk1) is necessary for the perpetuation of meristem populations throughout the plant, and in the formation of the leaf blade. Plants homozygous for the blk1-R mutant allele show a reduction in shoot apical meristem (SAM) size that often results in premature shoot termination. Additionally, blk1 functions in the initiation and maintenance of the meristem populations that give rise to the mature ear and tassel. Mutant plants often have a significant reduction in inflorescence size and in the number of initiated spikelet meristems. Interestingly, the meristem phenotype correlates with a progressive reduction in the length and width of the leaf blade. While leaves at lower nodes appear normal, upper leaves are often bladeless and consist of just sheath and ligule. Closer investigation of mature mutant leaves reveals that they accumulate extra epidermal cell layers on the upper/adaxial side of the leaf. Double mutant analysis suggests that blk1 may function in an overlapping pathway with abphyl1, a cytokinin response regulator that represses meristem size. Plants homozygous for the abphyl1-O and blk1-R mutations initially have characteristics of abphyl1 single mutants, including a first leaf with two midribs. However, later in development mature double mutant plants appear as blk1 single mutants. Longitudinal sections through double mutant meristems reveal additive effects on SAM size. In situ and qRT-PCR expression analysis show that abphyl1 transcript accumulation is down in blk1 mutants, suggesting a possible role for blk1 in cytokinin regulation. This regulation may be downstream of cytokinin synthesis as expression of Zmlog1, a gene implicated in activating cytokinin precursors, is normal. Efforts are currently underway to uncover the identity of the blk1 locus and to further examine the potential role of BLK1 in hormone regulation.

P77

Barren stalk fastigiate1 encodes a transcription factor required for inflorescence development in maize

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In the past few years several genes controlling the formation of axillary meristems in maize inflorescences have been isolated. Bif2, Spi1 and Ba1 are examples of such genes that have been shown to function either in auxin transport, synthesis or response. Mutations in any of these genes cause defective development of axillary meristems, in both tassel and ear. Here we characterize a maize mutant called barren stalk fastigiate1 (baf1). Mutant plants manifest dramatic effects on ear formation, and are often missing ears completely. On the other hand, the tassels of baf1 mutants show only mild defects, such as a peculiar upright tassel branch phenotype, due at least in part to the fusion of the branch base with the main spike, and the occasional occurrence of single instead of paired spikelets. When ears are formed on mutant plants, they develop fused to the main stalk but remain fertile. We positionally cloned the Baf1 gene and find it encodes a putative transcription factor containing an AT-hook DNA binding domain. Transient expression of a Baf1-YFP fusion protein revealed that Baf1 is nuclear localized, as expected for a transcription factor. Expression analysis of Baf1 transcripts reveals a highly specific domain of expression during inflorescence development. The role of baf1 in the development of tassel and ear will be discussed.

P78

CREUSA1 functions downstream of RGD2 during mediolateral and dorsiventral patterning of the maize leaf

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The maize leaf is delimited by three axes: proximodistal, mediolateral and adaxial-abaxial. RAGGED SEEDLING2 (RGD2) is involved in establishing mediolateral polarity during early stages of leaf development in maize. Recessive, loss-of-function mutations in rgd2-R often condition a broad spectrum of mutant leaf phenotypes on a single plant--narrow leaf blades reflect mild manifestations, whereas the more severely affected leaf is radial. Such radial leaves, however, retain adaxial-abaxial markers, such as collateral vasculature, making rgd2 unique among other radial-leaf mutants. Previous work in our lab utilized laser microdissection-micorarray to precisely dissect and quantify differential transcript accumulation in the meristems of rgd2-R mutants relative to those of their non-mutant siblings. This analysis yielded 170 genes implicated to function in a rgd2 fashion. Reverse genetics analyses were launched to investigate the function of creusa1 (cru1), a gene of unknown function down-regulated in meristems of rgd2 mutants. Loss of cru1 function results in a range of aberrant mediolateral differentiation similar to weak phenotypes of rgd2-R; some leaves display partial loss of lamina development, and others show partial bifurcation along the midrib. Abaxial ectopic outgrowths observed near the sheath-blade junction and reduction in xylem differentiation suggest that CRU1 also functions during the establishment or maintenance of adaxial-abaixal polarity. in situ hybridization reveals cru1 is expressed in the vasculature of leaf primordia at plastochrons 1-4 (P1-P4), a pattern which, in the case of P1, precedes and predicts vasculature development. This work demonstrates the potential of the RGD2-associated gene database for unraveling genetic pathways that function during leaf development.

P79

Cellular growth responses of maize leaves to cold and drought differ and correspond to specific transcriptional and metabolic changes

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Even in temperate climates, early season low temperatures and periods of drought affect the growth of maize seedlings and limit yield. To study the effects of cold and drought, we quantified leaf growth of maize seedlings at low night temperature (4C) and at limited water supply (soil water potential of approximately -1 MPa). Both treatments resulted in a significant 25% reduction of the steady-state leaf elongation rates, but kinematic analysis revealed a different cellular response. Both treatments inhibited meristem activity, but in a different manner: low temperature reduced the production of meristematic cells by prolongation of 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 process. 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 profiled the transcripts of dividing, elongating and mature cells of stressed and non-stressed leaves by micro-array experiments. In addition, we complemented the transcript profiles with the quantification of approximately 150 metabolites by GC-MS analysis. This allowed us to identify both transcripts and metabolites with profiles that are associated with the cellular growth responses to the environmental conditions tested. These results revealed new insights in the molecular, cellular and physiological responses to these agronomical important stress conditions.

P80

Characterization and cloning of vanishing tassel2 (vt2): a gene which functions in auxin biosynthesis in maize inflorescence development

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The plant hormone auxin plays a key role in many aspects of growth and development. Cell elongation, inflorescence and root development, and leaf initiation are all known to be controlled by auxin in maize. Disruption in auxin biosynthesis, transport, or response causes very severe effects on plant growth.

Here we present the characterization and cloning of vanishing tassel2 (vt2), a recessive mutation in maize found to cause significant defects in development. vt2 mutants have altered vegetative and reproductive phenotypes, including severely reduced plant height and almost completely barren inflorescences. Quantitative analysis has confirmed significant reductions in plant height, leaf number, ear number, spikelet and kernel number, and tassel and ear length compared to normal. In addition, scanning electron microscopy has confirmed that vt2 mutants produce fewer axillary meristems than normal in developing tassels and ears.

We have cloned vanishing tassel2 using a positional cloning approach and have confirmed cloning with four alleles. vt2 encodes an enzyme required for auxin biosynthesis. Expression analysis is currently underway to further elucidate the function of vt2 in maize development. Finally, double mutant analysis has further characterized the role of vt2 and has revealed surprising interactions between vt2 and other auxin-defective maize mutants, such as the barren class of mutants.

P81

Characterization of Brachytic tasselseed-1 (brts1); a regulator of growth, tillering and sex determination

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Manipulation of plant architecture is an important component of yield, regardless of whether yield is measured as harvestable grain or the overall biomass of crop plants. Many mutants that impact this trait have been identified in maize, although only a few genes underlying these mutants have been cloned. These include Brachytic-2 (br2), an ABC transporter responsible for auxin transport, and several genes involved in gibberellic acid (GA) biosynthesis/signaling.

The focus of this poster is a newly discovered recessive maize mutant which is not only compromised in growth and height but also exhibits a tassel seed phenotype to varying extents. As a result, we have named this mutant brts1. In addition to the severe reduction in phytomer dimensions, leaves of brts1 mutants show helical twisting. Surprisingly, we also found that the brts1 mutation completely suppresses the ability of maize to tiller. A combination of these phenotypes has not been described in maize mutants to date. Our results indicate that the pleiotropic mutant phenotype of brts1 is not due to a deficiency in GA or auxins, two phytohormones that play key roles in maize growth and development. Efforts to examine the involvement of brassinosteroids and jasmonic acid in the brts1 phenotype have been initiated.

Presently, we have two alleles of brts1, both of which were isolated from populations active in Mutator (Mu) activity. Both of these alleles have been advanced for cosegregation analysis to identify Mu elements that may have caused these mutations. Crosses have also been made to generate additional alleles of brts1 by directed mutagenesis with Mu. As an alternative approach to clone brts1 by map-based cloning, F2 populations of this mutant have been generated with B73, A188, A619 and Mo20W. These F2 populations are being evaluated with simple sequence repeat (SSR) primers to reveal the genomic location of brts1. Now that all these tools are in place, we hope to make rapid progress with the mapping and cloning of brts1.

P82

Characterization of ZmBIP2, a monocot specific bHLH transcription factor which interacts with BARREN INFLORESCENCE2 and BARREN STALK1

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Maize inflorescence architecture is determined by the activity of axillary meristems. Axillary meristem initiation and organogenesis is controlled by the polar transport and accumulation of the hormone auxin. BARREN INFLORESCENCE 2 (BIF2) encodes a serine threonine protein kinase, co-orthologous to PINOID. As in Arabidopsis, BIF2 phosphorylates the PINFORMED auxin efflux carriers, which are required for polar auxin transport. Additionally, BIF2 interacts with, and phosphorylates, BARREN STALK1 (BA1), an atypical basic helix-loop-helix transcription factor required for axillary meristem initiation in maize. bif2 mutants initiate few axillary meristems resulting in barren tassels with few branches and spikelets, as well as ears with few kernels. ba1 mutants also have barren tassels and double mutant analysis suggests that bif2 and ba1 function in the same pathway. Therefore, genetic and physical interactions suggest BA1 may be a target of BIF2 during axillary meristem development. Here, we present the identification and characterization of Zea mays BIF2 INTERACTING PROTEIN2 (ZmBIP2), an atypical bHLH protein which belongs to a monocot specific lineage with no co-ortholog in eudicots. We show that ZmBIP2 physically interacts and co-localizes with BIF2 and BA1. ZmBIP2 is also shown to homodimerize and heterodimerize with BA1 in vitro and in planta. Overexpression of ZmBIP2 in Arabidopsis results in a barren inflorescence with ectopic carpelloid tissue on the few flower-like structures that form. We propose that ZmBIP2 interacts with BIF2 and BA1 to function in auxin mediated control of proper inflorescence development in maize.

P83

Characterization of maize cellulose synthase (CesA) mutants and expression profiling of the CesA gene family at the whole-plant and cellular levels.

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The maize cell wall is a composite of numerous carbohydrate-based constituents. Elucidating the genetic mechanisms 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. Here, we characterize knockout mutations in three cellulose synthase genes (CesA7, CesA8, and a previously undescribed paralog of CesA7). Interestingly, CesA7 and CesA8 were previously found to be expressed at relatively high levels in tissues undergoing the transition from primary to secondary cell wall biosynthesis (Appenzeller et al., 2004). The mutants analyzed in the present work were generated in the transposon-mutagenic Uniform Mu population, thus providing a means to investigate their phenotypic effects in a uniform, inbred background. The CesA mutants are being tested for phenotypic associations under field conditions. Thus far, single knockout mutants have not been linked with a readily-detectable phenotype, however preliminary evidence indicates that double knockouts may be responsible for severe kernel phenotypes (defective embryo and/or empty pericarp). Developmental and morphological characterization is continuing, and a triple knockout mutant is being developed. In addition, gene expression analyses are being conducted for the entire maize CesA gene family at the whole-plant and cellular levels. Tissue-specific gene expression has been characterized throughout development, and a protoplast system is being used to evaluate the role of each cellulose synthase in de-novo cell wall biosynthesis. To accomplish this, protoplasts are sampled at designated time points throughout cell wall regeneration. Expression profiles from endosperm- and embryo-derived protoplasts will be compared to better understand differences in cell-wall biosynthesis by different cell types.

P84

Characterization of rough endosperm3 genetic modifiers

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The rough endosperm3 (rgh3) mutant causes developmental defects that are either seed or seedling lethal. Rgh3 is likely to encode a U2AF35 Related Protein (ZmURP), which is a predicted RNA splicing factor. U2AF35 proteins identify splice acceptor sites during RNA processing and function through protein-protein interactions. Genetic modifiers of rgh3 can identify genes that act within the same pathway as rgh3, and we investigated whether natural variation could modify the rgh3 phenotype. The rgh3 mutation was originally isolated from the UniformMu population in the W22 inbred background. We crossed rgh3 to the B73 and Mo17 inbreds. Mutant seedlings from the B73 F2 population showed a distinct, open leaf and dwarfed phenotype in comparison to a hooked, adherent leaf phenotype for rgh3 mutants in the W22 background. Mutant seedlings from the Mo17 F2 population were partially suppressed, but showed a more continuous gradient of phenotypes. In order to confirm these variations, we also backcrossed F1 plants to rgh3/+ in the W22 background. The backcross from the B73 F1 segregated for the B73-like seedling phenotype in a 1:1 ratio. These data are consistent with a single dominant modifier. We mapped the B73 modifier to chromosome 8 using approximately 120 SSR markers that are spaced throughout the genome and polymorphic between B73 and W22. These data suggest that map-based cloning of the B73 modifier is feasible and may identify a target or interacting protein of ZmURP.

P85

Developmental Stage, Reproductive Tissue, and Cytotype Effects on Transcriptional and Post-Transcriptional Regulation of Mitochondrial Genes

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Cytoplasmic male sterility (CMS) is maternally inherited and results from the interaction of nuclear and mitochodrial expressed gene products. We have profiled transcripts and protein products of mitochondrial genes in developmentally staged pollen and developing female reproductive structures (immature ears) from isogenic NB and CMS-S maize cytotypes that do not have CMS-S nuclear fertility restoring alleles. Western blots showed the accumulation of mitochondrial ATP synthase and respiratory complex subunits were clearly reduced in both CMS-S and normal microspores as compared to the immature ears. We examined RNA editing, a post-transcriptional feature of plant mitochondrial gene expression, to test a possible mechanism for producing the observed tissue and developmental stage specific phenotypes. We report on the editing patterns determined from sequence analysis of RT-PCR products of atp4, atp6, atp8, and atp9 for microspores and immature ears of our isogenic Mo17 CMS-S and NB cytotypes. In addition, the comparison of these results to publically available data of NB editing, and results from RNA editing prediction software will be presented.

P86

Dissecting Cis-Regulatory Code of Putative Bidirectional Promoters in Cereal Genomes

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A promoter regulating the expression of adjacent divergent genes organized in a head-to-head fashion can be defined as a bidirectional promoter. A subset of putative bidirectional promoters from rice, sorghum, and maize were identified by computational analysis based on intergenic distances of <1 kb and <250 bp between adjacent genes with divergent arrangement. Promoters whose adjacent divergent genes showed conserved gene order and orientation were identified. We have identified overrepresented cis-regulatory motifs in these promoters using Plant Cis-acting Regulatory DNA Element (PLACE) database. In addition, we have identified conserved motifs in orthologous promoters of rice, sorghum, and maize using phylogenetic footprinting. To identify regulatory regions with respect to bidirectional activity, rice promoters are being analyzed using deletion constructs. Five full length promoters and their deletion constructs were cloned in a binary Gateway vector to drive the expression of enhanced green fluorescent protein (egfp) and red fluorescent protein (rfp) genes in forward and reverse orientations, respectively. Transient expression assays were carried out on 15-20 day old rice seedlings using Agrobacterium by surface scratch method. We report the spatial and temporal expression patterns of reporter proteins in rice. Linker scanning mutagenesis will be used to identify cis-regulatory motifs involved in bidirectional activity. Bidirectional promoters might be involved in novel gene regulatory mechanisms. These promoters can be used in coordinating expression of multiple genes in metabolic engineering and molecular farming to produce vaccines, pharmaceuticals and plastics.

P87

EXPRESSION PATTERNS OF BEARDED-EAR ORTHOLOGS IN THE GRASS FAMILY

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ZAG3 (Bearded-ear) is a MADS-box gene of maize orthologous to the AGL6 and AGL13 genes of Arabidopsis. Many studies have shown that AGL6 is primarily a floral specific gene but its role during flower development is unclear in part because its expression pattern varies from one lineage to the next. Some authors have suggested that AGL6-like genes may have an important role in perianth evolution. The zag3 mutant (bearded-ear, bde) produces extra flowers and extra carpels suggesting that ZAG3 is involved in floral meristem determinacy and carpel development. We previously showed that the phylogeny for the ZAG3-like genes in grasses largely agrees with the species phylogeny. Two different duplication events were discovered: a) around the base of the grasses, leading to two paralogous clades (OsMADS6 and OsMADS17), and b) in Zea and Tripsacum (ZAG3 and ZAG5). Here we present an update of the phylogeny as well as data from mRNA in situ hybridization in distantly related grass species. In situ hybridization showed that ZAG3 orthologs in grasses are expressed initially in floral meristems. Later in development, mRNA was detected in lodicules and in the developing gynoecium, in which it becomes restricted to the inner integument. Expression in stamens was only detected in the basal grasses (i.e. Streptochaeta angustifolia) and in subfamily Ehrhartoideae (i.e. Oryza sativa and Leersia sp.). Expression in the palea was only detected in members of subfamily Panicoideae (i.e. Sorghum bicolor and Setaria italica). OsMADS6 and OsMADS17 showed overlapping expression patterns. These results suggest that ZAG3 may play a general role in the grasses during lodicule and carpel development. In addition, expression in stamens and paleas in some lineages suggests that ZAG3 orthologs have diversified in grasses and may have different roles in different species.

P88

Expression and Functional Characterization of 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. Modern maize is less sensitive to photoperiod than teosinte 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. It has been shown previously that regulation of flowering by CO and Hd1 is both transcriptional and post-translational. CO and Hd1 are transcriptionally regulated and exhibit diurnal patterns of expression in both inductive and non-inductive photoperiods. Furthermore, CO is regulated post-translationally, as CO protein is ubiquitinated and rapidly degraded in the dark. It is presumed that HD1 protein is stable, as it acts to promote flowering in inductive photoperiods and to repress flowering in non-inductive photoperiods. To begin to understand the photoperiod pathway in maize and the function of conz1, its expression was characterized. 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. The distinct expression pattern of conz1 in long-days cannot be explained by the expression of maize homologs of GIGANTEA (GI), the upstream activator of CO in Arabidopsis. Thus, I have shown that the expression of conz1 responds to photoperiod. To better understand the function of conz1, a 9.4kb genomic fragment containing conz1 was transformed into rice plants lacking functional Hd1. Flowering time of the transformants suggests that conz1 restores photoperiod sensitivity and complements loss of Hd1 function. These results suggest that the cis-regulatory regions of conz1 are conserved between rice and maize and that CONZ1 protein is functional in a heterologous system.

P89

Expression of the Fas1 Paralogs in Maize

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The FASCIATA1 (Fas1) gene encodes the largest subunit of chromatin assembly factor-1 (CAF-1), which is involved in DNA replication-associated chromatin assembly and is essential for proper development in plants. In Zea mays there are two Fas1 paralogs designated ZmFas1a and ZmFas1b, which encode proteins with approximately 90% amino acid similarity. ZmFas1a and ZmFas1b are located on chromosomes 8 and 3, respectively. Plant FAS1 proteins contain a charged domain rich in amino acids lysine (K), glutamic acid (E) and arginine (R) referred to as the KER domain, which is thought to form a histone interacting coiled-coil structure. This domain contains a highly conserved 25 amino acid long motif that is found once in Arabidopsis FAS1, while maize FAS1a and FAS1b have 3 and 4 repeat motifs, respectively. ZmFas1 as well as ZmFas2 and ZmMSI1, which encode the remaining two subunits of CAF-1, are expressed at a higher level in shoot apical meristem as compared to whole 14 day seedling. Analysis of 454 sequencing data derived from reverse transcribed B73 shoot apical meristem mRNAs indicates that ZmFas1a and ZmFas1b are expressed at the same level in this tissue. RT-PCR analysis of multiple tissues from B73 plants indicates that both ZmFas1 paralogs are expressed to a similar level in all tissues examined; tissues that are mitotically active exhibit a high level of expression, consistent with CAF-1 molecular function. In contrast, analyses of seedlings from 11 North American inbred lines, reciprocal hybrids of B73 and Mo17, and 5 open-pollinated Mexican land races demonstrated variable ZmFas1 paralog expression, with ZmFas1b generally being expressed at a higher level.

P90

Finding Direct Targets of Maize Transcription Factors

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Transcription factors (TFs) function in networks and play essential roles in many biological processes. Identification of direct targets for TFs is the cornerstone for understanding these regulatory networks, yet still remains a bottleneck in deciphering signal transduction pathways. Towards the goal of establishing the architecture of regulatory networks in important crops, and linking system components to agronomic traits, a main objective of this research is to identify direct targets of some well characterized TFs from maize, complementing the development of GRASSIUS (www.grassius.org) as a public web resource for regulatory information in grass crops.

To fulfill the objective, three strategies are being combined: 1) SELEX (Systematic Evolution of Ligands by EXponential enrichment) to identify transcription factor DNA-binding preferences in vitro, 2) ChIP (Chromatin ImmunoPrecipitation) to identify/confirm putative targets in vivo, and 3) ChIP-Seq (ChIP coupled with high through-put Sequencing) to identify genome-wide targets. Here, we report our progress on OPAQUE2 (O2). In vivo O2 binding to the -326 and -54 promoter of the 22Z-4 gene was confirmed by ChIP, providing confidence in our methodology and valuable material for our ChIP-Seq experiments. Progress on other transcription factors will be also described. This project is funded by NSF DBI-0701405.

P91

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 prerequisite for a normal development of the plant embryo. HD-ZIP IV transcription factors including FWA, GL2, AtML1 or PDF2 in Arabidopsis and OCL1 to 5 (Outer Cell Layer) in maize, seem to play crucial roles in the differentiation and maintenance of 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 at the apex and is seedling lethal. In maize, OCL1 is specifically expressed in the epidermis of the embryo, the endosperm and young organ primordia. To identify direct or indirect target genes of OCL1, the transcriptome of 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 qRT-PCR. Expression patterns of each gene in the maize plant were established by qRT-PCR and in situ hybridization. Annotations obtained by bioanalysis revealed that several target genes encode proteins involved in lipid metabolism, defense or cuticle deposition. A preliminary GC-MS analysis showed defects in the distribution of waxes in OCL1-OE juvenile leaves compared to wildtype. Whenever available, the promoter sequences were scanned for the presence of L1-box, the cis-element of HD-ZIP IV transcription factors in Arabidopsis. A L1-box was found in the promoter of three target genes which encode for a Lipid transfer protein (Ltp), an ABC transporter (ABCt) and a TPR protein. Biolistic trans-activation assay indicated that OCL1 may directly activate the Ltp and the ABCt promoters. Mutations in the L1-box of the ABCt promoter caused the loss of its trans-activation by OCL1 suggesting that it occurs by a direct binding of OCL1 on its L1-box.

P92

Genetic and Anatomical Analysis of Upright Tassel Architecture

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In grass species, the pulvinus is a structure found in the axil of inflorescence branches. The size and shape of the pulvinus affects the angle at which the lateral branches emerge from the main axis, and therefore has a large impact on inflorescence architecture. Through EMS mutagenesis we have identified four different recessive mutants in maize that cause the tassel branch angle to become more acute. In general, mutant plants have a much smaller pulvinus than their normal sib plants. Two of the four mutants we identified as alleles of ramosa 2 (ra2) and liguless1 (lg1). Ra2 encodes a LOB domain transcription factor previously shown to control inflorescence architecture by limiting growth of axillary meristems and affecting tassel branch angle (Bortiri et al., 2006). Lg1 encodes a conserved Zinc finger SBP-type domain and lg1 mutants are lacking the leaf ligules and auricles (Moreno et al., 1997). In addition, we have identified two new mutant loci, SLO271 and SLO365, and mapped them to bin 2.02 and 2.06, respectively. Positional cloning suggests that SLO365 encodes a transcription factor belonging to TCP family (TEOSINTE BRANCHED1/CYCLOIDEA/PCFs family). TCP proteins are plant-specific transcription factors which are shown to be involved in specifying plant morphologies and controlling biosynthesis of the hormone jasmonic acid. We are in the process of positionally cloning SLO271 with the hope of understanding how these different loci interact to promote pulvinus formation and inflorescence branch angle variation. Inflorescence branch angle varies in other grasses such as sorghum and rice, and has become fixed in various inbred lines. We will investigate the possible conservation in this program of inflorescence development using the genes we have identified in maize.

P93

Genetic control of aleurone differentiation in maize

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The aleurone layer, which coats the peripheral endosperm, helps accumulate storage molecules in the endosperm of maturing seeds and supplies secreted hydrolases to digest storage molecules in the endosperm when seeds are germinating. The aleurone is a good model system to study cell fate because of its simplicity, easy accessibility, and convenient markers. A hierarchical model for the regulation of aleurone development has been proposed with genes such as cr4 and dek1 functioning in cell fate specification and the naked endosperm (nkd) gene functioning downstream in cell differentiation. A new mutant, thick aleurone*, functions as a negative regulator of aleurone cell fate; thk* mutants have aleurone layers averaging 4-6 cells thick instead of the normal single cell layer. Surprisingly, thk* appears epistatic to dek1 suggesting that Dek1+ is a negative regulator of Thk+. The nkd mutant shows a partially differentiated aleurone layer and the mutant phenotype shows a 15:1 segregation ratio indicating it is caused by 2 recessive genes. Double mutant analysis suggest that Thk* functions upstream of nkd. Both genes function upstream of Vp1::GUS. We have begun mapping the nkd and thk genes using IDP markers and markers we developed. The nkd1 locus has been localized to chromosome 2L in an interval of 6 BACs between b019B15 and c0166I20. The thk* locus maps to the distal tip of chromosome 1S. Learning the molecular identity of these genes will provide key information for explaining how aleurone development is regulated and could lead to improved cereal grain quality.

P94

Genome-wide resolution of transcriptional changes in ramosa3 inflorescences using Illumina's digital gene expression analysis

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Branch patterning in maize inflorescences is an important trait relating to seed number and harvesting ability. Although genetic studies have identified key genes that regulate inflorescence architecture, links to associated metabolic and regulatory pathways have not been resolved on a genome-wide scale. In this work, we used Illumina's digital gene expression (DGE) platform to compare transcript profiles in B73 and ramosa3 (ra3) mutant maize. The RA3 gene encodes a trehalose-6-P phosphatase and mutants in this gene display a highly branched ear phenotype in the B73 background. Unlike the other known transcription factors in the RAMOSA pathway, the RA3 sequence is indicative of a metabolic enzyme, suggesting a putative link between developmental and biochemical processes. We used three immature ear samples from both B73 and ra3 mutant plants to construct six 3'-anchored, DGE libraries. Each library included DpnII and NlaIII digests, which were multiplexed during sequencing based on specificity of their respective 4-base restriction sites. Deep sequencing of all six DGE libraries, plus an additional ra3 technical replicate, generated ~27 million 20-mer reads with frequencies spanning four orders of magnitude. Based on a 3-read cutoff in B73 or ra3 samples, we identified 18,154 and 25,777 unique, consensus sequences from the combined DpnII- and NlaIII-generated libraries, respectively. Approximately 80% of these non-redundant sequences mapped perfectly to the maize reference genome while ~6% mapped to repetitive regions. Coordinates for these mappings were used to associate the unique sequences with gene models and annotations from a working set of 113,671 genes. Our analyses showed that 75% of DpnII- and NlaIII-tags mapped to corresponding regions of the genome and identified differentially expressed genes in B73 and ra3 libraries. Results from this study demonstrated the effectiveness of DGE-based transcript profiling in maize and resolved differential expression profiles that will help define roles of the RA3 gene.

P95

Histological study of Maize ear development

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Plants architecture and reproductive development rely mainly on a proper organization of meristematic tissues. Meristems are pluripotent cells able to regenerate and provide cells to form organs such as leaves, stems or inflorescences. Two types of meristems are generally described. Indeterminate meristems keep on generating cells until organs senescence. Determinate meristems stop producing cells following a determinate fate.

In maize, female inflorescence development is an excellent illustration of meristems determinism. Maize ear spikelets result from four successive meristems. As monoecious species, maize female immature meristem (IM) emerges on the axil of leaves from the shoot apical meristem (SAM). Then the indeterminate IM produce three successive determinate meristems; the spikelet pair meristems (SPM), the spikelet meristems (SM) and finally the floral meritems (FM).

As a combination of successive meristems, maize female inflorescence remains a useful tool to investigate meristem fate. To achieve this purpose, we are currently developing robotized facilities for mRNA in situ hybridization and immunolocalization. Homologs of SAM Arabidopsis genes such as Clavata, Wuschel, PIN, CycB1 ... are primarly used to evaluate the systems.

P96

Identification of ramosa1-63.3359 phenotypic modifiers using the IBM population

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ramosa1 (ra1) encodes a C2H2 zinc finger transcription factor that imposes determinacy on branch meristems in developing inflorescences. Mutants of ra1 display increased tassel branch number as well as branching on the ear. ra1-63.3359, an allele of ra1 possessing a frameshift mutation that adds 17 extra amino acids at the C-terminus, exhibits a background-dependent phenotype. When introgressed into B73, this allele shows a strong branched-inflorescence phenotype. However, the phenotype in Mo17 is much weaker, with tassels looking relatively normal and ears possessing few to no branches. Using the IBM population, we exploited these phenotypic differences to map modifiers of ear-branch number. Each RIL in the IBM-94 population was backcrossed to Mo17- and B73-introgressed ra1-63.3359 to generate two sets of F1BC1 populations that segregated ra1-63.3359. For each population, ear-branch number was used to map modifiers of branching. Using this method, we were able to identify several loci in Mo17 and B73. We will present the results of this analysis as well as preliminary data for the fine mapping of these loci.

P97

Inflorescence branching in maize: An unsuspected function of RAMOSA3?

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Inflorescence architecture is a defining feature of plant development that is critical for reproductive success, and thus crop yields. The architecture of the maize inflorescence is determined by networks of genes controlling the specification, maintenance, and determinancy of pools of stem cells called meristems. The classical ramosa (ra1, 2, 3) mutants have highly branched inflorescences, due to increased activity of spikelet pair meristems. RA1 and RA2 encode transcription factors, but RA3 is predicted to be a trehalose metabolic enzyme. RA3 is a functional TPP in vitro and in vivo in yeast; however, several lines of evidence suggest that it could have an additional unsuspected function as a transcriptional regulator in maize: 1) RA3 has a discrete expression pattern subtending axillary meristems, suggesting a developmental, rather than general metabolic role; 2) RA3 is genetically upstream of RA1 and regulates its expression; 3) RA3 immunolocalization shows both nuclear and cytoplasmic localization; 4) A Yeast 2-Hybrid screen suggests that RA3 interacts with several transcription factors, including those belonging to the Zinc Finger Homeodomain (ZF-HD) class. In order to test the hypothesis that RA3, like some metabolic enzymes in yeast, has an additional role in transcriptional regulation, we plan to confirm RA3 interacting proteins with immunoprecipitation experiments using tagged transgenic lines. We will also directly assay whether RA3 occupies the promoter of RA1 in vivo via Chromatin Immunoprecipitation (ChIP). In addition, we will conduct expression profiling of immature inflorescences in ra3 mutants and wild type backgrounds in order to identify potential targets and establish the steady-state effects of loss of RA3 function.

P98

Investigating DISCOLORED1 (DSC1) function during maize kernel development

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The discolored1 (dsc1) mutation, identified in a screen of defective kernel mutations arising from a Mutator stock collection, is being used to investigate kernel development. Mutant kernels are easily detected as brownish, malformed kernels on ears segregating kernels homozygous for the dsc1 mutation. Phenotypic analysis has illustrated a delay in both embryo and endosperm development in dsc1 mutant kernels from 8 DAP through 16 DAP followed by degeneration or complete deterioration of mutant kernels by 20 DAP. Partial sequence of the dsc1 locus was previously identified using Mu transposon tagging and subsequently used in 3' RACE RT-PCR to acquire a 2.469 kb cDNA. The partial genomic sequence from the dsc1 locus and the cDNA sequence both align to the genomic sequence on BAC AC197554. The dsc1 gene putatively encodes an 823 aa ACAP type ADP-ribosylation factor GTPase activating protein (ARF-GAP). ARF-GAPs function in endomembrane trafficking and belong to a large family of proteins that are conserved in many species including humans, drosophila, yeast, Arabidopsis, and maize. In maize, there are putatively 36 genes that encode ARF-GAPs based on in silico predictions using the maize gene space. ADP-ribosylation factors (ARFs) cycle between an active and an inactive state that facilitates cargo binding and vesicle formation or vesicle dissociation and cargo release, respectively, at intracellular membranes. ARF-GAPs are necessary to deactivate ARFs so that vesicles are able to fuse with their acceptor membrane to release their cargo. The DSC1 putative orthologue in Arabidopsis is VASCULAR NETWORK3/SCARFACE, an ARF-GAP that functions in the recycling of PIN1 and is necessary for auxin efflux. Currently we are using YFP tagged transgenic lines to determine if auxin efflux or other cargo is misdirected in dsc1 mutant kernels. Future prospects include performing complementation tests in Arabidopsis and generating additional mutant alleles through reverse genetics.

P99

Investigation of putative long-distance, flower promoting signals mediated by maize homologs of the Arabidopsis FT/FD regulatory module

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Higher plants use a multitude of perceptive measures to regulate the floral transition, a process that involves careful coordination of both environmental and endogenous cues. Recent molecular and genetic analyses in Arabidopsis, a dicot plant that flowers more rapidly in response to long day photoperiods, suggest that a long-distance florigenic signal may be the product of FLOWERING LOCUS T (FT). FT is activated in mature leaves by the CONSTANS (CO) regulatory protein and travels through the phloem to the shoot apex where it interacts with the transcription factor FLOWERING LOCUS D (FD). The FT/FD complex then activates a cascade of floral identity genes to promote the transition to flowering. In contrast to Arabidopsis, temperate maize is largely a Day-Neutral (DN) plant, while teosinte, the progenitor of maize, has an obligate requirement for short day photoperiods to flower. Recent discoveries of FD and FT homologs in maize (DLF and ZCN, respectively) support the notion of a universal, long-distance florigenic signal that induces floral transition. We are attempting to ascertain whether or not FT and FD functional orthologs are conserved in maize and, if so, whether a maize-specific FT/FD-like regulatory module functions to promote the transition to flowering. We are comparing the expression of these genes in wildtype vs. indeterminate1 mutant (severe late flowering) maize as well as florally-induced vs. uninduced teosinte. Preliminary evidence shows that several of the putative maize FT orthologs have unique expression patterns in comparison to Arabidopsis FT with respect to the floral transition. Other data suggest that ID1 acts upstream of the ZCNs.

P100

Mapping and Characterization of the Fascicled ear1 mutation

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To form stereotypical ears and tassels, maize inflorescence meristems must maintain organized, coordinated growth by timing organ separation precisely and restricting proliferation. Dominant Fascicled ear1 mutants fail to maintain a single terminal center of proliferation in their inflorescence meristems, resulting in bifurcation of the terminal growing points of both the tassel and the ear. Our previous studies show that this mutation does not affect vegetative traits. The reference allele was discovered in a Mexican maize population by Dr. Paul Weatherwax and reported in 1917. We have shown a second allele, Fas1-SH, to be independent using RFLP analysis. This allele was found in a targeted Mu tagging ra2. A putative third allele, Fas1* was identified in an EMS screen. Genetic mapping by David Jackson in 1998, placed Fas1 27cM proximal of Rld1 on chromosome arm 9L. Through positional cloning, the Fas1 locus has been determined to be within 2 non-overlapping BACs in bin 9.06. Progress sequencing candidate genes in the region is discussed as well as additional phenotypic analysis.

P101

Metabolic analyses of extremely late-flowering indeterminate1 maize mutants reveal metabolic traits that may be associated with the floral transition in maize

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The INDETERMINATE1 (ID1) transcription factor is a key regulator of the transition to flowering in maize. ID1 is expressed exclusively in immature leaves where it controls the production or transmission of leaf-derived florigenic signals. Previously, we performed microarray analysis of immature leaves of the severely delayed flowering time mutant id1 and wild-type maize plants in order to identify downstream targets of ID1. Intriguingly, we found that a significant proportion of genes up-regulated in id1 mutants compared to wild-type plants were associated with carbohydrate metabolism, photosynthesis, and carbon fixation. We are currently investigating the metabolic traits (starch, protein, total soluble sugar, metabolic profiles) of id1 mutant mature (source) and immature (sink) leaves at the point of transition to reproductive development. Starch levels in id1 mutant plants are not significantly altered compared to those in wild-type plants. This finding is consistent with transmission electron microscopy (TEM) examination of id1 mutant leaves which reveals no observable difference in plastid ultra-structure or starch granule size. Preliminary analyses suggest that ID1 activity is associated with significant carbon and nitrogen metabolic alterations in mature leaves, a trait which may be vital for the onset of the floral transition in maize. To further elucidate the link between plant metabolism and flowering time, we are performing metabolomic analysis of leaf tissues from id1 mutant plants and wild-type plants using gas chromatography/mass spectrometry (GC/MS). This approach enables the comprehensive examination of hundreds of metabolites, including intermediates and end-products of primary metabolism. Ultimately, this work should provide insights into the gene-to-metabolite networks operating during the transition to flowering in maize, and the involvement of ID1 in this transition.

P102

Molecular dissection of heterosis manifestation in maize roots and embryos

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Heterosis describes the superior performance of heterozygous F1-plants compared to their homozygous parental inbred lines. Heterosis in maize (Zea mays L.) is already manifested during early plant development. In this study, embryos of the inbred lines UH005 and UH250 and their reciprocal crosses (UH005xUH250; UH250xUH005) were subjected to proteomic studies (2D-PAGE) at different developmental stages (25 and 35 DAP) in order to identify proteins that are non-additively accumulated in hybrids. Moreover, comprehensive transcriptome profiling experiments of 3.5 day-old primary roots of four inbred lines (UH002, UH005, UH250, and UH301) and their twelve reciprocal hybrids were performed. This early developmental stage was selected in order to profile gene expression before morphological differences between roots of inbred lines and hybrids become manifested. At this developmental stage root hairs, which are a marker for cell differentiation, have been formed, whereas lateral roots have not been initiated. Identification and characterization of genes and proteins that are non-additively expressed in the reciprocal hybrids will contribute to the better understanding of the molecular basis of heterosis.

P103

Molecular interactions among regulatory factors influencing shoot maturation in maize

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Shoot maturation in maize is characterized by three major phase changes, the transition from juvenile to adult vegetative development, inflorescence initiation, and the specification of floral organ identity. Molecular genetic studies in maize and Arabidopsis have identified important factors for the general process of phase change and shoot maturation, including genes that function in small RNA biogenesis and metabolism, microRNAs, transcription factors, growth regulators (GA and ABA) and sensitivity to photoperiod. Detailed analysis of molecular interactions within the vegetative phase change pathway has clarified the functional relationships among some of these regulatory factors. Analysis of mutations and natural allelic variation affecting vegetative phase change indicates that the observed antagonistic effects of miR156 and miR172 are mediated by independent pathways that converge to regulate Glossy15. Experiments involving exogenous GA application and GA-deficient mutants demonstrate that GA both represses miR156 and promotes miR172 expression, but acts most strongly on miR172. We also show that the delayed shoot maturation observed in photoperiod-sensitive tropical maize lines is associated with prolonged Glossy15 expression, suggesting novel strategies to increase maize biomass.

P104

Morphological analysis of tassels replace upper ear1 in Maize

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The maize inflorescence develops from meristems, which determine inflorescence architecture. During development, spikelets transition from bisexual to unisexual due to the selective elimination of male or female developmental processes, resulting in two morphologically distinct inflorescences: the male tassel and female ear. Networks of genes and hormones are known to regulate these developmental processes. For example, the ramosa genes and barren inflorescence2 (bif2) are important in tassel and ear architecture while genes like tasselseed4 (ts4) and teosinte branched1 (tb1) regulate directly or indirectly the sex of tassel and ears. The tassels replace upper ear1 (tru1) gene regulates a distinction between tassel and ear identity for the axillary branches. We performed a morphological study of strong tru1-ws mutants where axillary branches are transformed from short and ear-tipped to elongated, staminate inflorescences. Axillary branch shank, inflorescence and prophyllum, were all elongated at 58 days after sowing(DAS58). The shank phenotype showed a shade avoidance response. Also, axillary branches showed increased apical dominance after DAS51. Moreover, mutants were shorter with reduced leaf blade growth, reduced central spike on the main tassel and fewer elongated internodes between root and tassel compared to sibling heterozygous plants at DAS70. Dynamics of axillary branch growth were also studied and will be described. So far, tru1 has been narrowed to a 5 BAC interval between contig 128 and 129 on chromosome arm 3L from our mapping population (total 2,971 individuals). Nine plants holding potential new tru1 alleles were generated by EMS-mutagenesis. Double-mutant analyses for tru1-ws with bif2 and barren stalk1 (ba1) mutants are 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 morphological analysis of mutant plants, to eventually elucidate tru1 function in regulating inflorescence development in maize. This work was funded by Iowa State University and NSF.

P105

New maize mutants defective in anther development

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We are interested in identifying key genes required for setting cell fates prior to meiosis and for regulating the switch in the cell cycle from mitosis to meiosis. To identify these genes, we examine male sterile mutants for cytological defects in early anther development. The five cell types in anther locules are analysed on cross-sections through anthers embedded in Spurr's resin. We use all sources of male sterile mutants available to the maize community including RescueMu mutants from the Maize Genetic Stock Center, EMS mutagenized maize mutants obtained by J.Hollick and mutants from the Maize-Targeted Mutagenesis project.

Of the 112 mutants screened in 2008, 2 were found to have defects in meiotic prophase (ms17P1-WR and RescueMu A55-25), 3 block meiocyte development after tetrad stage (ms8/mtm99-56, ms11 and RescueMu A60-35a) and 6 have defects in early anther development. Some mutants have defects in particular cell layers (ms*-6015, mtm99-66, ms9) while other mutants fail to differentiate all cell layers (ms-si*-355, M163131 and M163089).

It is the last class, which we are mostly interested in. Complementation tests showed that some of the newly found ms mutants are allelic to known maize ms mutants. Mutants defective in cell fate acquisition or maintenance will be subjected to transcriptome and proteomics profiling on anthers and dissected cell types.

This study is part of a NSF sponsored plant genome research project, Cell Fate Acquisition in Maize, which we call "The Anther Project" (PI: Virginia Walbot, Co-PI: Zac Cande).

P106

Nlr1 integrates plastid division and leaf blade development

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The flat leaf blade of higher plants is an important adaptation for efficient photosynthesis. The leaf blade provides a porous tissue with large surface area for light capture and gas exchange. We have recovered a maize mutation that impacts development in multiple tissues including distinct narrow leaf and rough endosperm (nlr1) phenotypes. The nlr1 mutant displays reduced blade expansion as a consequence of reduced minor vein number. We infer from this phenotype that blade growth is reduced late in organogenesis (~p4-p6). In addition, nlr1 mutants have a reduced the number of chloroplasts. In contrast to chloroplast division mutants in dicots, nlr1 chloroplasts do not show volume compensation, and the reduced number of chloroplasts results in a mottled, pale green leaf phenotype. Northern blots probed for chloroplast rRNA indicated that nlr1 mutants have no obvious defects in chloroplast transcription and translation. We have identified a transposon insertion from a Robertson's Mutator (Mu) flanking sequence tag that is linked to the nlr1 phenotype. We mapped the nlr1 locus to a 9 gene interval that is centered on the Mu insertion. The transposon disrupts a gene encoding a Type C, J-domain protein that is specific to photosynthetic eukaryotes, named DjC78. J-domains activate Hsp70 ATPase domains and have numerous functions including control of chloroplast division and gene expression. Chloroplast import assays with in vitro transcribed and translated ZmDjC78 indicate that the protein is not chloroplast-localized. Transient expression of a ZmDjC78:GFP fusion in Arabidopsis mesophyll protoplasts suggests that the protein may be localized to the nucleus. Further characterization of the nlr1 mutant is expected to reveal how chloroplast division and leaf blade expansion are connected.

P107

Pollen Shed Delay

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Darwin studied cross and self-fertilization in plants and found silk delay is normal in corn (Z. mays L.) varieties because crossing increases vigor of the resulting progeny. Corn silks are 90% water; they are further delayed by drought. Selection for strong silk emergence during inbreeding among plants grown at high plant density has greatly increased hybrid corn drought tolerance over the last 50 years along with more upright leaves to reduce self-shading. I selected the earliest 160 silking plants in each of six, 1600 plant backcross populations grown at 60,000 plants per acre including alleys near DeKalb, Illinois in 2008. Many plants silked strongly, and I had to wait from one to four days per plant for pollen shed to pollinate 960 selected plants. Shed delay is extremely rare. Our season was 30% short of heat units in May causing a cool, late season. These shed delay plants had their tassels encased in their uppermost one or two leaves. When unwrapped the tassels felt cool and moist--water cooled by plant transpiration. Apparently mitosis and meiosis for pollen production requires heat units directly to the tassel. By contrast, the developing female flower is normally encased in leaves (husks); yet, undergoes mitosis and meiosis to form the ovules on the cob.

P108

Regulation of the HD-ZIP IV transcription factor ZmOCL1 by a miRNA

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ZmOCL1 is the founding member of the maize ZmOCL (Zea Mays Outer Cell Layer) family encoding plant-specific HD-ZIP IV transcription factors. It is expressed in the epidermal layer of the embryo and organ primordia, suggesting a role in the differentiation and maintenance of epidermal cell fate. The existence of a conserved 21 bp sequence in the 3'UTR of the majority of the HD-ZIP IV genes from maize, rice and Arabidopsis suggested a potential regulation of these genes (including ZmOCL1) by a miRNA. This would be a novel miRNA since no small RNAs matching the 21 bp target sequence were found in existing databases. First experimental evidence for the existence of such a small RNA in maize was recently obtained by Northern blot. Hybridization of low molecular weight RNA with a radio-labeled probe complementary to the putative miRNA regulating ZmOCL1 revealed a band in the 22-24 nucleotide size range in several maize tissues such as tassel and ear. We called this small RNA miR1. RLM-5'RACE experiments did not allow us to detect any ZmOCL1 cleavage products within the putative miR1 binding site, suggesting that miR1 does not regulate its target genes by transcript cleavage. On the contrary, a preliminary analysis of miR1 activity in planta using a GFP sensor system, in which the 3' end of the GFP coding sequence was transcriptionally fused to the entire 3'UTR of ZmOCL1 (including the miR1 target sequence) suggests that miR1 could regulate its target(s) through translational repression.

P109

Shoot-borne root patterning is altered in ramosa1 mutants of Zea mays L.

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Maize shoot-borne roots represent the majority of the adult root system in maize. They provide physical stability, facilitate water uptake and acquire necessary nutrients. The above ground shoot-borne roots are often referred to as brace roots. Above and below ground roots develop around the node. A previous QTL study suggests the ramosa1 (ra1) gene is a candidate gene affecting brace root patterning. ra1 affects the development of maize tassels by suppressing tassel branching and promoting spikelet pair formation. Significant differences in shoot-borne root patterning were observed when comparing ra1 and wild-type plants with the ra1 mutants producing fewer brace roots at a node and fewer nodes with brace roots. In 2007 and 2008 tassel and root traits were measured on a group of 25 diverse maize lines. In 2007, a significant positive correlation was observed between average tassel branch length and four brace root traits: nodes with brace roots, brace roots at node 1, brace roots at node 2, and total number of brace roots. In 2008, a positive correlation was observed between average tassel branch length and both number of nodes with brace roots and total number of brace roots. Although the number of brace roots at node 1 and the number of brace roots at node 2 were not significantly correlated with average tassel branch length in 2008, scatter plots of these traits exhibit a positive trend. The differences in shoot-borne root patterning in ra1 vs. wild-type individuals indicate ra1 is a viable candidate underlying a QTL for shoot-borne root traits. Our results suggest that ra1 is involved in both the inflorescence and shoot-borne root development programs. Mutations in ra1 appear to have opposite effects on maize brace roots relative to tassels indicating that the role of ra1 may differ depending developmental phase. This research was supported by the National Science Foundation, the UMEB Program, Life Science Mission Enhancement, and USDA, ARS.

P110

The Maize *stunter1* Maternal Effect Mutation Affects Male and Female Gametophytes

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stunter1 is a maternal effect mutant that displays defective maize kernels. The small, stunted kernels exhibit a reduced but normal endosperm with a relatively normal embryo. In an attempt to identify the genetic lesion and better characterize the *stt1* mutant, we are using map-based cloning and conducting phenotypic analyses of the male and female gametophytes and the developing seed. The lesion is located in a 1.5 cM region on the long arm of Chromosome 2. The mutant displays incomplete and variable penetrance of the kernel phenotype and reduced transmission of the mutation through the male and female parents. In addition, the *stt1* mutation causes significant changes in the sizes of both the male and female gametophytes. The *stt1* pollen grains are smaller than wild type, and although the pollen nuclei appear normal, the *stt1* pollen grains do not germinate as well as wild-type grains. The reduction in size of *stt1* embryo sacs is largely due to differences in the central cell, which is approximately half the size in the mutant embryo sacs compared to wild type. Additionally, the antipodal cells of mutant embryo sacs appear larger, less cytoplasmically dense, and possibly fewer in number than wild type. The results strongly suggest that *stt1* is a maternal effect mutant and that the morphology of the mutant embryo sacs prior to fertilization influences endosperm development, ultimately leading to the production of miniature kernels from mutant embryo sacs.

P111

The effect of planting density on shoot-born root initiation, and correlated agronomic traits

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Shoot-born roots play many essential roles in the successful cultivation of maize. One role that the shoot-born root system fulfill is physical anchorage for the plant. A previous study which exposed early flowering French maize lines to an increased ratio of red to far-red light reported a depletion of shoot-born roots. The current study aimed to build on the previous research by including a broader germplasm base including lines representative of four maize population structure groups as well as performing the investigation in the field under conditions which are more closely approximate production conditions.. Shoot-born root traits as well as several traits regulated by phytochrome activity were measured. Increasing plant density resulted in reductions in the number of shoot-born roots at a node and the number of nodes with shoot-born roots. In additions, the phytochrome related traits of ear eight and stalk diameters were significantly reduced while average internode length significantly increased as planting density grew larger. The number of juvenile leaves, node numbers and average internode length were not significantly different across treatments providing developmental queues for the planting density response during plant growth. Trellis plots revealed evidence for treatment by genotype interactions modulating the correlated nature of shoot-born root initiation and light signaling traits. A model is presented postulating the role of identified quantitative trait loci polymorphisms in root growth response to light limiting conditions. The current study is assisting in the understanding of the biological mechanisms linking dense planting to a more frequent occurrence of lodging in the field.

P112

The maize tasselsheath4 gene regulates meristem boundaries by repressing ramosa2

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Maize floral development is dependent on the orderly initiation of several types of specialized lateral meristems that ultimately determine overall inflorescence architecture. The tasselsheath4 (tsh4) gene is an SPL transcription factor that regulates the partitioning of meristem primordia into lateral organs. In the absence of tsh4 activity, bract primordia subtending each lateral meristem are de-repressed and grow out at the expense of the meristem. We report that tsh4 functions to control this process by repressing the ramosa2 (ra2) LOB domain transcription factor. Using double labeling, we show that tsh4 and ra2 expression overlap early in inflorescence meristem development, and then resolve into complimentary domains that specify the subtending bract and spikelet pair meristem respectively. Moreover, using the knotted1 gene and branched silkless1 genes as meristem markers, we find that tsh4 defines a domain that marks the developing stem and bract adjacent to the spikelet pair and spikelet meristems. Finally, we show that ra2 RNA and protein is ectopically expressed in tsh4 mutants, further supporting that tsh4 represses ra2. The complimentary expression pattern of tsh4 and ra2 is conserved in related grasses, indicating that these two genes comprise a conserved regulatory network.

P113

The non-reductive mutant 1, a first step towards engineered apomixis in Maize

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To engineer apomixis (clonal seeds) in Maize, we try to dissect this process in its unique features and try to find mutants of every class: no reduction, no recombination and fertilisation-independent embryo development. The project aims at engineering apomixis by combining the different meiotic mutants with one of the parthenogenetic mutants.

In this talk I will focus on the non-reductive mutant 1 (nrm1), found in a screen of lines carrying active Mutator transposons that were pollinated by 4n^R-nj and screened for plump kernels with the R-nj staining. Since Maize has a strict ploidy requirement for correct endosperm development (2 maternal : 1 paternal), only unreduced embryo sacs should develop normally when pollinated by a tetraploid pollen donor.

The ploidy of the plump kernels from the screen was deduced by flow cytometry. The meiotic defects were visualized by dissecting the female gametophyte at different stages and analyzing them by confocal laser scanning microscopy. To test for recombination and paternal contribution, polymorphic SSRs were analyzed in the offspring from different crosses. Although it is a meiotic mutant, the genetics look very simple. Different cloning and mapping approaches were applied and are still ongoing.

P114

The phytochrome interacting bHLH proteins affect photomorphogenic development in Maize

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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.

P115

The shade avoidance syndrome in maize: Genetic and hormonal control of seedling elongation.

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In the dense stand of a typical maize field, the selective absorption of red (R) and blue lights by photosynthetic pigments creates a light environment characterized a reduced ratio of R to far-red (FR) light. The phytochrome photoreceptors perceive this R:FR reduction and trigger a series of morphological changes referred to as the shade avoidance syndrome (SAS). In maize, an increase in plant height and reductions of tillering and root development are among the phenotypes associated with the SAS. This repartitioning of resources mediated by the SAS is thought to result in lower grain yields. To gain a better understanding of the genetic and physiological mechanisms underlying the SAS, an assay mimicking canopy shade in growth chamber was developed. Specific roles for PhyB1 and PhyB2 in mediating the SAS were investigated in different backgrounds using singles and double mutants. Surveys of the phenotypic variation associated with the SAS were made using both a panel of genetically diverse inbreds and the intermated B73 x Mo17 (IBM) population. The roles played by the gibberellic acid (GA) and auxin pathways in the downstream hormonal control of the SAS was also investigated. Results suggest that GA is required for the shade-triggered elongation of the mesocotyl and that responses to shade are mediated in a tissue-specific fashion.

P116

The use of mixed models to fine map cis regulatory elements located upstream of teosinte branched 1 (tb1)

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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 branched1 (tb1). Our work is focused on studying the regulatory elements that produce a two fold higher expression of tb1 in maize when compared to teosinte. A cis regulatory region is located ~58-69 kb upstream of tb1. Introgression lines were made, containing teosinte fragments of the cis regulatory region upstream of tb1, in a maize inbred background. A mixed model approach was used to analyze the large data set that was generated by phenotyping 26 different introgression lines over five different field seasons. The breakpoints of each introgressed teosinte fragment were located by identifying sequence polymorphisms between the maize and teosinte alleles. This sequence data allowed us to test very specific regions for regulatory importance. Each introgression line was phenotyped for multiple traits so that the effects of the regulatory elements in various tissues could be analyzed. Information from this analysis has helped us to localize the regulatory region upstream of tb1 and has indicated that there are multiple functional regulatory element within the region. 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.

P117

Two redundant pathways of Trp-dependent biosynthesis of auxin and their possible relationship to sugar metabolism in developing seeds of maize.

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The most abundant endogenous auxin in plants, indole-3-acetic-acid (IAA), is at the highest levels in developing seed of all tissues in the maize plant. Although all IAA biosynthesis in developing seeds is Trp-dependent (T-D), very little is known regarding genes and enzymes of its biosynthesis. The loss-of-function mutation at the Miniature-1 (Mn1) locus that encodes an endosperm-specific cell wall invertase is associated with several pleiotropic changes in the mn1 seed mutant, most importantly the reduced levels of IAA in both endosperm and embryo of developing kernels (Phytochemistry 69: 692, 2008,). Attempts to understand the basis of IAA-deficiency have led us to two new maize genes each of which represent the two major T-D branches of IAA pathway in plants. The ZmTARelated-1 gene (Accession # EU872320) is a maize ortholog of the recently discovered Trp-aminotransferase in Arabidopsis – a key enzyme in the indole-3-pyruvic acid (IPA) branch, and the ZmYUC1 (Accession # DQ995287) that codes for a flavin mono-oxygenase-like enzyme in the tryptamine (TAM) branch in plants. Transcript levels of these two genes by q-PCR analyses were the highest at 8 - 12 DAP, coincident with a peak of IAA levels as well as cell division and elongation phase; however, only the ZmYUC1 levels were reduced in the mutant. Subsequent stages, 20 – 28 DAP, showed a steep decline in the levels of ZmYUC1; the ZmTAR1 showed only moderate down-regulation. As for the relative amounts, the TAR1 transcripts were nearly 10- and 20-fold higher in the Mn1 kernels and embryos, respectively, than the YUC1.
The invertase-deficiency also led to changes in sugar composition in both endosperm and embryo of the mutant. Additionally, reduced endosperm mass in the mn1 was seen as early as 10 - 12 DAP; more importantly, significant reductions were also seen in the mn1 embryos.

Overall, these data provide evidence of sugar-hormone crosstalk and embryo-endosperm inter-dependence in seed development in maize.

P118

ZmRAB2A1 partially co-localizes with ER and shows distributed expression through the cytoplasm

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Maize leaves grow in a predictable and repetitive pattern from the shoot apical meristem. Cell shapes in mature leaves reflect the patterns of division relative to cell expansion and contribute to the final leaf shape. To understand these growth processes, we have identified mutants with altered cell shapes/sizes due to lesions in genes that regulate division and/or expansion. From this screen, we identified Warty1, which encodes ZmRAB2A1, a highly conserved, small GTPase known to be involved in vesicle trafficking. Homologous RAB2 proteins in other eukaryotes are known to traffic vesicles within a restricted ER/Golgi cellular compartment. The ZmRAB2 sub-group bears high sequence similarity to HsRab2, although the maize sub-group has more members. We hypothesize that ZmRAB2A1 may have similar functions to HsRAB2A. Alternatively, ZmRAB2A1 may define different compartment boundaries in maize cells or the protein may have diversified to accommodate the vesicle trafficking unique to plant cell growth. To distinguish these hypotheses, we tagged ZmRAB2A1 with YFP to test for co-localization with other compartmental markers in transgenic maize. Additionally we are using GFP antibodies to immunoprecipitate the tagged FP fraction and we are developing FP-tagged Golgi markers for further testing of the hypotheses. Our results show ZmRAB2A1 partly colocalizes with the ER, as expected, but is also widely distributed through the cytoplasm. Density centrifugation results support the fluorescent micrograph localization results. Since live cell imaging of the native FP shows localization into the cortical cytoplasm as well, we suggest that ZmRAB2A1 may show both specialized function and plant-specific distribution, further implicating ZmRAB2A1 in cellular signaling during leaf cell expansion.

P119

sparse inflorescence1, barren inflorescence1 and barren stalk1 promote cell elongation in maize inflorescence development

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The plant hormone auxin plays a critical role in the development of the maize inflorescence. Multiple mutations with defects in inflorescence development including sparse inflorescence1 (spi1), Barren inflorescence1 (Bif1), barren inflorescence2 (bif2), and barren stalk1 (ba1) show defects in auxin biosynthesis or transport. spi1 functions in localized auxin biosynthesis and ba1 encodes a transcription factor while bif1 and bif2 regulate auxin transport. This report shows that cell length is significantly reduced in mature spi1 tassels suggesting a decrease in localized auxin biosynthesis leads to a decrease in cell elongation. We also show that Bif1 and ba1 mutants have reduced cell elongation in the inflorescence though bif2 did not show any significant defects. We are currently examining cellular defects in other tissues in these mutants. These results emphasize the importance of auxin biosynthesis and transport in cell elongation during inflorescence development.

P120

Effects of *rmr1* on *Pl1-Blotched*

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Pl1-Blotched is an epigenetically controlled allele of the maize anthocyanin regulatory gene, *purple plant1 (pl1)*. *Pl1-Blotched* leads to a variegated pattern of pigmentation, which contrasts with the uniformly dark purple pigmentation of plants carrying the dominant *Pl1-Rhodes* allele. *Required to maintain repression (rmr)* loci stabilize the transcriptionally repressed state of *Pl1-Rhoades* alleles (designated *Pl'*). The recessive mutations defining *rmr1*, *rmr2*, and *rmr6* destabilize the *Pl'* state and lead to an increase in *pl1* RNA levels, darker pigmentation in plant tissues, and meiotic transmission of *Pl1-Rhoades* revertant states. Due to the fact that *Pl1-Rhoades* and *Pl1-Blotched* are epialleles, we hypothesized that *rmr1* is also involved in the regulation of *Pl1-Blotched* and predicted that the mutant would release the epigenetic silencing of *Pl1-Blotched*, increase *pl1* RNA levels, and ultimately lead to greater anthocyanin pigmentation in tissues normally regulated by *Pl1-Blotched*. To test this hypothesis, a genetic cross was made to introduce *rmr1* into a *Pl1-Blotched* line. Anthocyanin levels of leaf sheath tissue were measured as a proxy for the effect of *Pl1*, and PCR-based SSR markers were used to screen for parental polymorphism at *rmr1*, *pl1*, and *b1* loci. Subsequent genotyping revealed an insufficient number of progeny from the cross with the desired genotype to draw statistically significant conclusions about the effects of *rmr1* on *Pl1-Blotched*.

P121

Genetic and phenotype characterization of maize transgene-reactivated mutants.

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The silent b1 genomic transgene (BTG) is a powerful marker for investigating epigenetic gene silencing in maize (McGinnis et al., 2006), and provides a system in which heritable changes in gene expression can be correlated with specific epigenetic marks and mechanisms. A forward genetics screen based on the BTG-reactivation phenotype was conducted using EMS mutagenized maize. Through this screen, ten mutants, designated transgene reactivated 1 (tgr1) through tgr 10, have been identified for further investigation. Genetic analysis to date suggests that Tgr2 is a semidominant mutation, whereas the remaining mutations are recessive. Dosage sensitivity within the pathway, heritability of phenotype upon outcrossing with wild type for some mutations, and the non-recessive nature of one mutation confounds classical genetic allelism tests and complementation analysis. A map based approach to determine chromosomal location of each mutation has been initiated. Additionally, in depth phenotypic characterizations are underway for each mutant, including DNA methylation analysis and characterization of each mutant's ability to maintain transcriptional silencing of other epigenetically regulated loci. Progress in each of these areas will be reported.

P122

How temperature and light can regulate heritable changes in gene expression

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Controlled light and temperature conditions defined critical stages of seedling development (first two/three weeks) and determined heritable epigenetic expression-states for the paramutated R allele. Changes in gene silencing were monitored in pollen test crosses from maize tassels, sampled from each of 75 single plants, each day, over an eight-day period. R allele silencing (paramutation) increased upward in the tassels; pollen sampled from the upper branches of each of 75 tassels showed less pigment than R from pollen sampled from the lower branches. Expression-states of R are related, therefore, to position in the tassel from which pollen was sampled provided seedlings are started under defined light/dark and temperature conditions. Silencing is heritable and can be incremented each generation; therefore, the environment is implicated in regulating heritable evolutionary changes in gene expression.

Prior reports on R paramutation treated variation as stochastic and reported variation as pooled means. Controlled conditions show variation as a developmental continuum responding to light/dark conditions.

We conclude that epigenetically determined gene expression-states require operational definition which includes, at least, environmental conditions (light/temperature), stage of plant development during which conditions were applied, genome context, and gene history.

"Relatively little is known about how the plant epigenome changes in response to developmental or environmental cues" (review Zhang, 2008, Science, Vol. 320; 489-492).

P123

RNAi-mediated Silencing of a Maize Histone H1 Linker Protein Confers Increased Transformation Efficiency in Maize.

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Chromatin remodeling plays a crucial role in gene regulation and expression. Therefore, we hypothesize that disruption of chromatin-associated genes that function to modify the accessibility of DNA to transgene integration will affect transformation efficiencies in maize. The maize chromatin gene HON102 encodes a linker histone protein within the Histone H1 homology group. In our study, a RNAi-inducing inverted-repeat (IR) construct targeting the maize chromatin-associated gene HON102 was bombarded in individual, replicated experiments along with the empty vector control, pMCG161, and the stable transformation efficiency was measured. The average stable transformation rate for the IR construct targeting HON102 was 7.5%, and was statistically significantly higher than the pMCG161 control efficiency of 4.2% over 11 independent, replicated experiments. Quantitative RT-PCR and Northern blot analysis confirms mRNA reduction in 40% of the events produced with this construct. Tissue expression analysis with 100bp gene-specific primers reveal that of the six HON family members in maize, only two others are expressed in the same tissue types as HON102. These genes, HON101 and HMGA102, are not affected by the IR construct targeting HON102 for reduction; thus supporting the hypothesis that HON102 has a unique role in transformation. Finally, three new IR constructs were constructed and used in primary transformation experiments to further elucidate the role of HON102 and the histone H1 family in maize transformation. Interestingly, the experimental variability observed in transformation frequency of these experiments is correlated with the ability of the RNAi construct to silence HON102. Our current interpretation is that the increased transformation frequency observed upon HON102-silencing is likely due to increased spacing between nucleosomes, i.e. a more relaxed DNA conformation and increased transgene integration.

P124

Temporal shifts in the heritability of Mutator transposons activity in Zea mays

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The Mutator (Mu) system of transposons in maize provides a well-characterized platform for classical genetic analysis of non-Mendelian inheritance patterns and epigenetic processes. We have derived a genetic system that includes a single regulatory MuDR element, a rearranged derivative of that element, (Muk) that can silence one or many MuDR elements via siRNAs and that triggers heritable transcripitional gene silencing. Following the loss of Muk due to genetic segregation, it is possible to examine changes in the epigenetic state of the silenced MuDR elements over multiple generations. One such state is the propensity of the silenced element to respond to a second, active element. Previous data suggested that this propensity can change over time, with additional rounds of meiosis acting to deepen the silenced state. Using a partially deleted version of MuDR called d107, we developed a screen to observe the effect of an active MuDR on a d107 that had been silenced for one or two generations. We found that although the active MuDR element can transcriptionally activate the silenced d107 element in both generations, that activity was only reliably heritably transmitted when the d107 had only been silenced for a single generation. We hypothesize that silencing of MuDR elements is a progressive process that involves successively deeper silenced states. Passage through meiosis may act as a checkpoint, during which provisionally established silenced states are made more permanent. Ultimately we seek to use this system to better define and distinguish transcriptional activity from epigenetically encoded silencing information.

P125

Unexpected Retrotransposon Expression Patterns in the Shoot Apical Meristems of the mop1 (RNA-dependent Polymerase 2) Mutant

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Mop1 (Mediator of Paramutation1), a putative RNA-dependent RNA polymerase (RdRP), has been shown to play key roles in paramutation (Alleman et al., 2006) and silencing of DNA transposons (Lisch, et al., 2002). To directly test the effects of Mop1 on gene expression, RNA-seq was conducted on shoot apical meristems (SAMs) from mop1 mutant and non-mutant seedlings. Several million RNA-seq reads were produced and mapped to a partial maize genome assembly (MAGI4, http://magi.plantgenomics.iastate.edu/). Over 10,000 genes were differentially expressed (FDR < 0.15) between the two genotypes; 7,000 of these are Fgenesh predicated protein-coding genes. Several gene annotation categories were significantly enriched in the differentially expressed gene sets, including chromatin modification, DNA methylation, nucleosome assembly and ribonucleoprotein, all of those are consistent with mop1's role in gene silencing. Additionally, almost 80% of ~ 400 chromatin-related genes (www.chromdb.org) were differentially expressed in the mop1 mutant. By combining RNA-seq data with published small RNA data from the mop1 mutant (Nobuta et al., 2008), we found that the expression patterns of individual genes are highly correlated with the accumulation of sequence similar small RNAs. Transcripts from ~100 characterized retrotransposons differentially accumulate in the mop1 mutant and Mop1 control. Contrary to the expectation that a functional Mop1 gene would silence retrotransposons, approximately half of these retrotransposon-derived transcipts are down-regulated in the mop1 mutant SAMs relative to wild-type SAMs. This finding is, however, consistent with our previous report that retrotransposon-derived transcripts accumulate to higher levels in SAMs versus seedlings (9% vs. 0.3%, respectively of all transcripts), even though Mop1 transcripts accumulate to higher levels (120-fold) in wild-type SAMs as compared to seedlings (Ohtsu, et al., 2007).

P126

Unstable factor for orange1 (Ufo1) induced histone modification of pericarp color1 gene in maize

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Epigenetic gene regulation is a global phenomenon among many organisms and is associated with DNA methylation, chromatin remodeling, and small RNA based silencing. Although studies exploring the mechanisms of epigenetic regulation are blooming, little is known about how these epigenetic marks cooperate with each other and lead to gene silencing. In this study, we address epigenetic mechanism involved in gene regulation of maize pericarp color1 (p1) gene. The p1 gene encodes a Myb transcription factor and regulates the accumulation of phlobaphenes (flavonoid pigment) in floral organs. P1-wr, a natural allele of p1, was shown to have a multicopy gene structure that is transcriptionally regulated by a mechanism correlated with extensive DNA methylation and results in tissue-specific pigmentation. The Unstable factor for orange1 (Ufo1), a trans-acting modifier, epigenetically regulates P1-wr gene expression, leading to enhanced accumulation of phlobaphenes in pericarp, cob glumes, husk, silks, leaf sheath, and tassel glumes. We have previously demonstrated that p1 x Ufo1 interaction leads to hypomethylation at P1-wr allele. Our recent evidence showed that the gain of pigmentation in P1-wr plants in the presence of Ufo1 was correlated with histone modification. Chromatin immuno precipitation assay (ChIP) revealed that histone 3 lysine acetylation (H3K9/14-Ac) was modified on the distal enhancer region of p1 in the presence of Ufo1. The other histone modifications (eg. H3K9- me2, H3K4- me2) are examined and the preliminary data will be presented. Our results suggest that Ufo1 plays a role in both DNA methylation and histone modification. This study will also address gene expression and penetrance differences between original Ufo1 and introgressed Ufo1 stocks.

P127

Effect of chromosome number variations on global gene expression

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Despite the widespread interest in aneuploidy, the molecular mechanisms that lead to phenotypic alterations in aneuploid organisms are still poorly understood. Moreover, it is not clear what gene interactions are involved in coping with gene dosage imbalance caused by aneuploidy on the global genomic level. Plants are more tolerant to aneuploidy than animals and present a good model for research. Here, we investigated aneuploidy effects on gene expression in the maize aneuploid that carried an extra copy of a small arm of chromosome 5 and exhibited several phenotypic traits, such as stunted growth, late development, partial tassel sterility, and knots in the leaves. We were primarily interested in understanding whether the phenotypic syndromes of aneuploidy could be attributed to a small number of affected genes or a complex network of gene interactions is involved. We also wanted to know whether different plant organs respond differently to aneuploidy. We compared expression levels of approximately 15,000 maize genes in meristems and leaves of aneuploid and wild type seedlings, to understand effects of aneuploidy on gene expression. Our microarrays experiments demonstrated that different sets of genes are affected in meristems and leaves and allowed identification of approximately 50 genes that changed their expression in response to aneuploidy. Analysis of expression for these genes in five different maize tissues will be presented and analyzed

P128

Evolution of *bz* haplotypes in maize and its relatives

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The organization of the maize genome is remarkably polymorphic. The length and make-up of a given intergenic region can differ greatly among lines because of various insertion polymorphisms. The main contributors to this difference are the retrotransposon clusters that comprise the majority of the repetitive DNA in maize. However, other transposons, such as the classical transposable elements of McClintock, as well as MITEs and the newly discovered *Helitrons* and *TAFT* elements, also contribute to it.

We recently carried out a vertical comparison of 8 *bz* haplotypes from Corn Belt and tropical inbreds and tropical land races and found a remarkable extent of variation1. In pairwise comparisons, the percentage of shared sequences ranged from 25 to 84%. Yet, variation is limited to the content of the intergenic space and introns, not of the genes themselves. The *bz* region is gene-rich and offers an excellent opportunity to examine how the gene content of the region has diverged since the rice-maize split roughly 50 MYA. Therefore, we have undertaken an analysis of *bz* haplotypes in several *Andropogoneae*, from teosintes (*Zea mays*, ssp. *mexicana*; *Zea luxurians*; *Zea diploperennis*) to *Coix* and *Sorghum*, and have compared them with rice. BAC clones containing *bz* haplotypes from these relatives have been isolated and sequenced. We find that the *stc1* gene, involved in an indirect defense response to herbivores, is evolving very fast: frameshift nonfunctional alleles are present in the maize KYS inbred and some individuals of *Zea diploperennis*; the seventh *stc1* exon is missing in *Coix*; different vestiges of two exons are present in *Sorghum propincuum* and *Sorghum bicolor*, and the gene is completely missing in rice. A partial analysis of haplotypes that do not clone as single BACs (e.g., *Tripsacum*) will also be presented.

1. Q. Wang, H. K. Dooner, Proc Natl Acad Sci U S A 103, 17644 (2006).

P129

Evolution of Cereal Storage Proteins

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With several cereal genomes sequenced or being sequenced, we can investigate the relationships of storage proteins in their chromosomal context and ancestry. We make the assumption that conserved gene order between two genomes did not occur by chance but ancestry, referred to as orthologous regions. If a third closely related genome deviates from this order, we would suggest that the non-collinear gene is a copy derived from a gene somewhere else in the genome. However, in some cases the donor got lost and only the copy remains. It appears from our studies that in cases where the donor was lost, lack of concerted evolution gave greater freedom to the copy to evolve in its structure. Seeking residual conservation of protein structure to construct alignment clusters permitted us to classify the prolamins, the major seed storage proteins in most cereals, into four groups, alpha, beta, gamma, and delta prolamins. Based on this division, the divergence node seems to have been fixed at the subfamily level of the Poaceae with examples from the Ehrhartoideae (rice), Pooideae (wheat, barley, Brachypodium), and Panicoideae (millets, maize, and sorghum), respectively. Furthermore, chromosomal alignments of orthologous regions from representatives of these taxonomic subfamilies and phylogenetic analysis indicate that water insoluble storage proteins originated from water soluble ones. Despite the divergence at the subfamily level, regulation seems to be more conserved, as O2-like transcription factor genes are syntenic across subfamilies, which is not surprising because the target genes in all these species are expressed in the endosperm of the seed.

P130

Mapping of Telomere Length Regulating Factors

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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. Mammalian studies have shown that proper regulation of telomere length is important in ageing, cancer and a diverse range of disease states. Quantitative Trait Locus (QTL) mapping is a powerful method for identifying loci that control traits with complex inheritance that can be precisely measured and exhibit a high degree of heritability. We are utilizing QTL mapping in the well-defined IBM population (302 RILs, >2,000 markers) to identify genes that regulate telomere length. Using Terminal Repeat Fragment (TRF) analysis via Southern blot hybridization, we can determine telomere length phenotypes. This method, however, is not without its difficulties and limitations, so we are also investigating slot-blot analysis to determine the relative abundance of telomeric hybridization as a method to determine telomere length. We will select the best combination of methods to obtain telomere length data and will perform QTL analysis to fine map allelic differences affecting telomere length. The physical map regions corresponding to stringent QTL confidence intervals will be used to generate lists of candidate genes. These candidate genes, as well as other a priori candidate genes, will then be amplified using RT-PCR in the Maize Diversity Lines in order to determine whether or not variation in expression levels for candidate genes correlate with variation in the telomere length phenotypes. The telomere length data will also be analyzed relative to other phenotypes collected for these lines to search for other potentially meaningful trait correlations. The multi-faceted strategy of QTL mapping in the IBM population and investigation of candidate genes in the diversity lines will be leveraged by the genetically-anchored sequenced genome of B73 to identify novel regulators of telomere length homeostasis in maize.

P131

Mitochondrial Genome Changes Associated with Reversions of S-Type Cytoplasmic Male Sterility

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In CMS-S mitochondria, male sterility is associated with high-level expression of a 1.6-kb RNA containing two chimeric open reading frames, orf355 and orf77. CMS-S mitochondria carry two double-stranded linear DNA plasmids that terminate in protein-bound inverted repeats. TIR sequences also precede orf355/orf77 in the main mitochondrial genome. Recombination between plasmid TIRs and the genomic TIRs leads to linearization of the main mitochondrial genome adjacent to orf355/orf77. It appears that the 1.6-kb RNA is only transcribed from a linear end, using the plasmid TIR promoter. Spontaneous mutations in the CMS-S genome have been found that cause the plant to revert to a fertile state. In some revertants, the orf355/77 sequences are lost; in other cases, the S plasmids are lost. One revertant tested still possesses S plasmids as well as integrated TIRs and complete orf355/77 sequences in the main mitochondrial genome, yet the plant is not male sterile. Our evidence supports the hypothesis that rearrangement and/or deletion events that alter the relative positions of the TIR region and orf355/77 cause CMS-S plants to revert to fertility.

P132

Phylogenetic Examination of Zea and Tripsacum

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Zea and Tripsacum are the two genera that form subtribe Tripsacinae in tribe Andropogoneae of the panicoid grasses. The closest wild relatives of maize, the teosintes of the genus Zea , and the more distantly related Tripsacum species, have held special interest for students of crop evolution and breeders seeking sources of new genetic diversity for maize improvement. One hypothesis for the origin of Tripsacum, which we attribute to Edgar Anderson and Walton Galinat, is that it was the result of a hybridization between an ancient form of Zea and an andropogonoid grass species. In this study we examine the phylogenetic relationships among three groups: diverse maize inbreds, all seven currently recognized teosintes and six diploid species of Tripsacum. Using primers originally developed to sequence a diverse set of nuclear genes in maize, we have targeted the Zea portion of the Tripsacum genome in this analysis. Future genetic studies of Tripsacum, based on whole genome sequencing, will provide unbiased access to the entire genome, and further evidence to test the allotetraploid origin hypothesis.

P133

Recombination across a gene-rich genetic interval in heterozygous maize haplotypes differing in *Helitron* and retrotransposon content

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To answer the question of how the remarkable variation in maize haplotype structures affects recombination, we have compared recombination across a genetic interval of 9S in two highly dissimilar heterozygotes which shared one parent. The genetic interval in the common haplotype is ~100-kb long and contains six genes interspersed with gene-fragment-bearing *Helitrons* and retrotransposons that, together, comprise 70% of its length. In one heterozygote, most intergenic insertions are homozygous, though polymorphic, enabling us to determine if any recombination junctions fall within them. In the other, most intergenic insertions are hemizygous and, thus, incapable of homologous recombination. Our analysis of the frequency and distribution of recombination in the interval revealed that: (i) Most junctions were circumscribed to the gene space, where they showed a highly nonuniform distribution. In both heterozygotes, more than half of the junctions fell in the *stc1* gene, making it a clear recombination hotspot in the region. However, the genetic size of *stc1* was two-fold lower when flanked by a hemizygous 25-kb retrocluster. (ii) No junctions fell in the *hypro1* gene in either heterozygote, making it a genic recombination coldspot. (iii) No recombination occurred within the gene fragments borne on *Helitrons* nor within retrotransposons, so neither insertion class contributes to the interval's genetic length. (iv) Unexpectedly, several junctions fell in an intergenic region not shared by all three haplotypes. (v) In general, the ability of a sequence to recombine correlated inversely with its methylation status. Our results show that haplotypic structural variability strongly affects the frequency and distribution of recombination events in maize.

P134

Whole Genome Profiling: a new method for sequence based whole genome physical mapping

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Whole genome sequences are a very important tool to identify the genes that are responsible for important traits in commercial plant breeding. However, the investments necessary to develop a comprehensive whole genome physical map and corresponding sequence assembly are economically unfeasible for many of these crops. Therefore we have developed a new cost effective method to construct high quality sequence-based physical maps, called Whole Genome profiling (WGP). Such maps are constructed by sequence-based fingerprinting of a 10x BAC library, pooled as individual BAC clones in a multi-dimensional format, followed by sequencing of short 30 bp tags spaced 2-3 kbp across each BAC clone using the Illumina Genome Analyzer II. Subsequently the BAC clones are ordered into contigs by using overlapping regions with identical sequence profiles. Unlike other profiling methods the WGP map has ordered sequence-based anchor points. The availability of a sequence-based map allows very efficient and low cost Whole Genome Sequencing (WGS) of your crop of interest whereby the quality of the WGS assembly dramatically increases. Following proof of principle in Arabidopsis (125 Mbp), we have successfully applied WGP in melon (450 Mbp) in combination with WGS sequencing, and are currently constructing a BAC map for an undisclosed 2.6 Gbp plant genome. Initial results indicate that WGP is also applicable to larger genomes. Clearly WGP offers an array of applications all of which are geared towards identifying and characterizing economically important genomic regions or genes in real crops that often have large complicated genomes.

P135

An Optical Map of the Maize Genome

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Maize is distinguished by its long history as a classical model system for genetic and cytogenetic studies, in addition to its pivotal role as a food crop. Although genetic and cytogenetic analyses have provided a global overview of maize genome structure, significant insights will emerge from the complete knowledge and comprehensive annotation of its sequence. As part of this effort, we have constructed a genome-wide restriction map for use as a scaffold for sequence assembly and finishing. The maize genome is notorious for harboring very complex and extensive panoplies of repeats that confound traditional sequence assembly approaches. To efficiently tackle such genomic elements, our map provides an independent, high resolution physical map guiding difficult sequence assemblies and gap closure operations. The current optical map build comprises 69 map contigs equal to or larger than 4 Mb, spanning 92% of the maize genome. We have developed new algorithms that have linked 60 optical contigs with 90% of the iMap span using FPC maps and unfinished BAC sequences. Such linkage places our optical map within the information-rich iMap resource that integrates a variety of genetic and physical map data. Because optical maps are directly constructed from the analysis of genomic DNA molecules, obviating clone libraries, we project that such maps will greatly complement and potentiate next-generation sequencing approaches dealing with complex plant genomes

P136

Characterization of a worldwide collection of maize lines through large-scale SNP genotyping

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Characterization of genetic diversity among maize (Zea mays L.) inbred lines is of great importance for maize breeding. To evaluate maize genetic diversity using single nucleotide polymorphism (SNP), a total of 730 maize inbred lines representing both tropical and temperate maize germplasm, were collected from breeding programs and germplasm banks including 436 from CIMMYT maize breeding programs in Mexico, Zimbabwe and Kenya, 96 from Brazil, and 198 from different regions of China. All the maize lines were genotyped by the Illumina GoldenGate assay using a SNP chip containing 1536 markers. A preliminary analysis was carried out using a subset of 188 maize lines, which included 12 from China and 176 from CIMMYT Global Maize Program (27 from Kenya, 74 from Zimbabwe, 75 from Mexico). A total of 849 of the 1,536 SNP markers showed a high level of polymorphism within this germplasm subset and provided high quality data. These 849 markers were then used for genetic diversity analysis, detecting 1,698 alleles (all with just two alleles at each locus). The average polymorphic information content (PIC) was 0.292 with a range from 0.104 to 0.375, and a peak distribution between 0.350 and 0.375. Gene diversity for each SNP locus varied from 0.110 to 0.500 corresponding to an average of 0.366. On average, the inbred lines exhibited heterozygosity at 4.7% of the genetic loci. The 188 inbred lines were grouped into three major clusters by the unweighted pair-group procedure with an arithmetic mean (UPGMA) based on the Rogers genetic distance. Cluster I consisted of 88 inbred lines predominantly from Zimbabwe, Cluster II included 56 inbred lines largely from China and Kenya, and Cluster III comprised of 44 inbred lines mainly from Mexico. Most of the lines that were clustered very closely to each other were adapted to similar geographical (environmental) areas. These results indicate that high throughput genotyping using SNP chips provides a powerful tool for large-scale germplasm evaluation. The outputs from this genetic diversity analysis are likely to provide an important guide for improved utilization of maize germplasm in breeding programs. A comprehensive overview of the analysis of all 1536 SNPs across the total of 750 maize lines will be presented at the conference.

P137

Comparative Analysis Of C3 And C4 Leaf Development In Rice, Sorghum And Maize

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C4-type plants such as maize, sorghum and several promising biofuel feedstocks possess a set of complex traits that greatly enhance their efficiency of carbon-fixation, water and nitrogen use, and performance in high temperatures and light intensities, in comparison to C3-type plants such as rice. The key C4 traits are (1) specialization and cooperation of two leaf photosynthetic 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 C4 traits appear to be regulatory enhancements of features already present in less-efficient C3 plants, but regulated in different patterns. We are comparing several leaf tissues (isolated using Laser capture microdissection) of rice (C3), maize (a moderate C4) and sorghum (an extreme C4 grass). We are surveying the transcriptome, proteome and metabolome along a developmental gradient from immature tissues at leaf base to mature tissues at the leaf tip. This gradient was defined using sink-source transition and cell wall specialization as markers for developmental time points. Two hypotheses will be tested by the comparative analysis of 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. This analysis should identify the regulatory points that are potential targets for the engineering C4 traits in C3 species. The project outcomes will be available at http://c3c4.tc.cornell.edu curated at the Gramene public database (http://www.gramene.org) and deposited at NCBI and EBI.
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P138

Comparative Genome Hybridization Of Maize Inbreds And Their Wild Ancestor, Teosinte

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A NimbleGen array containing ~ 2.1 million probes was designed based on the genomic sequence of the maize inbred line B73. Replicates of Comparative Genomic Hybridizations (CGH) were performed on this array using the inbreds B73 and Mo17, their recombinant inbred lines, and their wild ancestor, teosinte. A large amount of structural variation (SV) was observed between B73 and Mo17, including Copy Number Variation (CNV) and Single Feature Polymorphisms (SFPs). The distribution of SV across the genome is not random. We demonstrate that CGH-based probes with significant signal difference between B73 and Mo17 can be used as high resolution informative genetic markers. More SV can be detected via CGH between maize inbreds and their wild ancestor, as compared to inter-maize comparisons. Chromosome regions potentially involved in maize domestication were identified.

P139

Development of Chromosome-specific in silico SNPs in Maize

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National Center for Biotechnology Information (NCBI) contains a large number of bacterial artificial chromosome (BAC) clones, which were generated and deposited by the Maize Genome Sequencing Project. In this pilot study, we used BAC clone sequences of chromosomes_1 and _2 for development of chromosome-specific in silico SNPs. BAC clone sequences were downloaded from NCBI. Semi-automated pipeline involving masking the repetitive sequences within those BAC clones followed by construction of temporary contigs was developed. The consensus sequences of temporary contigs were used to blast dbEST and dbGSS at NCBI. The retrieved hits were aligned and in silico SNPs were discovered. Since e-SNPs represent “virtual” polymorphisms, they have to be validated by re-sequencing of the target regions in experimental lines. However, re-sequencing of every target region is a time-consuming process. The Illumina GoldenGate (GG) assay is capable of genotyping and validating 1536 SNPs in one reaction in a three day period. In this study, 595 e-SNPs representing 96 loci were discovered and submitted for the Assay Design Tool (ADT) at http://www.illumina.com. ADT rejected about 30% of the submitted sequences. The rest of the sequences had SNP scores ranging from 1 to 0.5, with “1” as the highest score. These sequences were submitted to Illumina for GG assay design. The GG genotyping results showed approximately a 65% success rate of eSNPs, which included both polymorphic and monomorphic markers. Finally, 13% of discovered e-SNPs were mapped mainly on chromosomes _1 and _2 as expected. The results of this pilot project demonstrated the usefulness and cost-effectiveness of e-SNP discovery coupled with high-throughput genotyping using the GG assay.

P140

Differential expression of miRNAs in response to salt stress in maize roots

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Maize (Zea mays) responds to salt stress via changes in gene expression, metabolism and physiology. This adaptation is achieved through the regulation of gene expression at the transcriptional and post-transcriptional levels. MicroRNAs (miRNAs) have been found to act as key regulating factors of post- transcriptional gene expression. However, little is known about the role of miRNAs in plants' responses to abiotic stress.

A custom mu paraflo (TM) microfluidic array containing release version 10.1 plant miRNA probes (http:// microrna.sanger.ac.uk/) was used to discover salt stress-responsive miRNAs between the salt-tolerant maize inbred line 'NC286' and the salt-sensitive maize line 'Huangzao4'.

MiRNA microarray hybridization revealed that a total of 98 miRNAs, from 27 plant miRNA families, had significantly altered expression after salt treatment. These miRNAs displayed different activities in the salt response, and miRNAs in the same miRNA family showed the same pattern. Interestingly, 18 miRNAs only expressed in the salt-tolerant maize line were found, and 25 miRNAs showed a delayed regulation pattern in the salt-sensitive line. A gene model was proposed to show that miRNAs regulate the abiotic stress-associated process and the gene networks coping with the stress.

Salt-responsive miRNAs are involved in the regulation of metabolic, morphological and physio-logical adaptations of maize seedlings at the post-transcriptional level. The miRNA genotype-specifc expression model might explain the distinct salt sensitivities between maize lines.

P141

Enhancer Trapping in Maize

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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 regulated by a minimal 35 promoter and a plant selectable marker (bar) cloned within a maize 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 hybrid maize by Agrobacterium mediated transformation. We have obtained transgenic plants from over 30 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. Unique transgenic insertions, identified by FISH are being introgressed into the B73 inbred line together with the immobile Ac to produce germinal transpositions of the Ds trap element. These lines will be screened for GFP expression (indicating trapped enhancers) and self pollinated to remove Ac. These enhancer trap lines will be useful for ploidy studies but will also serve as an important tool for functional genomics studies.

P142

Fine-scale analysis of heteroallelic expression in contiguous chromosomal regions in Arabidopsis and maize

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Evolutionary divergence in gene expression is central to phenotypic divergence and, ultimately, speciation. Nevertheless, very little is known about the myriad components that contribute to this expression variation in nature. Here we examine the relative contribution of parental alleles to the transcriptome in hybrids and report on the nature of the genetic components contributing to heteroallelic expression variation. We tested the heteroallelic expression variation of 30 genes in the an1-cer1 region on chromosome 1 from Arabidopsis cross Ler x Cvi. Over half of the genes show significant allelic expression variation in at least one tissue, that is, anywhere from 1.3 to 20 fold more expression from one allele than from the other in hybrid tissues. Eight genes preferentially expressed the Ler alleles, while another eight genes preferentially expressed the Cvi alleles. Fine structure recombination is now being used to dissect the components of this differential allelic expression. Similar studies are underway in the highly dynamic bz1-sh1 region on chromosome 9 of the maize genome. Preliminary analysis of this region recovered 13 candidate genes, of which at least 6 are expressed in every tissue examined. Expression of one gene, stc1, is restricted to leaf tissue. Heteroallelic expression analysis of these seven genes is underway, and is expected to yield informative results with respect to the effects of cis-regulation on gene expression in maize.

P143

Functional Genomics of Maize Gametophytes

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Our long-term goal is to understand the genetic control of the development and function of angiosperm gametophytes. Gametophytes require processes basic to all cells (e.g., mitosis, cytokinesis, and vesicle trafficking), as well as those specific to reproduction (e.g., gamete fusion, and pollen tube/synergid interactions). Mutations that are lethal to both the male and female gametophyte are not recovered even in screens directed specifically at only one of them. We are conducting a large-scale screen for mutants affecting maize (Zea mays) gametophyte development and/or function by leveraging the distinctive ability in maize to generate genomic duplications using translocations between the standard A chromosomes and the supernumerary B chromosome. In conjunction with the power of transposon mutagenesis and seed markers, this allows recovery of mutations lethal to the gametophyte regardless of mutant phenotype severity. In a genetic screen, these tools avoid bias against gametophyte-essential genes, because a subset of the gametophytes that carry an otherwise lethal mutation also carry the appropriate duplication with a wild-type allele and are therefore viable. Additionally, we are performing an analysis of the transcriptome of the mature male and female gametophytes using the Illumina platform of next generation sequencing and comparing those expression profiles to profiles of select sporophytic tissues. We predict this project will provide novel insights into the biology of gametophytes that can be readily extended to other plant species.

P144

Gene expression profiling and promoter motif detection in seedling maize roots and root hairs

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Our long-term goal is to reduce the amount of fertilizer needed for maize growth. We have two approaches: (1) accelerate early vegetative development in order for the plant to take up more soil nutrients in the Spring during peak litter decomposition; (2) optimize root hairs to take up more soil nutrients as the root hairs represent up to 70% of the root surface area. Our initial goals are to define promoter motifs specific for early maize development and root hairs in order to facilitate subsequent targeted transgenic improvement, and to discover candidate signalling genes for targeted breeding improvement of nutrient use efficiency. As a first step, we used microarray profiling to define expression clusters associated with early maize vegetative (shoot and root) development by comparison to a later stage of development. We developed a BIOPERL program to find over-represented promoter motifs using Seeder (Fauteux et al., 2008) and MEME (Bailey et al., 1994) algorithms. Here we present the results for root organs. To study the root hair transcriptome in response to nitrogen, we have first optimized a protocol for maize root hair growth and RNA isolation. We also have optimized a transient biolistic system for roots and root hairs to validate candidate promoter motifs using GUS reporter cassettes.

P145

Gene silencing during the domestication of maize

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Changes in gene expression patterns are associated with changes in plant form during evolution. The shoot apical meristem (SAM) gives rise to all above-ground structures. Hence, changes in gene expression in the SAM and/or young seedlings may be responsible for the morphological differences between maize and its wild ancestor, teosinte. Using a combination of 454 and Solexa sequencing a collection of ~10 million maize and ~7.2 million teosinte ESTs were generated from SAMs and seedlings of maize and teosinte. These ESTs were aligned to all genes predicted based on the B73 maize reference sequence. A set of 157 genes that are expressed in teosinte but not in maize was identified. These "mute genes" are hypothesized to have been silenced during the domestication of maize.

P146

Genetic Architecture of Maize and Teosinte

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

P147

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

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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 under three states of water-stress. The transcriptomes were compared at the three treatment conditions (before water stress, 24 hrs into the water stress, and 24 hrs into water-stress recovery) using a factorial, direct loop design applied to each genotype. A linear model of transcriptome abundance and the use of empirical Bayes shrinkage of the standard errors estimated significant changes in transcript abundance across treatments within each of the genotypes. The transcriptional profiles during the recovery condition show that water stress failed to induce lasting transcriptional changes: fewer than 120 genes of over 50,000 genes differed between control and recovery within genotype. In contrast, over 1,100 genes per genotype changed between the transcriptomes of control and stress treatments. On average, a genotype shared 10% of its stress-responsive genes with all other (six) genotypes, shared 60% of its stress-responsive genes with some (two to five) genotypes, while 30% of a genotype's stress-responsive genes were not shared but unique to the individual genotype. Metabolic pathways that responded to stress were affected in a genotype-shared and genotype- specific manner, with the majority of pathways in the former category. In addition, we observed that stress enhances transcriptional diversity among genotypes, consistent with the idea that environmental change can reveal cryptic genetic variation. We suggest abiotic stresses may induce large, genotype-specific transcriptional responses.

P148

High-resolution genome-wide mapping of recombination events in maize

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Meiotic recombination is one of the major forces affecting genome evolution and shaping its structure. However, very little is known about the genome-wide distribution of recombination events in maize. Factors affecting this distribution are also poorly understood. To examine where meiotic recombination events take place in the maize genome, we have developed a method to identify DNA fragments flanking the sites of meiotic double-strand-breaks (DSBs) in chromosomal DNA. DSBs are formed early in meiotic prophase and initiate meiotic recombination. To isolate these fragments, we use chromatin immunopurification (ChIP) with an antibody against the RAD51 protein. RAD51 is the key recombination protein involved in meiotic DSB repair and during most of zygotene forms distinct foci on the sites of DSBs, where it coats single-stranded DNA overhangs created by DSB resection. Following immunopurification, the DNA fragments associated with RAD51 are sequenced using the Illumina Genome Analyzer. This method allows mapping DSB hotspots genome-wide with a resolution of a few hundred bp. Our preliminary analysis of DSB distribution on the short arm of chromosome 9 revealed that meiotic DSBs form a large number of distinct hotspots and coldspots along the chromosome. Many hotspots are associated with the locations of genes but, overall, we also found a significant percentage of hits to repetitive elements in the maize genome. Fewer DSBs are seen in a ~ 6 Mbp region near the telomere than along the rest of the chromosome length. This map will permit elucidating if specific DNA sequence features are required for DSB hotspot formation, which, eventually, might allow us to design artificial recombination hotspots.

P149

Highly polymorphic and haplotype-based SNP markers for maize

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TraitGenetics has sequenced DNA fragments from more than 7500 maize unigenes in a panel of maize lines. Based on these panel sequences, we have identified SNPs and haploytpes in many maize genes. The information was used to design individual SNP assays that display a high allele frequency for the minor allele in this maize panel. These assays were subsequently used to determine the level of polymorphism in a set of maize lines and hybrids. Large numbers of SNPs were validated to contain an allele frequency of 0.3 to 0.5 in elite maize material. In a second experiment, SNPs specific for individual haploytpes were selected from the sequenced amplicon (on average 3 SNPs per amplicon) and also analysed in the large set of maize lines and inbreds. Using two Illumina 1536-plexes, we have validated assays sets of functional and haplotype-specific SNPs for nearly 1000 maize unigenes. These markers can now be used in mapping and genetic fingerprinting experiments.

P150

Hybrid specific changes in small RNA populations from maize seedling shoot apex

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Heterosis, or hybrid vigor, is the observation that progeny exhibit greater size, faster development, and higher fertility compared to their parents. Heterosis in maize is manifested in both vegetative tissues and reproductive organs and the hybrid advantage is visible throughout development. We used the Illumina deep sequencing platform to profile small RNA populations of shoot apices isolated from 11-day old seedlings of two inbreds (B73, Mo17) and their reciprocal hybrids (B73 x Mo17, Mo17 x B73). This tissue was chosen because it was expected to be enriched for small RNAs with regulatory functions, and it also corresponds to a stage of rapid leaf initiation and cellular proliferation within leaves and internodes. Amongst the four libraries, roughly 108,000 unique sequences with greater than 5 reads were cataloged, with nearly 75% of these sequences showing perfect matches to unique or multiple contigs of the draft Maize genome assembly (Release 3a.50, December, 2008). The relative proportion of 21-24-nt small interfering RNAs (siRNAs) did not vary significantly between the libraries; however, close to 40% of the total unique 21-24-nt sequences were found to accumulate specifically in either one or both of the hybrid libraries, indicating hybridization significantly increases the complexity of the siRNA populations within maize seedling shoot apices. Several conserved microRNAs (miRNAs) showed non-additive expression patterns in comparisons of inbreds and hybrids, which were confirmed via TaqMan qRT-PCR assays. One of the most interesting differences is a two-fold increase in the hybrids compared to their inbred parents for miRNA168 (miR168). MiR168 targets the mRNA for ARGONAUTE1 (AGO1), which encodes an essential component of miRNA-directed RNA cleavage and also functions in RNAi, the tasiRNA pathway, and can modulate chromatin modifications. The implications for observed changes in small RNA profiles to the molecular basis of heterosis in maize are discussed.

P151

Identifying essential genes for maize kernel development

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Cereal grain development consists of a complex array of interrelated processes controlling the specialized cellular activities required for grain function, and which are important for human exploitation of this tissue for nutritional and industrial purposes. Mutants that produce an empty pericarp (emp) or defective kernel (dek) phenotype identify genes that are essential for the various processes that contribute to grain development. While important and interesting genes are certainly represented in dek/emp mutants, these mutants are problematic to study because of their abundance and non-descript phenotypes. We suggest the best approach for these mutants is to clone first, analyze the mutant later. We have developed an effective, high-throughput transposon display and gene cloning system, called MuTA, which allows the efficient identification of Mu insertions genetically linked to mutations. In initial experiments, 16 mutants were analyzed and linked insertions were identified for 8. Subsequent analysis confirmed the identity of 2 mutants with independent alleles, and broke linkage with 1 other. Linkage has held with the remainder but they have not yet been confirmed. We propose to analyze dek/emp mutants in existing mutant collections from Mu lines to learn the identities of genes required for the development of viable maize grains.

P152

Influence of host genetics on the rhizosphere microbial community of switchgrass and maize

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The rhizosphere microbial community is known to play a crucial role in promoting plant growth, in controlling soil borne phytoparasites, and in nutrient cycling. The abundance and composition of the root-associated microbiota are influenced by several factors, including plant species and genotype. At least part of this effect is may be due to genotype-specific variation in the root exudates that may play a key role in the selective stimulation of some soil microorganisms. Determination of the influence of host genetics on the composition of the collective rhizosphere ”metagenome” may be useful in understanding how plants mobilize micronutrients and avoid parasitism, especially in the earlier stages of seedling development. In the current study, we examined root-associated bacterial, archaeal and eukaryotic communities. We analyzed partial sequences of amplified 16S and18S rRNA genes from bulk soil, rhizosphere soil, root exteriors and root interiors from two diverse switchgrass cultivars, Alamo and Summer and from maize. Dramatic differences in relative and absolute colonization were observed across this germplasm, and these differences were also microbe-specific.

P153

Isolation and characterization of resistance gene homologues (RGHs) from switchgrass germplasm

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We isolated Resistance Gene Homologues (RGHs) from the switchgrass accession Alamo corresponding to the NBS-LRR class of resistance genes by sequencing PCR products amplified using degenerate primers targeted to the conserved NBS region and by mining a recently developed expressed sequence tags (ESTs) database. Eighty eight switchgrass NBS RGHs were isolated with degenerate primers and 295 were identified by mining a database of 424,545 switchgrass ESTs. NBS RGHs isolated with a PCR based approach were grouped into 5 classes. In addition to the 5 classes identified by degenerate PCR, 5 more classes were selected by EST database mining. Each of these 10 classes of RGHs in switchgrass was found to have close homologues in other grasses, including maize. We designed specific primers for these 10 classes to screen an Alamo switchgrass fosmid library to identify clones carrying full-length NBS RGHs. Currently, twelve positive clones containing NBS RGHs have been identified and isolated for full-length fosmid sequencing.

P154

Large-scale SNP Discovery in Maize Using Genome Complexity Reduction Approach

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Increasing demand for maize as a food, animal fodder and a source of bio-fuel requires further improvement of this crop, which has been tremendously advanced by application of high-throughput molecular markers, including single nucleotide polymorphisms (SNPs). However, SNP discovery in maize is a challenging task because of the complexity of the genome. Recent studies revealed that reduction of the genome complexity is a viable option for SNP discovery. Complexity Reduction of Polymorphic Sequences (CRoPS™) is a novel approach developed by KeyGene N.V. for large-scale SNP discovery in complex genomes. CRoPS™ technology is a combination of AFLP with a massively parallel pyrosequencing technology commercialized by 454 Life Sciences Corporation. In this study, we leveraged the CRoPS™ approach to facilitate SNP discovery in maize. AFLP-pre-amplified genomes of two elite inbred maize lines were sequenced. Over 1100 homozygous SNPs were identified. The Illumina GoldenGate (GG) assay (Illumina, San Diego, CA) is capable of multiplexing up to 1536 SNPs in one reaction in a three day period. CRoPS-discovered SNPs (KG-SNPs) were used to design a 1536-plex maize Oligo Pool All. Putative SNPs were converted into GG assay with 92% success rate. Approximately, 70% of KG-SNPs were polymorphic and were mapped in several mapping populations. Results of this study demonstrated the universal nature of CRoPS™ technology and Illumina Genotyping platform in rapid SNP discovery and genotyping, respectively.

P155

Length of Selection around Candidate Genes for Artificial Selection during Domestication and Crop Improvement in Maize

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Genomic screens for artificial selection have been successful in identifying candidate genes for agronomic traits in maize (*Zea mays* L). However, the validity of the candidates identified requires that selection sweeps are very short, only containing the candidate gene with the nearest neighboring genes have diversity levels indicative of neutral genes. We analyzed nucleotide polymorphisms at neighboring genes of selection candidate genes in modern maize (*Zea mays* ssp. *mays*) and teosinte (*Zea mays* ssp. *parviglumis*) accessions. Our objective was to determine the selection status of the neighboring genes of candidate genes for artificial selection. For 10 of 14 candidates neither of the two neighboring genes exhibited signatures of selection indicating that for most candidates genes the selection sweep only contained the initial identified gene and therefore this gene represents the true selected gene. For two candidates, one of two neighboring genes was positive for selection, and for two candidate genes, AY107228 and AY107907, both neighboring genes were selected indicating more extensive selection sweeps. Both AY107228 and AY107907 were previously classified as improvement genes with most of the diversity loss occurring during recent crop improvement. This result suggests that longer regions of reduced diversity may remain for improvement genes compared to domestication genes.

P156

Molecular genetics of arbuscular mycorrhizal symbiosis in rice

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The arbuscular mycorrhizal (AM) symbiosis is the most widespread plant-fungal interaction between roots of terrestrial plants and fungi of the Glomeromycota. The association receives increasing scientific attention because of the nutritional benefit it confers to host plants, its ubiquitous occurrence among extant plants, its influence on plant diversity in natural ecosystems and its possible ancestral relationship to other plant interactions. With the ultimate goal to exploit the potential of the AM symbiosis for sustainable agriculture our research has focused on identification of molecular mechanisms underlying functional compatibility in the AM symbiosis using the crop plants maize and rice.

In rice we used a combination of transcriptomics and functional genomics as optimal corresponding resources are available: rice is currently the only mycorrhizal plant offering a sequenced genome, different platforms for whole genome transcriptomics and extensive mutant collections combined with large FST (flanking sequence tags) databases for functional genomics. We are therefore in the unique position to comprehensively perform gene discovery linked to assessment of gene function.

Transcriptome analysis of mycorrhizal rice roots revealed >200 induced genes. To assign function to genes we identified insertions within a total of 75% of these genes. Heritable phenotypes altered in AM have been recovered.

A subset of genes was expressed in a symbiosis-dependent fashion. Suitability of these genes as markers was defined by characterizing their activity with respect to temporal-spatial expression and local versus systemic induction. These markers were used as molecular phenotyping tools to dissect signaling in AM symbioses.

P157

Molecular-genetic polymorphism of loci, associated with maize high-temperature tolerance

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Abiotic stress, especially high temperature action, considerably decreases harvest of agriculture plants, including maize. Maize (Zea mays L.) is a traditional cultural plant of southern Ukraine, characteristic of this region is high summer temperature from +30 to +35 ° C. Modern heat tolerance maize evaluation technology is required to be developed for using in heat-tolerant maize genotypes breeding programs. Our research aim is PCR analysis of heat shock protein (hsp)-associated maize molecular-genetic polymorphism for creation of heat shock tolerance markers system.

DNA of 35 maize lines, differed for heat tolerance PCR-amplified with seven primer pairs, specific to the hsp- loci (umcl545, umcl546, umcl610, uaz171, phi071, hsp18a, hsp26/1). PCR analysis of hsp loci revealed polymorphism of maize lines, contrasting by the heat tolerance for six of seven loci. Alleles numbers varied from 2 to 5. Locus hsp26/1 not revealed any polymorphism within analyzed lines, allele size was 274 b.p. Set of alleles, specific for heat-tolerant and heat-susceptible genotypes of maize was established: 80 b.p., 129 b.p., 92 b.p., 84 b.p., 194 b.p., 266 b.p. and 76 b.p., 129 b.p., 92 b.p., 98 b.p., 194 b.p., 266 b.p., of loci accordingly.

P158

Physical mapping of the *Rf8* restoration of fertility locus in T-cytoplasm maize

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Cytoplasmic male sterility (CMS) in T-cytoplasm maize is caused by the premature degeneration of the tapetum. This degeneration can be attributed to the unique mitochondrial gene, T-*urf13*, and its product, the 13 kDa URF13 protein. Interestingly, the gene is also responsible for the plant's sensitivity to a toxin produced by the fungus *Cochliobolus heterostrophus*, to which T cytoplasm maize is highly susceptible. Full suppression of T-*urf13*-mediated male sterility is attributed to the combined action of two nuclear genes, *Rf1* and *Rf2a*. *Rf2a* is one of a family of mitochondrial aldehyde dehydrogenases (mtALDH). By contrast, the molecular phenotypes mediated by *Rf1* and two partial restorers, *Rf8* and *Rf**, are characterized by the differential processing of T-*urf13* mitochondrial transcripts and the concurrent reduction of the URF13 protein. *Rf8* post-processes T-*urf13* transcripts, resulting in mtRNAs of 1.4 and 0.4 kb that co-segregate with partial fertility restoration. To continue our characterization of Rf-mediated T-*urf13* transcript-processing, a segregating backcross population of 1,738 individuals was generated to fine map *Rf8*. Previous work positioned *Rf8* on the long arm of chromosome 2. Eight sequenced B73 BACs that encompass the mapped region were analyzed with TEnest (Kronmiller, 2008. Plant Physiology 146: 45-59) for their composition of transposons in order to more efficiently develop markers. Codominant markers are being developed within the region that span introns or 3' UTRs based on mapped ESTs. Characterization of *Rf8* will provide an integral step in the functional analysis of CMS and the restoration of maize fertility.

P159

Project update on fluorescent protein tagged maize lines for cell biology and functional genomics

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Maize cells consist of interconnected but discrete compartments that help to maintain cellular function and order. Since identifying proteins that localize to these compartments is critical to understanding maize physiology and development, with the support of the NSF Plant Genome Program, we have set out to generate 100 stable, natively expressed, fluorescent protein (FP) fusion lines to provide a useful molecular resource for the maize community and to ultimately uncover guiding information for crop improvement. The lines developed thus far have provided unprecedented views of maize cellular architecture and we can now study real time dynamics of cell structure, function and protein localization for ~50 tagged genes. Current available lines highlight most major compartments and allow us to study hormone signaling pathways, cytoskeletal behavior, vesicle trafficking and other important developmental and physiological processes. Eventually, the tagged proteins will provide complete marker coverage of all subcellular compartments. Where possible, these FP fusion lines are used to complement their respective mutant alleles indicating functional activity. Data on the characterization of these lines, including confocal micrographs and movies, are accessible on the website, http://maize.jcvi.org/cellgenomics/index.shtml. The website also includes a community submission form to request for your favorite gene to be tagged. Targeted proteins are selected for the tagging pipeline if they fit several criteria outlined on the website, including that: 1) the total tagged genomic sequence is less than 10kb and 2) the gene has supportable mRNA expression. In addition, constructs and seeds from verified T2 generation transgenic plants are available from the Jackson/Sylvester labs.

P160

QTL-directed candidate-gene NAM analysis to identify natural variations related with maize leaf traits

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Nested association mapping (NAM) strategy, combining the advantages of linkage analysis (robust and low marker density demand) and association mapping (high resolution and rich allelic diversity), provides a high-resolution and cost-effective approach to dissecting the genetic architecture of complex traits. A maize NAM population comprising 5000 recombinant inbred lines (RILs) has been developed by crossing 25 diverse founders with a common parent (B73). With genotyping data from 1106 B73 common-parent-specific (CPS) SNPs across 5000 RILs, about 100 QTLs related with three leaf traits (i.e., leaf length, leaf width, and upper leaf angle) were mapped by multi-population linkage analysis. Based on the genomic sequence of B73, we plan to select 200 candidate genes with purported function in leaf shape, shoot apical meristem, cell growth, and phytohormone biosynthesis, from the mapped QTL-peak regions. The target DNA of the chosen genes will be enriched by a custom hybridization-mediated microarray method for 25 founders and the resulting fragments will be deeply sequenced by a next generation sequencing platform to identify candidate gene SNPs. Information of the candidate gene SNPs will then be projected to each RIL from founder alleles based on CPS SNPs. Final association analysis will be performed across 5000 RILs to identify genes underlying the natural variation of the maize leaf traits.

P161

Rapid Plant Seed DNA Extraction and Improved PCR of Plant Tissues Including Seeds and Leaves

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There is demand for high-throughput PCR using plant seeds and leaves, but there are very few methods that do not require highly specialized equipment. To this end, Epicentre developed the QuickExtractTM Seed and Plant (leaf) DNA Extraction Solutions. One hundred microliters are added to ten mg or less of seed fragments, or a small leaf disc, and after two heating steps totaling eight minutes the extract is PCR-ready. Single-copy maize or rice loci were amplified from rapidly extracted seed materials, using endpoint or quantitative PCR (real-time) formats. TILLING (targeting induced local lesions in genomes), SCAR (sequence characterized amplified region) and other PCR based methods for plant mutation and marker discovery should benefit from the novel sample preparation methods.

Seed germination and sprouting delay the results of genetic tests. The QuickExtract Seed DNA Extraction Solution saves the time and materials needed to grow leaves. When the seed extraction method was used in conjunction with the 2X premix in the PlantAmpTM PCR System, consistent results were obtained with plant species including monocots and dicots. PlantAmp contains reagents specifically designed to overcome amplification problems caused by polyphenols and other plant components. Cotton, apple, and sunflower seeds, known as polyphenol-rich and challenging DNA sources, yielded superior PCR results when a combination of QuickExtract Seed extracts and PlantAmp reagents was used.

P162

Restorer of fertility-associated loci maize polymorphism

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Cytoplasmic male sterility (CMS) is complex feature, determined both by mitochondrial and nuclear genes. CMS is also important agronomic feature widely used in maize breeding programs. Nine fertility restorers genes, designated as restorer of fertility or rf are known now. Each type of sterility (S-, C- , T-) is restored by definite restorer of fertility gene - if plant posses T-type sterile cytoplasm, fertility could be restored only by Rf1, Rf2 and Rf8 loci, both cross-reaction between definite CMS types and restoration of fertility by non-specific restorer of fertility loci (for instance, plants with T-type CMS can not be restored by Rf3 locus) is impossible. Restorer of fertility loci mapped on chromosomes 2, 3, 8 and 9.

Our research aim is investigation of the fertility-associated loci polymorphism, analysis of interaction between the allele state of loci, associated with fertility restoration and sterility, and designing of PCR-based markers system to predict the sterile genotypes of maize without using of ground control to simplify the process of maize sterile genotypes selection. 86 lines of maize differed by the fertility-associated loci allele state and by the type of cytoplasm (N-, T-, S- and C- types accordingly) are used.

Lines genetic purity was investigated by PCR analysis of three microsatellite loci (phi065, umc1172, umc1203), selected from previous researches (as the most polymorphic loci). Lines are homogeneous and homozygote both within ones and between analogous.

Search of genes (both mitochondrial and nuclear) nucleotide sequences is conducted from MaizeGDB. Vector NTI Suite 9 program is used for necessary nucleotide sequences searching and for primer design. Developed PCR primers allow determining sterile genotypes of maize by the meaning of PCR-based technique.

P163

Sequence Diversity and Evolution of Recombination Genes in Maize

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Recombination is the main source of genetic variation, responsible for creating new gene combinations in the progeny. Even though a large number of genes involved in recombination have been identified, genetic factors affecting recombination frequencies remain poorly understood despite the evidence of variation in recombination rates between different genotypes in several species, including maize. So far, recombination genes have mostly been studied through severe loss-of-function mutations, which completely abolish the functioning of the pathway. However, recombination proteins do evolve due to selection and we want to examine the effects of these subtle changes on the recombination pathway. Studying natural variation in the sequence of recombination genes will give us insight into how these genes evolve and how they are affected by selection. In this study, we are examining the sequence diversity in 11 key recombination proteins involved in various steps of the recombination pathway, using a set of 31 diverse inbred lines and 7 teosinte lines. Homologs of these genes were identified in maize. Entire ORFs along with some intron sequences were amplified for all these candidate genes using PCR. Phylogenetic analyses were performed using PAUP and MrBayes. Our results show that different recombination genes show strikingly different evolution rates and patterns. We also found that duplicated gene copies (e.g Rad51A1 and Rad51A2) exhibit different levels of sequence polymorphism, suggesting that they are under different evolutionary constraints. We are analyzing the patterns of selection in the sequences of the recombination genes. Although we expect to see predominantly purifying selection, theoretical predictions suggest that populations that are subject to strong selection pressure, for example during domestication, are likely to evolve higher recombination rates. If this is indeed the case, we expect to see signatures of positive selection in the genes responsible for meiotic recombination frequencies.

P164

Some classes of stress response genes appear to be more mobile within both Brassicales and the grasses

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Recent research in our lab comparing the genomes of Arabidopsis thaliana and Papaya has led to the observation that between one-fourth and three-fourths of the genes within Brassicales appear to have been mobilized, a proportion much higher than previous estimates. In addition, it has been observed that certain gene families tended to have a larger percentage of transposed genes than other families, and that these families were almost always those implicated in environmental stress response, such as B3 transcription factors, NB-LRR disease-resistance genes, and F-box proteins. In order to see whether this pattern of gene-family-specific mobility was unique to the Brassicales or was a more general trend among angiosperms, we used the flanking gene method to compare synteny of the aforementioned genes between rice and its close relative, Brachypodium (using Sorghum as the outgroup). What we found was that the very same gene families that tend to be more mobile in the Brassicales also appear to have a higher proportion of transposition in rice and Brachypodium, suggesting that gene-family specific mobility is a general trend in plants, an observation that raises important questions concerning the causes and consequences of gene transposition.

P165

The Grass Transcription Factor (TF) ORFeome Project and Guidelines for Naming Grass TFs

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The control of gene expression is central to all cellular processes. Transcription factors (TFs) function in networks, in which a TF may control the expression of another, which in turn may modulate the expression of additional downstream TFs. An emerging theme is the identification of these regulatory networks in which TFs participate. It is estimated that the number of TFs in maize is about 7% of the genome, representing more than 4000. As part of a long-term effort to investigate and understand grass regulatory networks, we have initiated The Grass Transcription Factor ORFeome Project. We have already cloned more than 100 transcription factors from maize and more than 50 from rice, and plan to clone at least 25 TFs from both sorghum and sugarcane. Full-length ORFs or cDNAs (flcDNAs) for TFs are being identified and then cloned into Gateway® Entry vectors that will permit the facile recombination into plasmids for expression in plants or microorganisms. It is anticipated that at least 25 of these clones will be recombined into destination (pDest) vectors suitable for overexpression and protein production. These proteins will be used to raise antiserum to be employed in developing chromatin-immunoprecipiation techniques aimed at TF target genes in the maize genome. Clones for these TFs are being made publicly available to researchers. Information on available clones is being posted at the GRASSIUS (www.grassius.org) web resource. As part of the database development we have proposed a set of rules for naming TF proteins in the grasses (Plant Physiology 2009 149(1) p4-6). The project has a strong educational component that aims to Foster the Integration of Research with Education (FIRE). In Fall 2006 to 2008, almost 150 Molecular Genetics Laboratory students at the University of Toledo participated in this project and learned database mining and gene cloning skills.

P166

Transcriptional regulation of nitrogen metabolism in maize

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Nitrogen (N) is one of the most important nutrients for plants and is a primary determinant for their growth and yield. Asparagine (Asn) is a crucial molecule of N metabolism because of its metabolic stability and high N/C ratio. Asn plays a central role in intercellular nitrogen transport from source to many sink tissues, and a higher Asn/Gln is required for optimal kernel growth and protein accumulation. The level of Asn in plants is determined mainly by the activity of asparagine synthetase (AS) and asparaginase (ANS). We have recently shown that the expression of genes encoding specific AS and ANS isoforms is reciprocally regulated at the transcriptional level; however, the mechanism controlling this pattern is unknown. In order to gain insights into the regulatory network controlling the reciprocal expression of AS and ASN, we compared free amino acid and global RNA expression profiles of developing leaves from N- stressed B73, Illinois High Protein and Illinois Low Protein inbred lines, genotypes which differ significantly in N metabolism for both vegetative and reproductive tissues. Leaves were sampled from each genotype at seven time points that span the transition from a developing sink tissue to a remobilizing source tissue. Our RNA profiles revealed that the majority of differentially accumulating transcripts encoded proteins performing functions of general metabolism, carbon metabolism and transcriptional regulation. As expected, principal indicators of nitrogen metabolism, AS and ANS, showed differential accumulation among genotypes. Quantitative RT-PCR analysis validated the expression of a subset of genes and confirmed the reciprocal regulation of AS and ANS. In addition, a coordinated expression among AS, a bZIP transcription factor, and a stress related kinase throughout all the studied developmental stages was observed. We cloned the AS promoter and our comparative sequence analysis revealed the presence of sequence variants that may contribute to the observed expression differences. The amino acid profile especially the level of Asn mirrored the pattern of gene expression. More details on gene classes, promoter sequence analyses, and amino acid profiles will be presented.

P167

Transcriptome analyses of developing ear-shoot in selected genotypes of maize

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Subtle differences in the balance between carbon and nitrogen metabolism dictate the agronomic performance of maize. Several field level studies were conducted to understand and alter this balance. The developing ear-shoot at pollination represents the most important organ level sink in maize. Pollination triggers the remobilization of nutrient resources into the ear-shoot that will be influenced by genotype and various environmental factors. We used two microarray platforms to elucidate changes in the transcriptome of ear-shoots among genotypes with contrasting grain yield responses to nitrogen supply. For the B73 X Mo17 hybrid and the Agilent microarray, 33 transcripts were up-regulated in nitrogen-sufficient condition and 18 were up-regulated in nitrogen-limiting condition. Similar experiments with the Arizona Oligonucleotide Array Project microarrays identified 153 transcripts up-regulated in nitrogen-sufficient condition and 162 transcripts up-regulated in nitrogen-limiting condition. The transcript profiling was then extended to ear-shoots of three IBMRI x IHP1 hybrids with strong grain yield responses to nitrogen fertilizer and three IBMRI x IHP1 hybrids showing no N response for grain yield. This experiment identified 283 transcripts with differential accumulation. A separate study surveyed ear-shoot transcript profiles among four tropical hybrids prevalently grown in Nigeria, where three high-yielding varieties under N-limiting conditions (4001/4008, KU1409/4008 and KU1409/9613) were compared to Oba super 1, a low yielding variety. Six hundred and forty eight transcripts were up-regulated in Oba super 1 and 638 transcripts were up-regulated in a combined analysis of the other three genotypes. A wide variety of changes in transcript profiles were observed among the genotypes and nitrogen treatments in different experiments, which was expected given the diversity of genotypes and growth environments studied. However, a large proportion of photosynthesis and carbon metabolism genes showed increased accumulation in high-yielding IBMRIL varieties in nitrogen-sufficient condition compared to the low-yielding varieties. Overall, this study provides a baseline data set for the interactive effects of genotype and N supply on mRNA expression variation in the maize ear-shoot.

P168

Use of Illumina-HTS to identify Mu-insertions underlying mutant phenotypes in the “Photosynthetic Mutant Library”

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The biogenesis of the chloroplast is a complex process involving thousands of nuclear genes. Many aspects of the process remain poorly understood, and the functions of a large-fraction of predicted chloroplast proteins are unknown. Forward-genetic analyses of chloroplast biogenesis have consistently led to recognition of unanticipated players and the establishment of new paradigms, but the power of phenotype-driven gene discovery for understanding chloroplast biogenesis has not been thoroughly exploited. Our collection of non-photosynthetic maize mutants, the “Photosynthetic Mutant Library” (PML), in conjunction with “next generation” sequencing provides an opportunity to discover most genes in maize that are required for photosynthesis. The PML collection consists of ~2100 independently-arising mutants culled from ~28,000 Mu-active lines; each mutant has an accompanying pedigree, and many have characterized chloroplast protein and RNA “fingerprints” that elucidate gene function. Allele-frequencies suggest the collection is near saturation for genes whose disruption causes a photosynthetic defect.

To fully exploit the PML collection for phenotype-driven forward-genetics, we developed a method that uses Illumina sequencing to identify the Mu insertion causing a phenotype-of-interest in high copy Mu lines. The approach entails sequencing (virtually) every Mu flanking sequence in a mutant, and comparing insertions among suitable genetic backgrounds to identify the insertion underlying the phenotype. Our method for enriching Mu-flanking sequences for sequencing differs from previously-described methods, by combining random DNA shearing with hybridization-enrichment via a biotinylated Mu-TIR oligonucleotide. This method, coupled with the large number of Illumina sequence reads, samples virtually all 32-mers within ~400 bp on each side of each Mu insertion, yielding large “peaks” of sequence reads marking each insertion. We will describe results of proof-of-concept experiments that document the success of the approach. This method adds to the available tools for phenotype-driven Mu-tagging/cloning. A byproduct of the approach is the identification of sequence-indexed heritable Mu insertions.

P169

What's new at PlantGDB for maize researchers?

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PlantGDB (http://www.plantgdb.org) is a plant genomics database featuring species-parsed sequence databases for Viridiplantae, annotated transcript assemblies for >160 species, online tools, and genome browsers displaying splice-aligned transcripts and gene models for 16 species (NSF#0606909). This poster will describe resources that may be useful to the maize research community on our newly redesigned website (viewable at http://beta.plantgdb.org; production release planned for March 10, 2009). (1) Gene Structure Annotation and Curation: PlantGDB's maize genome browser, ZmGDB (http://www.plantgdb.org/ZmGDB) currently displays the latest sequenced maize BACs with high quality spliced alignment to transcripts and transcript assemblies and will soon include gene models published by maizesequence.org, together with a core set of curated models. In addition, ZmGDB offers an online tool (yrGATE) for curated community annotation of gene structure based on the comparison of gene models and transcript evidence. We hope interested maize researchers will adopt this tool to further improve the annotation of the maize gene space. (2) Portable display of aligned sequence: A new DAS (Distributed Annotation Service) server at ZmGDB will allow our spliced alignments and annotated genes to be displayed on a remote genome browser such as MaizeGDB; conversely, ZmGDB can be configured to display DAS-served tracks from another genome database. (3) Sequence download: User can search by region or by functional annotation/ID and then download all aligned sequences, gene models, or upstream/downstream genomic sequence from the specified region in a variety of formats. (4) Transposon-tagged sites: Detailed genomic placement, sequence, and seed order information for Ds transposon insertion sites (see NSF# 0501713) can be accessed at http://www.plantgdb.org/prj/AcDsTagging. (5) Workflow management: PlantGDB's BioExtract Server (http://www.bioextract.org) provides an online platform for developing custom sequence analysis workflows. (6) Outreach: PlantGDB supports outreach to American Indian students each summer in addition to hosting a plant genomics research outreach portal (http://www.plantgdb.org/PGROP) for research and teaching.

P170

A Novel Approach for High Throughput Mapping of Transgenic Insertion Sites

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The ability to identify the location of putative transgenes quickly and with a high degree of certainty is a requirement for the success of any marker assisted introgression program. The objective of this study was to establish a functional protocol to map positions of transgenic events while balancing several key criteria associated with commercial timelines including throughput, use of resources and production of high quality data. We utilized the technique of bulk segregant analysis followed by selective genotyping to maximize success of identifying transgene locations. A panel of polymorphic SSR markers was used to genotype samples from pools representing plants that were either null or homozygous for the selected transgenic event. Comparison of genotypes between both BSA pools, the donor line and the elite isogenic line enabled researchers to map transgenic events with a high degree of efficiency using observations collected from segregating progeny. Furthermore, this procedure is amenable to automation producing a means for characterizing transgenic insertion sites that is far superior to traditional methods.

P171

A brown midrib mutant, bm2, decreases Western Corn Rootworm feeding on maize roots

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A previous microarray experiment determined that many maize defense genes are down-regulated by feeding of western corn rootworm (WCR) harboring Wolbachia. Wolbachia is a gram-negative endosymbiont that can alter reproductive patterns in its insect host. It infects all native populations of WCR as well as many other insects. In an effort to further understand the effect of Wolbachia on maize defense genes we evaluated WCR feeding damage in three brown midrib (bm) mutants. The bm mutants are single gene mutants characterized by alterations in lignin content. Genes in the lignin biosynthetic pathway are up-regulated during insect attack leading to increased lignin content and making the plant less palatable to the insect. bm1, bm2, and bm3, along with their wild-types were assessed for root damage due to feeding by WCR. Mutants and wild-types were grown to the V3 to V5 stage of development and then infested with 50 neonate WCR larvae. The larvae were allowed to feed for 48 hours after which the apical 10 mm of the root tip was collected for damage analysis. Images of the damaged roots were taken with a Leica-Eppendorf Microinjection Microscope using QCapture Pro software or with a Fisher Micromaster Microscope with Micron software. Damage areas were calculated and subjected to a statistical analysis to determine significant differences at the 0.05 significance level. Data analysis revealed no significant differences between bm1 and Bm1 wild-type and bm3 and Bm3 wild-type. These results are consistent with down-regulation of lignin biosynthetic genes observed on the microarray experiment. RT-PCR experiments to confirm changes in expression level are in progress. bm2 had significantly less damage than it's wild-type counterpart. This result is interesting because the bm2 mutant has been characterized as a component of the RISC complex, which is involved in miRNA processing perhaps suggesting that Wolbachia manipulates maize defense gene expression through miRNAs.

P172

A maize population, CRW3 cycle six, is resistant to Western Corn Rootworm at the V3 to V5 stage

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Western Corn Rootworm (WCR) is a significant threat to maize. The larval stage of WCR destroys the root system, preventing it from acquiring needed water and nutrients to support plant and reproductive development. The CRW3 population was selected for WCR resistance by field infestation with WCR eggs. To determine if CRW3 is resistant at the V3 to V5 stage of development, root feeding damage on CRW3 cycle 6 was compared to a susceptible check, B37xH84, and a resistant check, KWU7203xCRW3 cycle six. Plants were grown to the V3 to V5 stage then infested with 50 neonate WCR. 48hrs after infestation, four 1-cm seminal root tips were harvested from each plant. Images were captures of the root tips and analyzed by Image J to determine total root surface area. A Fisher Micromaster microscope with Micron software was used to quantify root damage areas. Duncan and Tukey means analysis revealed that CRW3 and the resistant check were statistically different from the susceptible check for both the size of feeding area and percent damage to the root surface area. However, CRW3 and the resistant check were not statistically different from one another. The number of feeding areas was not significantly different between the three maize lines. These data suggest that seminal roots of cycle six of the CRW3 population display WCR resistant as indicated by a reduction in feeding damage per feeding site. Further studies are needed to identify the factors in the CRW3 plants responsible for reduced WCR feeding damage.

P173

Allele Mining Via Phenotypic Selection in High Carotinoid Derived Maize Inbred Lines

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Maize that has been bred for high carotenoid content could serve as a source of biofortified dietary supplementation in countries that suffer from diseases from malnutrition. Recent studies have suggested that selection for various carotenoid profiles based on phenotype alone would be problematic and that marker-assisted selection (MAS) would be a more efficient strategy. To examine the amount of underlining allelic diversity from phenotypic selection alone, a set of high carotenoid ("Hi-C") inbred lines developed from crosses between 6 orange flint accessions and 3 yellow dent inbred lines, were characterized for the lycopene epsilon cyclase (lycE) gene at the molecular level. Lycopene epsilon cyclase has been shown to have a significant effect on the partitioning of carotenoid precursors, which ultimately lead to the synthesis of either lutein or zeaxanthin. The haplotype profiles of lycE for 34 Hi-C inbred lines and 6 yellow inbred lines where determined by both diagnostic PCR assays as well as sequencing of 2kb of lycE. All Hi-C lines possessed the exotic lycE alleles with previously reported lycE haplotypes represented in this set, as well as a number of other structural changes not previously identified. Finally it appears that phenotypic selection for intense orange kernels is sufficient to mine superior lycE alleles.

P174

Analysis of Inflorescence Architecture Traits in Context of Creating a Commercially-Viable Maize Population

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Genetic effects are well known to be population dependent. Hence, marker and QTL effects estimated in diverse, relatively unimproved populations may have less selection value in a translational genomics program aimed at improving commercially-viable germplasm. For greater relevance, genetic analysis should occur in populations closely related to current commercial germplasm. We are developing a commercial-grade mapping population to meet this need. The population is based on commercial lines that have recently come off Plant Variety protection. The lines included in this Ex-PVP population represent elite germplasm from hybrid development. Examination of the Plant Variety Patents data base reveals the lines we have selected are the direct progenitors of approximately 90% of current commercial germplasm. The lines included in this experiment are B73, Mo17, PH207, PHG39, LH82, PHG35, LH123, PHJ40, PHG84, PHG47, LH1, and PHZ51. We feel that this population will be a key component in the maize translational genomic pipeline.

The Ex-PVP population was grown in three replication experiments in three environments: Urbana, IL in 2007 and 2008 and West Lafayette, IN in 2008. A variety of traits have been phenotyped on the Ex-PVP lines in the parental, F1, and F2 generations with the intent of estimating genetic effects, specifically epistasis and heterosis. Inflorescence traits were a primary focus of this study. We collected data on specific traits related to ear and tassel architecture, flowering, leaf characteristics, plant architecture and grain yield. Results will be presented in the poster.

P175

Analysis of hybrid trait variation in intermated and non-intermated recombinant inbred line populations of Zea mays L.

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One explanation for hybrid vigour is that favourable 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. The RIL and IRIL populations were mated to an unrelated inbred line to develop hybrids. The hybrids were evaluated for grain yield, ear length, kernel row number, 1000 kernel weight and early biomass traits in three locations with two replications per location in year 2007 and 2008. As expected, the differences between trait means of RIL and IRIL populations were not significant (P=0.05). Although we expected hybrid trait variances to increase with inter mating, few trait variances differ significantly between RIL and IRIL populations. We identified a number of marker alleles consistently associated with ear length, test weight, and grain yield across years. As expected, fewer QTL were detected in the IRIL population compared to RIL population.

P176

Association Mapping Analysis of Resistance to Northern Leaf Blight in Maize

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Northern Leaf Blight (NLB) is one of the most important fungal diseases in maize that reduces yield both nationally and internationally. Resistance to NLB is controlled by several race-specific qualitative resistance genes as well as quantitative resistance loci. There is great interest in pursuing quantitative resistance for NLB in maize as it is more stable and less likely to break down over time. Many mapping studies for quantitative trait loci (QTL) using biparental recombinant inbred lines, recurrent selection mapping and nested association mapping strategies have reported the identification of QTLs for resistance to NLB. In this study, we use association mapping in approximately 300 lines of the maize diversity panel to identify loci across the genome that are associated with resistance to NLB. We compare these regions with previously reported QTL hotspots as a way to add additional resolution to the QTL region or identify novel QTL targets. We present the analysis of one region that was identified in the tasselseed2 (ts2) gene. An SSR specific to ts2 was analyzed in a set of six novel F2 populations derived from a large recurrent selection population. There is growing evidence that links ts2 to quantitative disease resistance, with emphasis on a role in jasmonic acid signaling.

P177

Association of *Dwarf8* Polymorphisms with Variation in Flowering Time in a Diverse Population of Maize (*Zea mays* L.)

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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*, located on chromosome 1, is a gene reported to be involved in the gibberelin pathway. Association studies have been challenging to reproduce in human studies and are not yet well-documented in plants. Nevertheless, 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. In our study, we have reevaluated a subset of the polymorphisms from the initial study: an 18 bp deletion in the promoter region, two insertion/deletions, a 3bp insertion and a 6 bp deletion using a different, significantly larger association population and new statistical methods (i.e. Q+K model of Yu et al. (2005)). The association between the 6 bp deletion and the association with variation in flowering time have been confirmed by Andersen et al. (2005) and Camus-Kulandaivelu et al. (2006).

Our results confirm the association between the 6 bp deletion and flowering time variation, although the association is not as strong as previously reported. This can be explained by the close relationship between flowering time and populations structure. The Q+K model explains 80% of the variation in flowering time. It is therefore nearly impossible for a SNP to explain the association with the phenotype. It is also likely that previous results were overestimated due to the pedigree LD between *Dwarf8* and *vgt1* (previously reported to be associated with variation in flowering time in maize).

Analysis of a linkage population detects a weak QTL for flowering time at the same general location on the NAM map as *Dwarf8*.

P178

Bulked segregant analysis using the high throughput maize GoldenGate SNP genotyping assay reveals multiple genomic regions associated with kernel hardness and tryptophan content in quality protein maize

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Quality protein maize (QPM) is a nutritionally enriched form of maize that contains twice the amount of essential amino acids (lysine and tryptophan) as compared to regular cultivars. Breeding for enhanced QPM necessitates simultaneous manipulation of two polygenic genetic systems in addition to the major gene locus opaque2. These modifier genes influence kernel characters and tryptophan content. The objective of this research was to rapidly and cost-effectively identify genomic regions associated with kernel hardness and tryptophan content using a combination of bulked segregant analysis (BSA) and genome-wide SNP scans. We used a 1536-SNP GoldenGate assay to perform BSA in seven F2 populations derived from QPM X QPM crosses that segregated for kernel hardness. Backcross-derived contrasting progenies ('high versus low' tryptophan lines) were then generated from four QPM X normal crosses. A typical positively associated marker identified through BSA has a distinct polymorphism between the parental genotypes as well as 'Class 5' (100% opaque) versus 'Class 1' (completely modified) bulks. BSA of 'Class 5' and 'Class 1' bulks in seven QPM X QPM crosses were used to identify several genomic regions such as bins 1.04/1.05 (1S), 7.02 (7S), 8.06 (8L), 9.04/9.05 (9L) and 10.04 (10S) that were associated with modification of kernel hardness. In each case, more than three SNP markers were identified in at least four populations. The genomic region associated with bin 7.02 was consistently identified across all the seven populations. Genome-wide SNP scans of the backcross-derived 'high versus low' tryptophan lines revealed polymorphic regions at bins 2.07, 5.03 and 10.03, which are currently being validated using SSR markers and segregating populations. The bin 10.03 was consistently associated with high tryptophan content in three of the four populations tested. As a cross validation, we generated whole genome SNP profiles of five isoline pairs of QPM and the respective normal-type, which confirmed the association of these genomic regions with hardness and tryptophan content. Once validated using targeted SSRs and segregating populations, these markers will greatly aid in designing a comprehensive MAS system for cost-effective QPM hybrid cultivar development.

P179

COGENFITO: a composite genotype finder tool that allows optimization of breeding schemes according to principles of genomic selection

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Determining the phenotypic consequence of natural allelic variation at particular loci is a common goal of plant breeders and geneticists. Careful use of molecular marker data can reduce the number of generations required to create stock populations to address this need. When several-to-many QTL affect a trait, assessing the additive, dominance, and interaction effects of a single QTL requires a breeding approach. Traditionally, contrasts of nearly isogenic line (NIL) pairs were used to eliminate variation at non-target loci. In previous work, we demonstrated that effectively isogenic line (EIL) pairs can be useful as well. EIL pairs fix the alleles at "control loci", allowing the alleles at "target loci" to vary. Because the use of EILs alleviates the need for introgressions, substantial breeding time is spared.

Here we introduce COGENFITO (beta version), an interactive, web-based tool that sorts and sifts through a given genetic mapping population's marker data set to identify lines with user-defined informative haplotypes. This tool will be launched at MaizeGDB in 2009 and will interrogate all mapping populations for which genetic maps have been built and genetic stocks exist. The researcher will specify the mapping population and either the centimorgan or molecular marker range corresponding to the genomic regions of interest. For each region specified, the desired genotype will be indicated. After a query is submitted, an output screen will show the researcher which stocks best match the desired parameters. Graphical display of haplotypes for user-selected lines will further facilitate experimental and breeding design optimization. COGENFITO will be applicable to a wide range of research pursuits, including QTL cloning, dissection of epistatic interactions, and control of genetic background in selection studies.

P180

Characterizing seed storage proteins in teosinte and tripsacum

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Seed storage proteins in modern maize varieties, called zeins, are deficient in three essential amino acids: lysine, tryptophan, and methionine. Attempts have been made to improve levels of these amino acids in a number of ways, including breeding of modern inbred lines with maize landraces and relatives of maize such as teosinte and tripsacum. HPLC analysis of seed storage proteins in 27 inbred lines, 17 landraces, and 11 teosintes showed large differences in types and amounts of these proteins, including novel proteins that were not present in the inbred lines (mean number of unique proteins was 22 in inbreds, 25 in landraces, 36 in teosintes). The alpha zeins, making up the largest percentage of total zeins, showed the greatest amount of variation (mean number of unique alphas was 15 in inbreds, 17 in landraces, 25 in teosintes). Preliminary analysis of tripsacum varieties had similar results, with some significant exceptions. Tripsacum was missing some zeins that were present all other samples, and had novel seed storage proteins that were not present in any of the other samples.

P181

Characterizing the selection response by selection and association mapping

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Recurrent selection (RS) is a method of population improvement used for crop species to increase the frequencies of favorable alleles and allelic combinations conditioning quantitative trait variation. We are developing an approach, using already-existing or custom-made RS populations, to simultaneously map loci controlling specific traits associated with population improvement and characterize the allele frequency response at those loci. Our study system is a maize population selected for two components of quantitative resistance to northern leaf blight, as we are equally interested in understanding the genetic basis of maize disease resistance. We will present the concept of our approach and current results from two years of phenotypic analysis along with some initial marker analysis. By combining the strengths of RS, population genetics and association analysis, we seek to provide insights into the genetic basis of quantitative disease resistance in maize, and more generally, to provide a new experimental approach for the analysis of quantitative traits applicable to many plant species.

P182

Combined QTL analysis of three maize-teosinte backcross populations

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Maize differs dramatically in morphology from its wild progenitor teosinte. Multiple QTL analyses of maize inbred - teosinte backcross populations have been used to identify the genetic changes that underlie these morphological differences. Using this approach, two major domestication genes have been identified: one controlling glume morphology (tga) and another controlling branching (tb1). However, other observed QTLs do not exhibit the same large effect size and narrow peaks as the QTL around tga and tb1. Furthermore, while the QTL for tga and tb1 appear consistently across studies, the placement and existence of many other QTL vary. This variation is due to temporal and spatial differences between the growing environments of the crosses and the genetic diversity of teosinte parents. Combined analysis of multiple crosses addresses both of these problems. Only QTL controlling differences between all maize and all teosinte in all environments should be identified by joint analysis. Combining studies increases the number of individuals analyzed, therefore increasing the number of recombination events potentially observed and narrowing the peaks of the QTL. With the goal of choosing domestication QTL with narrow peaks for positional cloning, three QTL populations were analyzed in combination. All three share a maize inbred parent, two share a teosinte parent and all three were grown in different fields at different times. Each population was analyzed individually using a multiple QTL model, then all three were combined into one population with cross as a covariate. The joint population was analyzed using composite interval mapping and multiple imputation mapping for nine traits selected during domestication. Joint analysis limited the identified QTL to those present in multiple populations with large effect and LOD score, as well as narrowing the QTL peaks. This narrowing was limited by a number of markers which only segregated in some of the populations.

P183

Confirmation of QTL for Reducing Preharvest Aflatoxin Accumulations in Hybrid Topcrosses

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Aflatoxins, a natural carcinogen produced by Aspergillus flavus in maize, are a chronic problem in Texas and southern United States. No elite germplasm offers true resistance to the infection and subsequent production; however, germplasm has been identified that can reduce aflatoxin accumulation. Previous research efforts have revealed quantitative trait loci (QTL) on different chromosomes for reducing aflatoxin accumulation in lines per se, but these have not been tested in hybrid combination. Our objective was to evaluate 132 Recombinant Inbred Lines (RILs) in hybrid testcross with LH195 for confirmation of QTL and to estimate agronomic performance and desirability at three locations. Aflatoxin was estimated by using the Vicam Aflatest. A second objective was to identify QTL associated with composition of the seed; starch, oil and protein were measure in the hybrid testcross RILs at three locations. Seed composition was determined using a Infratec Grain Analyzer. Significant differences were detected for College Station (CS), Weslaco (WE) and Corpus Christi (CC) for all four traits. A total of thirteen QTL were identified. Two QTL for reducing aflatoxin overlap the same map area on chromosome 1 as the QTL identified in per se evaluations. QTL for oil were detected on four chromosomes at CS, WE and CC; one of these flanks a QTL for aflatoxin resistance. Two QTL were detected for starch at CS. Grain yield was determined at each location by hand and combine harvest. Significant differences in grain yield confirmed that RIL hybrids did not yield as much as the commercial checks used. However, while these lines did not have agronomic performance similar to that of the commercial hybrids, several of the testcrosses accumulated fewer aflatoxins (RIL 115: 7.9 ppb, DKC66-80 54.41 ppb).

P184

Development and Marker Characterization of Maize-Teosinte Introgression Libraries

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Genetic analysis and improvement of crops relies on variation in genes controlling agronomic traits. In maize, artificial selection during domestication starting 7500 years ago and/or modern plant breeding over the last century has diminished this critical genetic variation. This is especially true for key genes responsible for traits that define differences between maize and its wild ancestor teosinte (Zea mays ssp. parviglumis). We have developed ten teosinte introgression libraries, each derived by backcrossing a different parviglumis accession into B73. Eight of these libraries, comprising 640 maize lines at the BC4S2 stage, were characterized with 768 SNP markers to define the introgressed teosinte chromosomal regions. Each line contains an average of 3 chromosomal segments encompassing ~4% of the teosinte genome. The development and evaluation of these maize-teosinte insertion libraries will enable us to evaluate allele series, test the impact of domestication on trait variation, and reintroduce valuable genetic variation into maize germplasm.

P185

Development of informative markers through association mapping in maize to improve drought tolerance in cereals

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Drought is a major limiting factor for cereal-crop production in developing countries. To identify superior drought tolerance alleles, we used association analysis which samples large allelic diversity and, in maize, has superior mapping resolution. To identify genes that confer tolerance to water deficit at the reproductive stage we identified SNPs in candidate genes for drought related traits. Phenotyping was conducted on more than 400 diverse inbreds in Mexico-TL and on 350 hybrids at 5 locations; two years at each. Plants were subjected to water deficit at flowering. Three tissues (ear tips, silks & leaves) were sampled at two time points (0 & 7 d after anthesis) for two years in the inbreds and analyzed for sugars, starch, abscisic acid (ABA) and metabolites. Hybrids were produced by crossing 349 lines with the tester line CML312 and phenotyped for yield and secondary traits in irrigated and drought (at flowering) and in five international locations. Forty six SSR markers distributed randomly across the genome were used to evaluate population structure of the lines. More than 500 candidate genes, about half related to drought, were selected for association tests. SNPs were used to scan the association mapping panel using an Illumina genotyping system. More than 36 SNPs from 30 genes were significantly associated with yield, metabolite and field morphological traits. Several ABA metabolite traits were associated with ABA-related genes. Many transcription factors and regulatory proteins were among the significantly associated SNPs. SNP associations with flowering date were abundant. This project has added to our understanding of the genes related to drought response and identified strong candidate genes that can be used to develop markers for future marker assisted selection for drought tolerance.

P186

Disease Lesion Mimics of Maize as a potential source of resistance to Fusarium ear rot

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Fusarium ear rot of maize is a major problem worldwide often resulting in poor quality grain and contamination with a family of mycotoxins called fumonisins. Fumonisins are produced by Fusarium verticillioides and related species and are acutely toxic to certain livestock. They function by inhibiting ceramide synthase thereby disrupting sphingolipid biosynthesis. A major biological effect of fumonisins is the induction of programmed cell death (apoptosis) in various in vitro and in vivo model systems. Resistance to visible symptoms of Fusarium ear rot can be selected by traditional maize breeding methods; however, this resistance is not correlated with toxin accumulation nor asymptomatic infection. In order to identify maize germplasm with resistance to fumonisin accumulation we initiated a project to screen maize lesion mimic mutant lines for ear rot and fumonisin accumulation. Lesion mimic mutants of maize are characterized by the formation of disease-like symptoms in the absence of pathogens and are associated with expression of programmed cell death mechanisms that are thought to play a major role in plant defense. Because a majority of the characterized mutations are dominant, they may serve as a model to identify disease resistance pathways for ear rot and fumonisin formation. This paper will describe our experimental protocol and preliminary data.

P187

Dissecting Resistance to *Aspergillus flavus* in Maize

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Aspergillus flavus causes Aspergillus ear rot of maize and contaminates developing or mature kernels with aflatoxins. These potent toxins cause liver cancer, nutritional interference and immunosuppression when consumed by humans or animals. As a result, the strict regulation on the commerce of contaminated maize causes economic burdens on farmers in the United States and other developed countries. In developing countries, where regulations may be nonexistent or unenforced, people commonly ingest this toxin. One of the best methods of aflatoxin control in maize is the development of host resistance. However, low levels of heritability and being a quantitatively inherited trait make this endeavor difficult to achieve. In order to identify loci controlling complementary mechanisms that could be combined to produce superior resistant lines, we have studied tissue specific components of resistance in silk and developing kernel assays. Using the most appropriate components of resistance, we are mapping QTLs that are associated with resistance to *A. flavus* in maize. We are evaluating a population of recombinant inbred lines from the cross of B73xCML322 and near isogenic lines with introgressions of Tx303 and CML52 using in-vitro assays in New York and field assays in Mississippi. Our preliminary results show that maize inbreds differ in infection frequency, latent period, and the extent of sporulation on silk tissue. Interestingly, developing kernels of inbreds were different only for sporulation. Two years of data on young kernel studies of the B73xCML322 population indicate the presence of QTLs for resistance to *A. flavus* sporulation in chromosomes 3 and 8. Other preliminary results based on two years of data on components of resistance, including resistance to colonization determined by qPCR and resistance to aflatoxin accumulation using NILs will be presented.

P188

EVALUATION OF STALK STRENGTH QTL BY NESTED ASSOCIATION MAPPING

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Lodging may be defined as rupture of the stalk below ear level. This phenomenon reduces yield 5-20% worldwide, raises drying costs, and exacerbates problems of volunteer plants emerging the following season. To address this issue, we have further elucidated the genetic architecture of stalk strength in maize using the Nested Association Mapping Population (NAM). Previous analyses of four biparental populations of F2:3 families by Flint-Garcia et al. (2003) revealed the utility of rind penetrometer resistance (RPR) in phenotyping stalk strength as well as the complexity of its genetic architecture. Surveying the allelic diversity, and utilizing the statistical power and marker density available in the twenty-five biparental populations of NAM, we have sought to confirm and supplement earlier analyses by implementing the same RPR phenotyping method. Current data from a single evaluation environment have mapped eleven QTLs accounting for 30% of the phenotypic variance across the twenty-five recombinant inbred populations. All QTL are shared by more than four NAM populations and eight of the eleven possess a series of positive and negative alleles with respect to the B73. Future analyses to enhance our understanding of stalk strength include replicated evaluation and genome-wide association mapping of RPR to exploit historical recombination in NAM founders.

P189

Effect of Plant Morphology and Planting Methodology on Biomass Production and Compositional Characteristics in Maize

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The ability to increase plant biomass and compositional traits such as digestibility are ever-growing concerns as the biofuel industry continues to grow. Altering plant morphology and planting methodologies are two potential methods to increase biomass per acre. It is hypothesized that both a tillering maize morphology and a planting regime which maximizes light interception by all plants will result in increased biomass production per unit of land. To test these hypotheses, genotypes with varying degrees of tillering were evaluated at two different densities and under two different planting methodologies. The two planting methods used were: traditional row-crop planting with plants 0.2 meters apart within a row and approximately 0.8 meters between rows and equivalent distance planting with equal distance between plants within rows and between rows while still maintaining the same planting density. Results of these replicated field trials will be presented. In addition to evaluating biomass yield, it is also important to evaluate digestibility and the effect that increased digestibility has on overall performance. To test this hypothesis hybrids with variable corngrass penetrance were also evaluated in the previously described trial. Cell wall composition and digestibility were determined. Identifying developmental mutants that increase digestibility while maintaining adequate biomass yield will be crucial for the efficient production of biomass for the biofuel industry.

P190

Effects of lfy1 gene on some characteristics of an inbred line and hybrids

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To improve yield and digestibility of corn stover, we have focused on the leafy (extra-leaf) gene (lfy1) and found out that lfy1 gene was located on the maize chromosome 3 by combination of AFLP, SSR technique and bulked segregant analysis (BSA) using F2 population (our unpublished data). In this study, we introduced lfy1 gene to our own inbred line KK11 and compared the characteristics between KK11 and its leafy near-isogenic line (NIL).

Introduction of lfy1 gene to KK11 was achieved by 3 generations of back-cross after crossing with the leafy inbred line 914L (from LFY, L. L. C.) and following selfing. The genotype of lfy1 locus was confirmed every generation by two SSR markers, mmc0001 and umc1578, which linked closely to lfy1 locus. By introduction of lfy1 gene, the NIL showed leafiness and average leaf number above ear was increased from 4.7 to 8.0 comparing to KK11. The silking date and the flowering date of the leafy NIL were delayed for 1 day and 4 days, respectively.

Two hybrids using KK11 as a pollen parent were compared the agronomical characteristics with the hybrids that replaced KK11 with the leafy NIL. Two leafy NIL hybrids showed the increased leaf numbers above ear, and the dry matter yields of stovers were also increased to 127% and 138%, respectively. However, little difference was observed in the ear yields. On the other hand, increase of the leaf numbers caused the elongation of stalk above ear, and as a result, slight increases of stalk breaking and insect damage were observed. We have determined the nutritional compositions of the hybrids and discussed the effects of leafiness on feeding value.

P191

Evaluation of a Gene Specific Marker in a Population of F2:3 Families for Resistance to Aflatoxin Accumulation in Maize

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Aflatoxins are carcinogenic and toxic metabolites produced by the fungus Aspergillus flavus during infection of maize and other seed oil crops. Climatic conditions in the South favor A. flavus infection and aflatoxin contamination in maize, making it a major issue for farmers in the region. Farmers suffer economic losses when aflatoxin levels in contaminated grains are deemed unsafe. Therefore, reduction of A. flavus infection and aflatoxin contamination are important from both economic and human health perspectives. One of the most promising avenues to combat aflatoxin contamination is the development of resistant maize lines. However, this has proven difficult due to a lack of gene specific markers for resistance. In a previous study, we identified candidate genes that were differentially regulated in response to A. flavus infection. Sequence analysis was performed on several candidate genes to identify polymorphisms between resistant and susceptible lines. One gene, a chloroplast precursor, was found to contain multiple polymorphisms, which were used to design a marker that could differentiate between the "resistant" and "susceptible" forms of the allele. This marker was used to screen a population of F2:3 families of Mp313E (resistant) x B73 (susceptible). Individuals with one or two copies of the "resistant" allele were found to have accumulated significantly less aflatoxin than individuals with no copies of the "resistant" allele. The marker was then mapped to chromosome four in the F2:3 population, and was found to have significant phenotypic effects for aflatoxin resistance. This marker can now be integrated in to existing marker assisted selection programs aimed at incorporating resistance into elite maize production lines.

P192

Evaluation of starch concentration, grain yield, and response to nitrogen levels in ILP90xB73 derived maize hybrids

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To continue our evaluations of starch concentration and grain yield in maize, materials derived from the cross of ILP90 to B73 and backcross to B73 were utilized. The (ILP90xB73)B73 BC1S3 line with the highest average starch concentration in testcross evaluation was crossed to the BC1S3 line with the highest grain yield. The F2:3 progeny derived from this cross was used to produce a new testcross population that were evaluated over four environments along with several commercial hybrid checks. The best lines from this testcross population appear to have relatively higher starch concentration than commercial hybrid checks, and notably have generally comparable grain yields. The best testcross lines showing the highest yields, and the best testcross lines showing highest starch concentrations, were selected and grown in four environments in 2008 along with more and newer commercial hybrid checks. The experiment included two environments with normal levels of nitrogen applied and two environments to which no nitrogen was applied. Our goal was to evaluate our experimental hybrids performance compared to commercial hybrids under normal and low nitrogen levels. Results showed our collective experimental hybrids to have equal average yield when compared to top commercial hybrids under no nitrogen applied, and comparable yields under normal nitrogen application levels. Starch concentration was very similar between the two in both conditions. Other traits such as kernel components and chlorophyll content were also evaluated. Correlation analysis revealed different patterns among traits within the experimental hybrid materials in comparison to within top commercial hybrid check materials. Additionally, testcross materials of F3s derived from crosses of PVP lines were grown in an adjacent experiment to investigate their hybrid potential compared to our experimental hybrids. PVP testcross materials showed very similar yields compared to our experimental hybrids, and both sets of materials had lines with yields similar to some of the commercial hybrids.

P193

Fitness comparisons of Bt-resistant and Bt-susceptible western corn rootworm colonies

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Western corn rootworm (Diabrotica virgifera virgifera LeConte) is an important pest of maize in the US. Since its introduction, acreage of western corn rootworm-targeted Bt-corn has increased dramatically. As with all single gene resistance mechanisms, the level of selection pressure applied towards development of resistant insects is a concern. To evaluate the longevity of resistance of western corn rootworm to rootworm targeted-Bt we conducted evaluated a number of fitness related parameters in a Bt-resistant (Cry3Bb1) and a Bt-susceptible colony of western corn rootworm. Each colony was derived from a cross of feral and non-diapausing strains. Newly emerged adults of each colony (both colonies reared on isoline corn the previous generation) were observed until death to examined traits such as longevity, egg production, and egg viability. In a second experiment we followed newly emerged first instar larvae of each colony, reared on isoline corn, until emergence as adults. There was no significant difference in time to emergence, total weight, number of larvae recovered or head capsule width between the Bt-resistant and Bt-susceptible colonies. Female longevity was not significantly different between the two colonies (81 days for Bt-susceptible and 71 days for Bt-resistant); however, Bt-susceptible males lived significantly longer than Bt-resistant males (70 days vs. 57 days). In addition, Bt-susceptible females produced significantly more eggs (754 eggs vs. 515 eggs) and had higher egg viability than Bt-resistant females. Furthermore, eggs of Bt-susceptible females were significantly more likely to hatch than eggs of Bt-resistant females. Although previous experiments documented that resistance is maintained for at least six generations without additional selection, fitness costs associated with Bt resistance would tend to delay pest resistance.

P194

Foxtail Millet: Developing a Model for Vegetative Branching in Grasses

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Plant architecture diversity in grasses derives from variation in patterns of branching. Maize has been particularly productive in elucidating the genetic control of branching, and comparative genomics has allowed information from maize and other models to be applied to other grass systems. We are particularly interested in the foxtail millet model system because of the great variation in branching patterns that results from segregation in the offspring of crosses between domesticated foxtail millet and its wild progenitor, green millet, as well as the imminent prospect of a draft genome sequence for foxtail millet. We present a preliminary analysis of vegetative branching variation in 157 recombinant inbred lines, derived from a cross between foxtail and green millet, which has allowed new insight into the trade-offs between branching types and the development of plant architecture over the growth period of the plant. These RILs, along with the two parental lines, were evaluated in a greenhouse in the summer of 2008 in Stillwater, OK. Traits evaluated were height, number of tillers and number of axillary branches, and these were recorded at three developmental stages: two weeks after planting (all vegetative), at flowering, and at harvest/maturity. We find segregation of tillering (basal branching) and axillary branching (branching along the distal extent of the culms and tillers), and correlations between height and various branching patterns. Building on prior QTL analyses of an F3 population of the same cross, we tested correlation between trait segregation and genotype for various candidate genes, including teosinte branched1, and found significant correlations. However, the correlation between genotype and phenotype appears to vary over the three growth stages measured, suggesting that mechanisms of genetic control of branching phenotypes varies over the life cycle of the plant.

P195

GENETIC CHARACTERIZATION OF A QUANTITATIVE TRAIT LOCUS CONFERRING RESISTANCE TO SOURTHERN LEAF BLIGHT OF MAIZE

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Dissecting quantitative resistance loci (QRL) in plants is the first step into understanding its nature. In our lab, we have developed near isogenic lines (NILs) that derived by repeated backcrossing of an elite source of southern leaf blight (SLB) resistance NC250P to the susceptible line B73. Furthermore, we have generated and evaluated NILs with each of the different subsets of the NC250P introgressions. Introgression 6A located on Chromosome VI, bin 6.01 had a significant effect on juvenile plants during greenhouse experiments and provided the largest effect in adult plants. Also, F2 individuals segregating for this resistance, yielded a 3:1 ratio with resistance being recessive. This introgression is a good candidate for fine-mapping and cloning of the gene responsible for this effect. Our main goal is to identify and characterize this locus. For this purpose, F2:3 families derived from a cross between a susceptible B73 and B73 containing introgression 6A will be used to fine-map this introgression. A combination of bioinformatics and transposon tagging techniques will be used to clone this locus and identify its biochemical function on resistance.

P196

GENOME-WIDE SCREENING FOR CIS AND TRANS REGULATION OF ALLELE-SPECIFIC GENE EXPRESSION IN MAIZE

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Inferred changes in cis- and trans-regulatory sequences have been shown to be associated with evolutionary divergence of gene expression between Drosophila species. Presumably, divergence in phenotypic expression of many quantitative traits accompanied the divergence in gene expression and that the divergence in gene expression was driven in large part by natural selection acting upon variation in regulatory DNA sequence. Based on these findings, we hypothesize that artificial selection on regulatory sequence variants could aid plant breeding efforts to improve crop productivity. Screening for allele-specific gene expression would seem to be a logical first step.

Quantitative genetic theory ascribes variability of general combining ability (GCA) to cumulative single-locus and intra-gametic espistatic variance, while variability of specific combining ability (SCA) is explained by cumulative single-locus and inter-gametic epistatic variance. We will apply a new approach to perform a Genome-wide analysis of Allele-Specific Expression Differences (GASED, Kiekens et al. 2006, Vuylsteke and van Eeuwijk 2008). In the GASED analysis mRNA concentration from an expressed gene is regarded as a phenotypic trait for which GCA variance is attributed to variability in cis-acting regulatory elements, whereas SCA variance is explained by variation in trans-acting or a combination of cis- and trans-acting elements. The GASED approach was formulated based on a diallel mating design. We extended the approach using general genetic effects models presented by Eberhart and Gardner (1966) to estimate dominance as local inter-gametic trans-element interaction effects, additive×additive epistasis as non-local trans-element interaction effects, and the recombination frequency between the locus in question and the locus of the regulatory element. The knowledge gained in this project will be used to develop allele-specific DNA markers located in regulatory regions of the maize genome.

P197

Gene expression analysis of european maize inbred lines for the prediction and characterization of heterosis

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The molecular basis of heterosis or hybrid vigour is still widely unknown. A reliable molecular prediction method would help breeders to save time and costs which are associated with traditional methods depending on extensive field trials to test for hybrid vigour in new hybrids. For a better understanding of the phenomenon heterosis and for its prediction we applied a microarray analysis to gain a genome-wide transcriptional overview of european parental inbred lines (Dent, Flint). We examined the transcriptome of 21 parental line seedlings using the 46k oligo arrays (www.maizearray.org), grown under controlled conditions for 7 days. The resulting gene expression data were correlated to heterosis data gained from extensive field trials over 3 years.

We observed a high correlation of differentially expressed genes and heterosis of “grain yield” and “grain dry matter content”. Thereby, different sets of genes were correlated with heterosis of the two examined traits. A strong correlation of several genes to heterosis is the first evidence towards a good prediction potential of inbred lines expression profiles. Furthermore, GO-based gene set enrichment analyses indicate that different biological pathways contribute to the heterosis of the two analysed traits.

P198

Genetic Analyses and Selection for Carotenoids using Single Kernel Genotyping with Allele Specific Markers

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Carotenoids are natural sources of yellow and orange pigmentation as well as antioxidants in maize grain. The carotenoid biosynthetic pathway is well understood biochemically, and genetic analysis linking color and carotenoid profiles to specific alleles and genes in the pathway is ongoing. Segregation analyses are being performed to understand inheritance patterns of visual qualitative differences in shades of yellow and orange in maize kernels. This is being complemented by single kernel, single plant, and bulked segregant analysis focusing on candidate genes. The carotenoid pathway has become a model system for the study of quantitative trait loci involved with metabolite synthesis, conversion and degradation. To date, several genes encoding enzymes of the biosynthetic pathway have been either directly mapped or are candidates for significant carotenoid QTL and a synthesis of available QTL and candidate genes will be presented. Allele specific markers based on functional polymorphisms in carotenoid biosynthetic genes have been developed and used on DNA extracted in a non-destructive manner from single maize kernels. This allows for color phenotyping, DNA analysis and subsequently, planting of the maize kernel, a significant advantage over leaf genotyping. The ears resulting from plants from genotyped single kernels provide an immediate progeny test and can be phenotyped for carotenoid levels. Using the available allele specific markers, a related applied focus of this research is to enhance plant breeding selection by combining the favorable haplotypes for three biosynthetic genes in order to enhance breeding for improved carotenoid and provitaminA content. This breeding approach takes advantage of the increasing knowledge of the carotenoid biosynthetic pathway and technological advances such as allele specific single kernel genotyping.

P199

Genetic Architecture of Multiple-Stress Responses

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Combinations of stress factors normally occur in the field, and both crop stress and general environmental ecological data suggest that combination stress effects are not easy to predict from single stress experimental analyses. Stress-combination differences may also explain why correlation of specific climate variables with chromosome location (QTL) across environments has been difficult. Many QTL analyses have been completed for drought stress, and we have completed the first QTL analysis for ultraviolet radiation effects. We have now completed the first QTL mapping experiment explicitly comparing multiple-stress QTL to those detected in single-stress environments.

We measured root and shoot biomass, change in plant height, and change in leaf length in the IBM94 recombinant inbred lines under control, drought, UV stress, and combined UV and drought stress. The stress levels chosen for this experiment were mild, to create realistic stress levels and to allow combined stress comparisons. The drought treatment regime resulted in a stress treatment of 70%FW and the ultraviolet radiation regime was similar to average values recorded outdoors.

Stress-specific QTL were localized using a mixed model with P values calculated for each marker x stress interaction. There are many more markers than lines for the IBM94 population; this is useful in that nearly all the recombination junctions are defined. We used a linear modeling method to detect loci and model treatment effects at significant loci. Analysis of plant height and root biomass data localized different QTL for individual and combined stress treatments. Identification of genetic architecture (how many loci and how they interact) is key for future application of combination-stress QTL to breeding.

P200

Genetic Diversity within Sweet Sorghum and Association Mapping of Brix and Height

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Sweet sorghums [Sorghum bicolor (L.) Moench] are a specialty type of sorghum that have been selected to accumulate high levels of edible sugars in the stem. Sweet sorghums also are generally tall and produce high biomass but relatively little grain. Little has been known about the genetic relationships and diversity within sweet sorghums or the relationships of sweet sorghums to the grain sorghum racial types. We screened a diversity panel of 125 sorghums, mostly sweet, with 48 SSRs and 384 SNPs. We identified three main genetic groupings consistent with phenotype and origin: historical and modern syrup types, modern sugar/energy types, and amber types. Using markers shared with an available large grain sorghum panel, we found that these three sweet types clustered with kafir/bicolor, caudatum and bicolor grain types, respectively. Relatedness information was then used to perform whole genome association mapping for height and brix (stem sugar). Three significant associations for height were detected. Only one significant association for brix was detected. Relevancy to maize work will be presented.

P201

Genetic Mapping of QTLs Associated with Planat Regeneration in Maize

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Genetic engineering can be utilized to study the structure and function of plant genes, introduce novel traits, and regulate expression of endogenous genes. Utilization of this tool in the genetic characterization and improvement of maize is hindered, however, by the requirement for tissue culture as part of the genetic engineering process. As with other cereals, only a few genotypes of maize demonstrate high culture and regeneration response. The genotype-dependent tissue culture response of maize greatly impedes progress in functional genomics studies, and adds years to genetics research and crop improvement efforts. The goal of our research is to identify and characterize maize genes having significant effects on tissue culture response to provide a better understanding of the genetic factors underlying embryogenic tissue culture regenerability. This, in turn, should lead to the development of new culture and transformation systems that are genotype-independent. Two inbred maize lines formed the basis of the mapping populations used in this research A188 (high culturability and regenerability; poor agronomic performance), and B73 (poor culturability/regenerability; excellent agronomic performance). A recombinant inbred line (RIL) population comprised of 101 individuals derived from crossing A188 by B73 was screened to identify lines with high plant regenerability. Seven lines displayed significantly higher plant regenerability vs. B73 and were designated A188-Regenerative Callus (ARC) lines. The ARC lines were genotyped with 140 genetic markers to identify inherited A188 genomic regions. Several of the ARC lines inherited an A188 genomic region on chromosome 3 that has previously been identified as being associated with improved maize tissue culture. To fine-map the chromosome 3 A188 region, a mapping population generated from crossing B73 to ARC60 was produced. Results indicate chromosome 3 A188 region contains a genetic factor contributing 12.5% of the variation in plant regenerability. Further fine mapping will be conducted to identify the specific genetic factor(s) associated with improved plant regenerability, and those factors will be transferred to agronomically superior lines.

P202

Genetic mapping of head smut and mosaic virus disease resistance and drought tolerance through high-throughput SNP genotyping of a large number of maize introgression lines

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Introgression lines (ILs), developed by recurrent backcrossing to the same parental genotype, could provide a unique genetic stock for quantitative trait locus (QTL) mapping. Using maize lines from six heterotic groups adapted to different ecological zones, Chinese Academy of Agricultural Sciences (CAAS) have developed more than 500 BC2F2 IL sets by crossing 11 testers widely used in China (as recurrent parents) with more than 200 local maize inbred lines (as donor parents) (Hao et al. 2009, Plant Breeding, in press). These maize IL sets have been screened for various types of biotic stress resistance and abiotic stress tolerance. From 51 IL sets, 235 introgression lines (ILs), selected for their stress tolerance, which included different generations of BC2F3, BC2F4, BC4F1 and BC4F2, were genotyped by the Illumina GoldenGate assay using a SNP chip containing 1536 markers. Out of these 1536 SNPs, 924 were found to be polymorphic among 235 ILs. Base changes involved A/C(168), A/G(583), A/T(69) and C/G(104). For the four different generations, heterozygosity rates ranged from 9.65% in BC2F3 to 4.46% in BC4F2. Marker-trait association for head smut and mosaic virus disease resistance and drought tolerance was determined using a ¦Ö2 test of the difference of allelic frequencies between groups of resistant versus susceptive lines. Loci conferring head smut disease resistance were identified on chromosomes 1, 3, 6, 8, 9 and 10. Loci contributing to mosaic virus disease resistance were found on chromosomes 1, 3, 4, 6 and 9. Loci associated with drought tolerance were located on chromosomes 1, 2, 3, 5, 7 and 9. Five SNPs in bins 1.00, 4.05, 6.04-6.05, 9.01-9.02 and 9.04 showed a high correlation for the three types of stress tolerance. Recently, a relatively small number of stress inducible genes have been identified and mapped in maize. Identification of SNPs from these genes will contribute to the identification of functional variation. The SNP-chip based high throughput genotyping system used in this study provides an efficient means of achieving genome-wide genetic mapping of complex traits that may quickly and cost-effectively provide functional markers for application in molecular breeding programs.

P203

Genetic, Genomic, and Breeding Approaches to Improve Yield and Starch in Maize

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Different approaches were taken to further characterize the genetic basis of starch concentration and grain yield in maize. The Illinois Low Protein cycle 90 (ILP90) strain was crossed to B73 and the F1 backcrossed to B73. The BC1 was selfed and BC1S1 progenies were advanced to BC1S3 and crossed to a tester. The rationale for using the ILP90 strain is that it shows the highest starch concentrations in maize. However, it has poor agronomic performance. Thus evaluating in a backcross to B73 enables the investigation of favorable alleles present in ILP90 in a relatively more elite background. Starch concentration and grain yields were evaluated in the testcross population over 4 environments, both showing a wide range of variation. Gene expression profile analysis was performed on high and low starch families from BC1S1 and testcross populations. Selected families were grown and self pollinated in 2005 and pollinated ears were sampled at 10, 15, and 20 days after pollination. Microarray analysis on these sampled materials revealed several differentially expressed genes. One gene particularly stood out and was selected for validation through RT-PCR. This gene is relevant to the overall starch biosynthetic process and accumulation, and provides a potential new insight on the basis of the relatively higher levels of starch in some lines of the ILP90xB73 backcross derived materials. Also, identification of the genomic location of loci controlling the traits was performed in the testcross population, revealing three QTL for starch and one for grain yield. One of the QTL identified for starch coincides with a QTL region detected previously for per se populations.

P204

Genome Dosage Effects on Heterosis in Triploid Hybrids of Maize

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Heterosis refers to the phenomenon that hybrid progenies perform better than both inbred parents for characters such as plant biomass, development rate and fertility. To test the influence of genome dosage on heterosis, triploid maize hybrids derived from B73 and Mo17 that differ in their genome dosages were analyzed. Heterosis was evaluated by measuring eight plant characters (height at four weeks, adult height, leaf length and width, ear length, tassel branch number, anther emergence time and silk emergence time) in reciprocal triploid and diploid hybrids, Mo17 x B73 (MBB and MB, respectively) and B73 x Mo17 (BMM and BM, respectively), as well as the corresponding inbreds, B73 (BBB and BB) and Mo17 (MMM and MM). For seven of the measured characters, the average values showed superior performance in both the reciprocal triploid and diploid hybrids than the corresponding inbreds, although such heterotic effect was found in only MBB and BM hybrids for the silk emergence time. Correlated with the fact that the B73 inbred is better than the Mo17 inbred for most of the measured traits, the MBB hybrid performed better than the BMM hybrid, while such a relationship does not exist between the two diploid hybrids. Indeed, the MBB/BMM ratio for all the measured characters is positively correlated with the BBB/MMM ratio ($r = 0.74$, $P = 0.014$) and the BB/MM ratio ($r = 0.84$, $P = 0.0022$), but not the MB/BM ratio ($r = -0.56$, $P = 0.092$). In addition, the two reciprocal diploid hybrids did not differ significantly for all the measured characters, while a significant difference was found between the two reciprocal triploid hybrids for the tassel branch number ($P < 0.05$). These results suggest that genome dosage affects heterosis in triploid hybrids.

P205

Identification of QTLs Regulating Heterosis for Seedling Dry Weight and Cob Weight

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There is limited understanding of the mechanisms responsible for heterosis. The inbred lines B73 and Mo17 produce a hybrid that exhibits “high parent heterosis” for many traits, including seedling dry weight and cob weight. These inbred lines are the parents of the Intermated B73 and Mo17 (IBM) population of Recombinant Inbred Lines (RILs). To better understand the mechanisms responsible for heterosis, seedling dry weight and cob weight were measured from multiple plants per replication in multiple replications of 291 IBM RILs and their crosses with B73 and Mo17. This design provides a contrast of homozygous and heterozygous genotypes at all loci that segregate between B73 and Mo17. The experimental design also included the B73 and Mo17 parental lines so that heterosis could be accurately measured for each B73xRIL and Mo17xRIL hybrid. Using our IBM SNP map that contains 1,016 genetic markers, QTL mapping was conducted for “high-parent heterosis” for both seedling dry weight and cob weight. Two and five QTLs were identified that regulate heterosis for seedling dry weight and cob weight, respectively. Analyses of the genes responsible for these QTLs are expected to help elucidate the mechanisms responsible for heterosis.

P206

Identification of chromosome regions controlling shoot-borne root diameter.

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Shoot-borne roots fulfill many roles in maize (Zea mays L.). Acting as conduits between the root hairs in the surrounding soil and the rest of the plant, these roots transfer water and nutrients required for growth and survival. Shoot-borne roots are capable transporting relatively larger amounts of water and nutrient than embryonic roots because of their larger diameters and surface areas. Besides providing maize plants with essential nutrients and water, they also form buttresses that help maintain a plant's upright position. Previous researchers identified root diameter at the first node above ground as significantly influencing vertical root pulling resistance, a measure of lodging tolerance. Given the agronomic relevance of shoot-borne root diameter little research has been done in maize to identify chromosome locations influencing the girth of these organs. Our goal was to identify chromosome regions controlling shoot-borne root diameter and to use these regions to identify candidate genes potentially underlying their quantitative inheritance. Two hundred and fifty lines from the intermated B73xMo17 (IBM) recombinant inbred mapping population were grown in the field in 2003. Four shoot-borne roots were sampled from the first node above the ground from up to five plants per line. Diameters were measured 5mm from their origin of stem emergence as per previous investigations. Initial QTL mapping was perform in Rqtl against 643 molecular markers evenly spaced throughout the maize genome. Following initial QTL identification, fine mapping was performed with a more complete set of molecular markers in the regions of interest. Candidate genes were identified using the molecular maps available in MaizeGDB and Gramene. This research was supported by MU Mission Enhancement and USDA, ARS funds.

P207

Integrative analyses of genetic variation in metabolic pathways associated with nitrogen utilization in maize

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Improving the nitrogen utilization of maize hybrids is key to maximizing crop yields, reducing nitrogen fertilization, and improving the energy balance for maize production. The accumulation of free amino acids, particularly asparagine and glutamine, is a metabolic indicator of plant N status that is closer to gene action compared to agronomic parameters typically measured in field trials. We investigated genotype-phenotype associations for the pathway controlling free asparagine and glutamine in hybrids where the Illinois High Protein1 (IHP1) inbred line was crossed to the IBM recombinant inbred lines, the source for the high density maize genetic map. Robust QTL were detected for levels of amino acid metabolites and the mRNA expression of amino acid assimilatory and catabolic enzymes. Further investigation of the genomic regions defined by these QTL suggest a regulatory network of cis- and trans-acting factors controlling free asparagine accumulation that also links to QTL controlling agronomic measures of N utilization.

P208

Iron concentrations within kernels vary between inbred lines and in response to low nitrogen and drought conditions

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Iron content in 25 NAM parent inbred lines exposed to four different growing conditions was measured with the following objectives: 1) to measure relative iron concentrations within genotypes, 2) to compare relative iron concentrations between genotypes with the hopes of identifying useful alleles, and 3) to compare relative iron concentrations between growing conditions.. Twenty-five inbred lines were grown in each of the four conditions – ample water and nitrogen, low water, low nitrogen, and a combination of low water and low nitrogen. Upon harvesting and drying of the corn, five kernels from each genotype grown in each condition were selected as a representative sample. Each kernel was split in half and one half of the kernel was then exposed to Perl's Prussian Blue (PPB) solution, a mixture that, upon exposure to iron, produces a blue color. Photographs of the kernels were taken 7 days after initial exposure to the dye and analyzed using Adobe Photoshop by calculating the number of blue pixels within the photograph, greater amounts of blue pixels corresponding to higher concentrations of iron.

After an initial run of this experiment, it was observed that there were differences in iron concentrations across the genotypes and across the various stress environments. It was also observed that there were staining differences between the embryo and the endosperm of the kernel. More strikingly, however, it was observed that there were distribution and staining differences within hard and soft components of the endosperm. We are currently measuring iron content in milled samples. The hard and soft endosperm proportions will be correlated with PPB staining.

P209

Joint Analyses of Test-cross Hybrids with Inbred-lines Increase Power of Association Under Unified Mixed Model Framework

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Although maize is one of the few plant species that possesses extensive resources for association mapping, there are only 302 lines in the current maize recombinant inbred line collection in the publicly available association population. Due to the small size of the population, this panel lacks the statistical power necessary for dissection of adaptive traits that consist of large numbers of small effect QTL such as flowering time. The lack of power is not only due to the small size of the population but also due to the reduction in accuracy of the phenotyping, which inflates measurement error because of large variation within population. Provided that a statistical framework to manage changes in scale of phenotypic variances is available, test-crosses of diverse inbred lines (hybrids) can be included in the association study framework. When hybrids and inbreds were jointly analyzed in a unified mixed model framework, our results indicated that the potential increase in power was between 21-43%. Furthermore, we also observed that the potential increase in power was contingent not only on the genetic architecture of the trait analyzed, but also on the population frequency of the alleles contributed by the selected tester. Results from the analyses of several traits investigated in current maize association population will be reported.

P210

Large-scale screening for drought tolerance at vegetative stage as revealed by biomass measured under water-stressed and well-watered environments, compared with other selection criteria

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A total of 550 lines collected from breeding programs across the world were evaluated under both well-watered and water-stressed environments (each with two replications) during the 2008 dry season at Tlaltizapan (State of Morelos, Mexico). Water stress at vegetative and flowering stages was imposed by stopping irrigation for four weeks at 6-leaf stage and 2 weeks prior to anthesis, respectively. Vegetative stage screening was based on multiple measurements of biomass before and after the drought stress was applied using the normalized difference vegetation index (NDVI) measured with a portable spectroradiometer (GreenSeeker). Other drought tolerance-related selection criteria evaluated include anthesis-silking interval (ASI), leaf senescence, chlorophyll content, root capacitance, drought tolerance index (DTI, final grain yield under stressed condition compared with the well-watered), and final grain yield. Under both well-watered and water-stressed conditions, DNVI was significantly positively correlated with chlorophyll content and final grain yield, while negatively correlated with leaf senescence. However, there was no significant correlation of DNVI with ASI or root capacitance. The relative DNVI measurements for the water-stressed environment compared to the well-watered environment did not show a significant relationship with any other selection criteria. In addition, none of the selection criteria were significantly correlated with DTI. The 45 lines with the best level of drought tolerance produced an average of more than 700 kernels per plot. All the tested lines have been genotyped using a 1536 SNP marker chip, allowing association mapping for drought-related traits to be carried out.

P211

Marker assisted breeding for desirable thinner pericarp thickness and favorable ear traits in fresh market waxy corn germplasm

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The lack of genetic information on waxy corn for fresh consumption in U.S. germplasm, and the increasing Asian-American population prompts the need for breeding and genetic research on fresh waxy corn for human consumption. Thin pericarp is one of the major characteristics for increasing tenderness of fresh waxy corn. Large ear and kernel size are also desirable traits for consumer appeal. This research is using marker assisted selection to pyramid the favorable alleles for thinner pericarp without losing favorable ear traits. We are investigating the inheritance and relationships of pericarp thickness and ear traits. QTL analyses were conducted on five pericarp thickness and ten ear traits in 264(BH20XBH30)F3 families from 2004 to 2006 to identify favorable alleles controlling the traits. Nine QTL, which explained the highest phenotypic variation for thin pericarp thickness, were found within bins 1.10, 2.06, 3.00, 4.01, 4.07, 6.00, 6.05, 8.05, and 9.04. Fifteen flanking markers were selected and screened through the mapping population to select the best families with the most favorable QTL alleles for thin pericarp thickness. To maintain the favorable phenotypic traits and to pyramid favorable alleles for pericarp thickness, 4 families which had most of the favorable QTL alleles for thin pericarp were cross pollinated with 2 other families having early flowering, relatively thin pericarp, and larger ear and kernel sizes. From these crosses, 485 F3 families were created, consisting of eight subpopulations. They are being assessed based on genotypic data, and phenotypic data on flowering dates, pericarp thickness, and ear traits. The subpopulations will be evaluated individually and through joint analysis. This will assess QTL regions to validate effects, to find possible QTL interactions, and to estimate the overall genetic and phenotypic improvements in these materials.

P212

Modification of Maize Carotenoid Composition through Degradation by ZmCCD1

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Enhanced production of carotenoid compounds through modification of plant secondary metabolism has the potential to increase the nutritive value of cereal grains by augmenting provitamin A (ProVA) and antioxidant levels. Successful manipulation of the pathway in maize grain has been achieved by coupling the biochemical model for carotenoid biosynthesis in homologous species with an understanding of the genetic variation that regulates this pathway in maize. Carotenoid production is quantitatively inherited, and is dependant upon the interplay of synthesis, conversion and degradation; therefore our ability to accurately manipulate the pathway prompts investigation of multiple enzymatic targets.

The maize carotenoid cleavage dioxygenase 1 gene product (ZmCCD1) is homologous to an Arabidopsis gene family shown to reduce total carotenoid levels through catabolism of specific carotenoid compounds. A survey of natural genetic variation at this locus in maize reveals a polymorphism that significantly affects ZmCCD1 function and is associated with a 35% decrease in lutein. The identified allelic series explains much of the variation observed in several QTL analyses for grain carotenoid traits, including the F2:3 population of A619 x SC55 in which a CCD1-specific marker mapped to the location of a major QTL for xanthophyll concentration. Biochemical profiles of developing maize kernels for genotypes representative of the CCD1 allelic series suggest that the onset of ZmCCD1-specific degradation causes a marked change in lutein concentration over time, and to a lesser extent in carotenes and zeaxanthin. The observed degradation profile appears to be temporally separated from peak synthesis; as such, correlation of this activity with biological parameters of the developing seed including water status and seed/sink size is being explored. In an attempt to forecast genetic pairings, we are re-evaluating how carotenogenesis is affected by combinations of allelic variation for ZmCCD1 with previously characterized pathway genes lycopene epsilon cyclase (LCYE) and beta carotene hydroxylase (CrtR-B1).

P213

Mutant-assisted exploration of natural variation underlying R gene-mediated immunity in maize

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This project seeks to refine the genetic architecture of the HR (hypersensitive response), one of plant kingdom's most important immune responses. However, instead of using artificially induced variation to do so, we propose to make use of the variation that is present naturally in the maize germplasm. Our rationale for this derives from the fact that, despite the use of exhaustive mutagenesis screens in many plant species, our understanding of how HR is triggered and executed remains incomplete. One way forward is to exploit natural variation, which has been generated and selected over millions of years of evolution. However, a major challenge to this approach is how to sift through the enormous diversity available. To this end, we have devised a simple yet effective method to discover and characterize useful alleles. This method, a variation on enhancer/suppressor screening that we have called MAGIC (for Mutant-Assisted Gene Identification and Characterization), makes use of the phenotype of a mutant (for a gene affecting the trait of interest) as a reporter to discover and analyze relevant, interacting genes present naturally in diverse germplasm. Using a constitutively-active (semi-dominant) allele of the of the Rp1 disease resistance gene in a MAGIC screen of the diversity panel, an amazing amount of variation capable of enhancing, suppressing, or altering the HR response in other ways was revealed. Since B73 had a moderately suppressing effect on this HR and Mo17 enhancing, it prompted us to conduct MAGIC on the IBM population. This led to the identification of a major QTL, which we have named Hrml1 (HR modulating locus-1). Our next goal is to explore the entire NAM resource with MAGIC not only to uncover additional Hrml loci but also to clone the genes that underlie these Hrml loci, including Hrml1.

P214

Nested Association Mapping of Gray Leaf Spot Resistance in Maize

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Gray leaf spot (GLS) is a foliar disease caused by Cercospora zeae-maydis and Cercospora zeina that flourishes in warm humid weather. GLS is a serious problem in several regions of the world, including North America and Africa. In the United States it is currently considered to be the most common and destructive maize disease. Expansion and increased severity of this disease corresponds to the increased use of conservation tillage practices, which leave spore containing residue on the soil surface. Genetic based quantitative resistance to GLS is important for crop production but little is known about the genetics of resistance. We are utilizing the nested association mapping (NAM) maize population, consisting of 26 populations of recombinant inbred lines (RIL), to identify quantitative trait loci for GLS resistance. Trials for GLS resistance in NAM were conducted in Blacksburg, VA in a location with high incidence of GLS provided by natural inoculum. Lines were evaluated for disease severity at 4 time-points and a multivariate mixed model was used to give best linear unbiased predictions (BLUPs) of the disease severity ratings. To model effects of quantitative trait loci (QTL), a general linear model was selected for genetic markers with a selection threshold at LOD > 5. Preliminary analysis has identified 11 QTL distributed across the maize genome.

P215

Nonmetric Multidimensional Scaling Corrects for Population Structure in Association Mapping with Different Sample Types

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Recent research has developed various promising methods to control for population structure in genome-wide association mapping of complex traits, but systematic examination of how well these methods perform under different genetic scenarios is still lacking. Appropriate methods for controlling genetic relationships among individuals need to balance the concern of false positives and statistical power, which can vary for different association sample types. We used a series of simulated samples and empirical datasets from cross- and self-pollinated species to demonstrate the performance of several contemporary methods in correcting for different types of genetic relationships encountered in association analysis. We proposed a two-stage dimension determination approach for both principal component analysis and nonmetric multidimensional scaling (nMDS) to capture the major structure pattern in association mapping samples. Our results showed that by exploiting both genotypic and phenotypic information, this two-stage dimension determination approach balances the trade-off between data fit and model complexity, resulting in an effective reduction in false positive rate with minimum loss in statistical power. Further, the nMDS technique of correcting for genetic relationship proved to be a powerful complement to other existing methods. Our findings highlight the significance of appropriate application of different statistical methods for dealing with complex genetic relationships in various genome-wide association studies.

P216

QTL analysis of nitrogen and carbon metabolites using the maize nested association mapping panel

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The regulation of plant growth and development is largely mediated by nitrogen and carbon metabolism. However, little is known about the genetic architecture of those metabolic pathways and networks. The maize nested association mapping (NAM) design, which integrates both linkage and association approaches, provides high resolution and statistical power to dissect complex quantitative traits. The NAM population is comprised of 5000 recombinant inbred lines (RIL) derived from crosses between B73 and 25 highly diverse maize lines (200 RILs for each cross). In this study, we applied a robotized platform to measure 12 nitrogen and carbon metabolites on the NAM panel, IBM, and the association panel: malate, fumurate, glutamate, chlorophyll A, chlorophyll B, nitrate, sucrose, glucose, fructose, starch, total amino acids, and protein. In total, about 12,000 samples were obtained for this study. Most of the metabolites showed high heritabilities. By sampling end and middle plants, significant differences in metabolite content were observed. End plants had higher content for 10 metabolites than middle ones, while middle plants contained more starch and there was no significant difference for glucose between middle and end plant. Significant correlations were detected between different metabolites especially for those metabolites located in the same pathway, such as fumurate and malate in the TCA cycle, or different but related pathways, such as fumurate in the TCA cycle and glutamate in the nitrogen pathway. By adding all QTL across the NAM panel, 21-40 QTL were identified for each metabolite. We found a large number of metabolite QTL with moderate phenotypic effects. Most of the QTL appear to be shared in 2 to 14 of the 25 populations of NAM, with alleles having different functional effects at each QTL distributed across founder lines. Many QTL from different metabolites co-localize on the chromosomes especially for those correlated metabolites, suggesting that a set of core QTL control metabolites from same pathways or from different but related pathways.

P217

NAM QTL for Armyworm and Banvel Herbicide Susceptibility

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Although maize accounted for approximately 86 million acres in the U.S. in 2008, limited options are available concerning insect pest and weed control in these production systems. Utilizing superior germplasm can provide the means to eliminate the need for selective control methods concerning insect pests while reducing the possibility of crop tolerance issues in respect to herbicide applications. The maize Nested Association Mapping (NAM) population was grown in Ponce, PR in the winter of 2006 while B73 Nearly Isogenic Lines (NILs) with flowering time introgressions were grown in PR in 2009. Fall armyworm (Spodoptera frugiperda) damage was scored at these PR field sites under natural infestation pressure. NAM was also grown in 2009 in Aurora, NY with herbicide susceptibility being scored following damage to Recombinant Inbred Lines (RILs) from two populations by an application of an auxin-type growth regulator in the Benzoic acid family (Banvel). It is our intention to detect QTL that confer resistance to the armyworm and also susceptibility to the benzoic acid-based herbicide chemistries. Further use of this knowledge could provide a more efficient means of introducing resistant traits into maize lines through marker-assisted selection.

P218

QTL mapping for amylose content in maize (Zea mays L.) using SSR markers

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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 extender1 (ae1) gene, raises the amylose starch 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 location of modifier genes via quantitative trait loci (QTL) analysis to investigate gene interactions among genes and QTLs leading to the development of the high amylose inbreds. The F2 population derived from a cross of (H99ae X GEMS-0067) was planted in South Dakota in 2005. Amylose content was measured by amyose-iodine colorimetry while the genotypes of individual F2 ears were scored using SSR-PCR by agarose gel electrophoresis. The results showed the major QTL was detected on the short arm of chromosome 5 indicating that the different alleles of Sbe1 played the main role in the increase of amylose content with the background of ae1 mutation. The QTL was present on the short arm of chromosome 5 between umc1784 and umc2400, both 8.7cM apart. It explained 43% of the total variance. The SSR marker, umc2400, was 2.7cM apart from Sbe1 and could be the indicator for the modifer gene. Another QTL was found on the long arm of chromosome 6 and the contribution to amylose content was minor, only 6% of the total variance explained.

P219

Sample size for QTL effect detection – a simulation study

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In maize breeding, we are increasingly faced with the challenge of testing for the effects of putative QTL for applications in marker assisted breeding programs. Many factors can affect the ability to detect putative QTL in segregating experimental crosses. The genetic background of the individuals in a population can mask the effect of the putative QTL depending on the number of individuals that are genotyped (sample size) and the magnitude of the QTL effect. The effects of these variables upon the ability to detect a putative QTL were investigated in a simulation study. For this study, 1,000 F2 individuals were drawn from a hypothetical population with 10,000 loci with small additive effects (genetic background) accounting for 50% of the observed total phenotypic variance, a major bialellic QTL (alleles A and a) accounting for either 1.5, 2.5, or 4.0% of the observed total phenotypic variance, and an error term. The simulated F2 population was selfed and 5, 10, or 15 individuals homozygous for either the A or the a QTL allele were test-crossed with a common tester (4 crosses per individual) and the mean difference between the two F3 groups (AAF3:F2 x tester, aaF3:F2 x tester) was tested using the z statistics. The results show that QTL effects accounting for at least 4.0% of the observed total phenotypic variance were detectable given a minimum sample size of 10 individuals.

P220

Spatial Corrections of single replication field trials using autoregressive and two-dimensional spline models.

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A simulation study was conducted to evaluate the performance of several models for spatial corrections of single replication field trials arranged in randomized complete blocks (RCB). The models evaluated included two-dimensional, separable autoregressive models of the first (AR1), second (AR2), and third order (AR3), as well as, two-dimensional spline interpolation (2DS) and RCB. Spatial effects were simulated from a multivariate normal distribution (MVN) using several different correlation structures for pass and range. The order of the spatial structures ranged from first to fourth, with correlations between pass and range varying between .4 and .85. Genotype effects were simulated as MVN using a relationship matrix based on actual maize genotypes. Residual error terms were sampled as iid normal. For each model, the correlations between true and estimated spatial effects (Rs) were calculated, as well as, the correlations between estimated spatial effects and the true genotype effects (Rg). When using all available data, Rs ranged from .73-.84 for AR1, .73-.83 for AR2, .74-.84 for AR3, .45-.68 for 2DS, and .3-.54 for RCB models. Although the AR models consistently provided the best estimates of spatial effects, the Rg values were greater than those of the 2DS model (.16-.31, versus .04-.06). Implementing AR models using only check records (ARC) reduced Rg but at the cost of a substantial decrease in Rs (.35-.6). These results indicate that, when using AR models on single replication field trials, input data should be restricted to check records. Overall, the 2DS model performed consistently across all data sets, making it a good choice for spatial corrections in single replication field trials.

P221

The Genetic Analysis of Quantitative and Multiple Disease Resistance in Maize

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We are interested in the genetic and physiological basis of quantitative disease resistance in plants, with emphasis on the necrotrophic foliar fungal diseases of maize southern leaf blight (SLB), northern leaf blight (NLB) and gray leaf spot (GLS).

Since many necrotrophic fungal pathogens share aspects of their pathogenesis strategies, we hypothesized that some QTL might confer “multiple disease resistance” (MDR). Syntheses of previous disease resistance QTL studies in both rice and maize indicated that disease resistance QTL were non-randomly distributed and often occurred in “QTL hotspots” where QTL for resistance to several different diseases co-localized. We observed highly significant genetic correlations for resistance to SLB, GLS and NLB in a diverse set of 300 maize lines. We also observed that specific allelic variants of a member of the glutathione S-transferase (GST) gene family were associated with resistance to multiple diseases. In our QTL mapping of SLB, GLS and NLB resistances, we frequently observed significant correlations between resistances to different diseases in the same population. Most large-effect QTL were disease-specific however, while smaller-effect QTL frequently conferred MDR.

We have also mapped resistance to SLB, GLS and NLB in the 5000-line maize Nested Association Mapping (NAM) population and have identified loci affecting resistance to multiple diseases. Surprisingly, at these loci, the allelic effects on different diseases are not always correlated. In other words some alleles conferring increased resistance to NLB (relative to the reference B73 allele), confer increased susceptibility to SLB and vice/versa. We will discuss the possible reasons for this.

The production and use of specialized segregating populations and near-isogenic lines to identify, validate, characterize and fine-map quantitative trait loci (QTL) for resistance to these diseases will be discussed. In particular we will discuss the fine-mapping of a QTL for resistance to SLB on chromosome 3 to sub-centiMorgan resolution

P222

The Gramene Genetic Diversity module: A resource for comparative genome analysis in plants

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Characterization of genetic diversity within and between species is a central activity in biology today. With the introduction of an increasing number of high-throughput sequencing and array-based genotyping technologies, identifying variation at the molecular level is becoming routine, while the ability to associate this type of data to variation at the phenotypic level continues to present significant challenges. The bottleneck encountered by studies aiming to elucidate genotypic-phenotypic associations is most acute in the areas of data post-processing, data storage, and in the computationally-intensive analyses of results. The Genetic Diversity module of the Gramene database (gramene.org/db/diversity/diversity_view) is specifically designed to handle these data and to facilitate data integration and analysis. It uses the Genomic Diversity and Phenotype Data Model (GDPDM; maizegenetics.net/gdpdm) to store RFLP, SSR and SNP allele data, information about QTL, passport data for rice, maize, wheat, and Arabidopsis germplasm, and quantitative phenotypic data for some of these accessions. The module has direct connectivity with analysis packages such as TASSEL (maizegenetics.net/tassel), as well as other tools and potential external data sources through the Genomic Diversity and Phenotype Connection (GDPC; maizegenetics.net/gdpc). Of increasing importance for the Diversity module is the effort to develop associations between rice, maize, and Arabidopsis, which will allow users to integrate information from each of these model genomes, building on the advantages and compensating for the disadvantages of each system and dataset. In this way, Gramene's Genetic Diversity module aims to increase the efficacy of comparative genome analysis in the plant kingdom and to provide plant researchers with a platform for managing and interpreting diversity data.

P223

The genetic architecture of maize leaf

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In nature, different species exhibit remarkable diversity, which is the result of adaptation to local environment for optimization of photosynthesis. Leaf shape, leaf size and leaf angle are important determinants of plant architecture, which significantly affect the photosynthetic potential. However, little is known about the genetic basis of natural variation of leaf traits. The Nested Association Mapping (NAM) panel, a novel community resource comprised of 5000 RILs derived from crossing 25 diverse inbred lines to the common inbred line B73, has been implemented in maize to dissect complex quantitative traits. NAM simultaneously integrates the advantages of linkage analysis and association mapping to provide high resolution and statistical power. We conducted a joint linkage analysis using the NAM panel for three traits: leaf length, leaf width and upper leaf angle. The phenotypes for these three leaf traits were collected in 5000 NAM lines in 12 environments across two years. Best linear unbiased predictions (BLUPs) of leaf traits for each line were obtained and used to identify QTL. A total of 39, 37 and 24 QTLs were detected for leaf length, leaf width and up leaf angle, explaining 73%, 76% and 67% phenotypic variation, respectively. All QTL identified are shared among populations. Most of them also showed allele series pattern, with both positive and negative effects relative to B73. We also investigated the genetic sharing among these traits. No significant genetic overlap was detected among them, suggesting independent set of QTL controlling these three different leaf traits. This result is consistent with the developmental study of leaf in Arabidopsis. We also found that many known leaf development genes are under the QTL peaks. Candidate gene association analysis could be applied to test these QTL effects.

P224

To use percent checks or not: the impacts of trait transformation on genetic parameters and the prediction of future performance.

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The use of percent checks (%C) is an intuitively appealing measure of performance because it gives a standardized (adjusted based on the magnitude of the trait) idea of the performance of hybrids relative to proven commercial lines; however, %C represents a transformation of the trait of interest, and as such, can introduce “noise” (unwanted variation) into the selection process. Using derivations of the expected heritability of %C and the untransformed trait (UT) values, it is shown that the heritability of UT will always be greater than or equal to that of %C. To determine the extent to which heritability differs between %C and UT, estimates of heritability were obtained for five datasets. Results showed that estimates of heritability were substantially higher for UT when compared to %C, resulting in more reliable estimates of genetic merit for UT. To determine the impact the use of UT might have on selection decisions, when compared to %C, rank correlations were calculated. In general, these correlations showed good agreement between methods, but examination of the top performing lines indicated the use of UT versus %C can impact decisions on line advancement.

P225

TED, an Unusual *Mutator* Like Element in Maize

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The new mutable allele *bz-m175* arose in a High-Loss x High-Knob stock, which carries transposons from different superfamilies. The transposon in *bz-m175* is an autonomous member of the *Mutator* superfamily, which we have named *TED* (Transposon Ellen Dempsey). *TED* is 3959 bp long, ends in 191-bp terminal inverted repeats (TIRs) and causes a 9-bp target site duplication. *TED* is predicted to encode a 704-amino-acid protein, TEDA, that is highly homologous to MURA, the *MuDR* transposase. However, unlike *MuDR*, *TED* does not encode a second function (B), which has been postulated to play a role in *MuDR* reinsertion after excision.

To assess if the absence of a B function affected *TED* reinsertion, we have isolated and characterized Bz’ germinal revertants from *bz-m175*. Revertants were identified as fully purple kernels in *bz-m175* ears. Among 15 concordant kernels (Bz endosperm and embryo), 6 represented transposition events. Most Bz selections were nonconcordant (Bz endosperm and bz-m embryo), and arose from megagametophytic reversions. The frequency of such events is high: > 1 per 1000 kernels. If *TED* reinserts after excision and reversion occurred in the megagametophytic division that produces the egg and a polar nucleus, one could recover a *trTED* element in the bz-m embryo of nonconcordant kernels. Indeed, we have found that two of eight nonconcordant Bz’ have a *trTED* element. Furthermore, out of 150 bz-s derivatives from *bz-m175*, 11 had a *trTED* element and 29 had a defective *TED* (*dTED*) element at *bz*. All the *trTED* elements can drive the transposition of *dTEDs* at *bz*, suggesting that none of the new *trTED* insertion sites are silenced, and that a B function may not be required for *TED* transposition. We are currently isolating the insertion sites of *trTED* elements, and molecularly characterizing the *dTED* derivatives from *bz-m175*.

P226

A Sequence-Indexed Collection of Ds Transposable Elements in Maize: Platforms for Regional Mutagenesis

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We have generated a collection of over 2,000 Dissociation (Ds) -containing pedigrees, each carrying a Ds element(s) for use in forward and reverse genetic screens. This NSF-funded project allowed us to create this sequence-indexed germplasm, which is currently the largest collection of its kind in maize, and is viewable at http://www.plantgdb.org/prj/AcDsTagging/. The number of annotated pedigrees continues to increase daily, as we further refine the efficiency of our high throughput molecular and bioinformatics pipeline. To create the collection, two Ds elements were chosen as donors that allowed us to easily score for unlinked Ac immobilized (Ac-im) –mediated Ds transposition events. Progeny were then screened molecularly for reinsertion events. Thus far, analysis of these newly transposed Ds flanking sequences indicates a nearly uniform distribution of Ds in the genome, with reinsertion events located on every chromosome arm with few large gaps. Importantly, most pedigrees are available as either stable (+Ac) or unstable (-Ac) insertion lines to enhance gene characterization studies. Past studies have repeatedly demonstrated the propensity of both Ac and Ds to transpose to nearby, genetically linked sites. To demonstrate the efficacy of our system, several pilot local mutagenesis studies were conducted utilizing Ds elements that were tightly linked to the target loci. Putative Ds reinsertion events were characterized using a PCR-based assay and insertion sites determined. These studies suggest that a two-component Ac/Ds tagging system will greatly accelerate functional genomic studies in maize.

P227

A set of maize lines with marked *Ds** transposons for localized mutagenesis

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A sequence-indexed reverse genetics resource is considered essential to fully exploit the maize genome sequence. To fulfill that need we are creating a set of 120 roughly equidistant transgenic *Ds** launching platforms consisting of a *c1* mutable reporter carrying marked *Ds** elements. The set will allow simple visual selection of element transposition from any region of the genome. Most genes in the set will be within 7 cM of a launching platform and are, therefore, realistic targets in localized transposon mutagenesis.

We are using *Agrobacterium* and have engineered two binary vector constructs based on *c-1-m2(Ds)* . *Ds** is marked by *Lc* in one and by *GFP* in the other, the former being more robust. Germinal transpositions of *Ds*(Lc)* can be identified in single-copy transgenotes as fully purple kernels that produce red plants. We have generated 46 transgenic events in Hi-II (*c1*), of which 42 showed kernel spotting when crossed to *c1 wx-m7(Ac)* . The c-m spotted phenotype segregates 1:1, suggesting transgene integration at a single locus. Southern blot analysis revealed 1 to 2 T-DNA copies per line, as in other maize transformation experiments with *Agrobacterium*. Most (38/42) c-m transformants gave confirmed *C'* purple revertants at an average frequency of 1%, sufficient to serve as sources of transposed *Ds** elements. About half of the *C'* revertants carried new *trDs** bands, a result consistent with the one-half fraction seen in other screens based on *Ac/Ds* excision. The germinally active single-copy T-DNA platforms are being mapped by isolating the adjacent DNA and comparing its sequence with the maize genome sequence. Of 14 sequences isolated so far, 11 have been mapped and 3 matched repetitive DNA. We are in the process of generating the remaining *Ds** transgenic lines that will make this a valuable resource for localized transposon mutagenesis across the genome.

1 Supported by NSF-PGRP grant DBI-03-21494.

P228

Ac/Ds elements induce genome rearrangement in transgenic maize and rice

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We have found that alternative transposition can induce deletions, inversions, duplications or translocations. Here, we want to test whether alternative transposition can be used to develop a genome rearrangement tool.

In maize, 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. To enable genome-wide distribution, we inserted the c1::Ds segment within a second transposon (I/dSpm), which itself was inserted into a maize p1 gene for kernel pericarp color. The transgenic stock was crossed by En/Spm transposase lines, and transposition of the engineered I/dSpm element resulted in red pericarp sectors. Kernels with red pericarp were selected, grown into plants, and screened by PCR and inverse-PCR to identify those in which the engineered I/dSpm element had transposed. To date we have obtained approximately 50 independent lines in which the I/dSpm element has inserted into different maize chromosomes. The lines containing transposed I/dSpm elements were crossed with a stable Ac transposase source (p1-vv5145), and candidate genome rearrangements were detected by loss of c1 function (colorless kernels). From one case with reversed Ac ends (R28B1), we obtained a variety of chromosomal rearrangements, including deletions, inversions and translocations.

To assess ability of Ac/Ds to induce genome rearrangement in other plants, we transformed a reverse-orientated Ac/Ds construct together with an Ac transposase gene into rice (Oryza sativa). A green fluorescence protein (GFP) marker between the reversed Ds termini was used for screening. From seedlings with a loss of GFP expression, we obtained some germinal deletion and inversion lines. These results indicate that alternative transposition-induced rearrangements can facilitate plant genome analysis.

P229

Evolutionary Origin of Retrotransposons Inserted in Rice Promoters and Genes and the Effect of Retrotransposon Insertions in Promoters on Gene Regulation

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Retrotransposons are abundant in higher plant genomes. Their contribution to the host genome evolution, whether deleterious or advantageous, has been debated for long time. At the same time, little is known about their evolutionary conservation at the level of species and subspecies. To better understand these two questions, evolutionary origin of eleven long terminal repeat (LTR) retrotransposons, long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) was investigated in model monocot rice. Seven of these insertions were in genes and four were in promoters. Eight out of eleven retrotransposon insertions appear to predate the ancestral Oryza AA genome. The effect of retrotransposons inserted in promoters of four rice genes is being investigated by evaluating promoter-reporter gene constructs in transgenic Arabidopsis plants. Eight promoter-reporter gene constructs, four with retrotransposon insertions and four without retrotransposons, were transformed into Arabidopsis to drive expression of reporter gene egfp. The eGFP expression in different tissues and developmental stages is being evaluated qualitatively with a fluorescent microscope and quantitatively using real time RT-PCR. Correlation between the conservation / polymorphism of retrotransposon insertions in promoters and their impact on gene expression will be evaluated. This study will provide insights into the role of retrotransposons in gene regulation in rice.

P230

Helitrons: Their Impact on Maize Genome Evolution and Diversity

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Gene movement by the newly-described Helitron family of transposable elements apparently has significantly impacted the evolution of the maize genome and has contributed to the lack of gene collinearity between different maize inbred lines. The abundance of these elements and the extent of diversity among them remain largely undetermined. Several hypotheses have been proposed to explain their transposition and the mechanism by which these elements prolifically capture and mobilize gene sequences, but each lacks supporting experimental evidence. To gain insight, we have used the short conserved terminal ends of the known maize Helitrons to search and discover other family members. We annotated these Helitrons based on the sequence and structure of the captured genes and performed PCR analysis to monitor their presence in different maize inbred lines using Helitron specific primers. Our data provide strong evidence that Helitrons are highly abundant, still active and have played significant role in reshaping maize genome through evolution.

P231

Mu Mapping in Maize and Teosinte Inbreds

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Conservation and/or proliferation of transposable elements during the evolution and domestication of maize tells us much about those processes and about differences between maize inbred lines. Mu transposons are found throughout maize germplasms and have been an invaluable genetic tool for identifying gene function via forward and reverse genetics. Maize inbreds theoretically lack Mu activator elements (MuDR transposase), thus their complement of Mu elements are expected to be stable and distinct. Through bioinformatic and PCR analyses we found that three inbreds (B73, W22, and Mo17), together with five teosinte inbreds (courtesy of J. Doebley), not only contain stable inserts from classic members of the Mu family (Mu's 1-9), but also the group of more diverse Mu's (Mu's 10 and 12). Phylogenic and sequence analysis showed that the Mu 10 element is very similar to Mu 9 (MuDR), and that both contain MurA-and MurB-like domains. Also of note is that Mu 12 inserts in B73 are approximately as abundant as all the Mu 1-9 elements together. Data from Mu-anchored 454 sequencing thus far indicates distinct profiles of ancestral Mu-inserts and different evolutionary histories for diverse maize and teosinte inbreds. Further analyses of these insert sites may also shed light on the specific physiological and genetic characteristics of the maize inbreds and the mutations they carry.

P232

New mutants available through MaizeGDB: Stable, sequence-validated Mu inserts in specific UniformMu lines

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New mutants listed at MaizeGDB include the first 2,727 stable, sequence-indexed Mu-insertions from the UniformMu Reverse Genetics Project at UF. Each insert is carried by a specific, defined line. The lines, in turn, are identified by their Mu-insert sites, thus allowing a given mutant to be selected by BLAST search (at MaizeGDB) and can be requested by a direct link to the Maize Genetics Cooperation Stock Center. Mu-inserts in each of the lines have been sequence-validated by interception of at least two axes in a DNA grid of 24 x 24 pooled samples. For a subset of the deposited lines, including 690 insertions, germinal inheritance was verified by 454-sequencing of Mu insertions in their F2 progenitors. Still further validation of mutants included PCR analysis (with products sequenced) for 85 selected lines. Of this 85-mutant sub-sample, 100% were found to carry the sequence-indexed Mu-insert. Each line is represented by seed from one to two F3 sibling ears and carries an average of 4.7 unique inserts. Insert numbers are greater in those lines that segregate for visible-kernel or seedling mutants.

P233

Nonrandom Insertion Patterns of Mutator Transposons in Maize Genes and Chromosomes as Revealed by 454 Pyrosequencing

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Due to its high rate of germinal transposition, the Mutator (Mu) transposon has become an important tool for forward and reverse genetics, as well as for gene cloning. A novel method, DLA (Digestion-Ligation-Amplification) was developed to amplify sequences flanking Mu transposons. Using DLA, Mu flanking sequences (MFSs) were amplified from Mu stocks and sequenced using 454 technology. 94% of ~965,000 reads carried Mu Terminal Inverted Repeat (TIR) sequences, validating the specificity of DLA. Among these TIRs, 21 novel Mu TIRs were discovered. Additionally, based on 43,571 unique Mu insertions, non-random patterns of Mu insertions were observed within genes and across chromosomes. Specifically, Mu elements prefer to insert in the first exons (especially the 5' UTRs) of non-repetitive genes. In addition, most chromosomes tend to have more insertions at their termini and fewer in their peri-centrometric regions. Non-repetitive genes have similar chromosomal distributions. However, even when controlling for gene density, Mu insertions still exhibit a pronounced preference for chromosome termini, indicating that gene density does not fully explain the chromosomal distribution of Mu insertions. We also note that the distributions of Mu insertions and meiotic recombination sites within genes and along chromosomes are similar, suggesting that common features may be involved in the selection of meiotic recombination and Mu insertion.

P234

Sequence Acquisition by Mutator Elements in Maize

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The capacity of transposable elements to induce various chromosomal rearrangements such as deletions, inversions, translocations and duplications suggests their role in genome evolution. Mutator (Mu) elements belong to DNA transposons and are the most active and mutagenic family of transposons in plants. Non-autonomous Mu and Mu-like elements (MULEs) have been shown to capture gene fragments, which may result in the creation of novel genes. Mutator and MULEs that acquire gene fragments have been referred to as Pack-MULEs. To study the mechanism of sequence acquisition by Pack-MULEs, a genome-wide approach was developed to screen for possible new acquisitions by Mutator elements. Two non-autonomous Mu subfamilies in maize, Mu1 and Mu8, were used for identification of sequence variants. Elements with additional genomic fragments or rearrangements were identified using a PCR-based approach that is able to detect variants of individual copies in the genome. With this approach, we were able to detect sequence variants involving rearrangements and deletions. A considerable number of amplifications were non-specific and further effort has been conducted to enhance the specificity of PCR amplification.

P235

Tame transposons and wild genes: Blurring the lines between hosts and parasites

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Genomes are often seen as being divided between DNA sequences that provide selective benefit to the organism and those that exist and proliferate simply because they can. The later category of sequences is larger composed of transposable elements, whose primary selective advantage arises from their ability to out-replicate the rest of the genome. The sometimes vast differences in overall DNA content between relatively closely related organisms suggests that this is, in large measure, an accurate view of genome organization. However, here we provide evidence for mobility of a number of classes of genes in the Arabidopsis lineage over evolutionary time, suggesting that some gene families, like transposons, may have the propensity to move. In contrast, we present analysis of various clades of transposases that have become domesticated and that do not show evidence of mobility. We suggest that the existence of a blurry line between transposons and hosts has been an important creative force in the evolution of genomes, because many of the intermediate steps in the evolution of new functions need not be selectively beneficial at the level of the host.

P236

Transposon Regulation of A1 Gene Expression

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The maize A1 gene is regulated by the flavonoid pathway regulators P1 and C1/PL1+R/B. Activation of A1 by C1/R is associated with histone modifications in a proximal promoter region to which the C1/R complex binds. Transposon insertions in the proximal region of the A1 promoter differentially affect A1 gene expression, in a transposase-dependent or –suppressible manner, suggesting an interplay between the insertions, the autonomous elements and the host regulatory machinery. In the Mu1 insertion allele a1-mum2, the A1 gene is expressed (at a low level compared with the wild type) in the absence of MuDR transposase, but suppressed when MuDR is provided in trans and concomitantly with the Mu1 TIR demethylation. Transient assays in maize BMS cells indicate that the Mu1 TIR, fused to the A1 promoter, does not abolish the activation of a luciferase reporter by C1+R, functioning instead as a weak enhancer. Since the control of A1 gene expression by either the flavonoid pathway regulators or transposons is suggested to be closely related to chromatin structure, which can not be investigated using transient assay, the current experimental focus is on ChIP and nuclease chromatin sensitivity assays. Results from these experiments will be presented.

P237

Uncovering Helitrons in the maize genome

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Helitrons are a class of mobile DNAs, recently discovered, in a broad range of eukaryotes, that are predicted to transpose by a rolling-circle mechanism. They are present in most but not all 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. Gene fragment acquisition is usually in the same orientation as Helitron transcription, such that these acquired fragments are often fused into chimeric transcripts after intron processing. Hence, it appears that Helitrons are particularly active in the use of exon shuffling to create chimeras that could evolve into novel genes. We developed and employed a structure-based approach to identify novel Helitrons in the maize genome. Over 2000 intact elements (8 families [5 of these families are new]) and a great number of truncated elements were identified. Sixty percent of maize Helitrons were found to have acquired gene fragments, with as many as 10 different genes represented in one particularly acquisitive Helitron. Analysis of these Helitrons has uncovered modes of element evolution, including sequence acquisition, and has provided insights into their transpositional history and secondary effects on genome structure.

P238

A New Outreach Program Trains Undergraduate Students to Mentor and Teach Plant Biology to High School Students

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The Carnegie Institution of Science, Department of Plant Biology has partnered with Stanford Science in Service to create a unique outreach program designed to train undergraduate interns in plant biology and science curriculum development. Through this program two undergraduate students work in Dr. Matt Evans lab each summer and learn many molecular and cellular biology techniques, including DNA extractions, PCR, and confocal microscopy of maize gametophytes. In addition to conducting their own independent research projects, the interns work with Kelly Beck of the Haas Center for Public Service to develop teaching units centered on the big ideas of plant biology. These lesson plans, which relate to Dr. Evans research and are designed to enhance the typical high school plant biology curriculum, are taught to the teen population at the local Boys and Girls Club. This is the first time such a research-teaching internship program has been implemented through Science in Service and Carnegie Institution. This new approach provides undergraduate students an opportunity to connect their own research experiences with civic engagement. The main goals of the project are to encourage future generations of scientists to consider teaching, training, and mentorship as integral components of the scientific endeavor and to introduce high school students in under-served communities to a science community and to plant biology. We hope that future iterations of this novel program will not only generate more civic-minded scientists but also learning modules that can be adapted for other outreach projects.

P239

Coming of Age of the iPlant Collaborative

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The iPlant Collaborative (iPC) is supported by NSF PSCIC to “create a new type of organization – a cyberinfrastructure collaborative for the plant sciences - that will enable new conceptual advances through integrative, computational thinking.” iPC is designed to be fluid and dynamic, utilizing computational science and cyberinfrastructure solutions to address an evolving array of grand challenges in the plant sciences and to develop innovative approaches to education, outreach, and the study of social networks.

Grand challenge questions in the plant sciences are large, currently intractable and require major cyberinfrastructure development. The selection of grand challenge questions is community-driven. To facilitate the community’s choices, we hosted five Grand Challenge Workshops in our first year on topics proposed by the community. Self-forming Grand Challenge Teams from the community are now proposing collaborative projects to develop Discovery Environments--the cyberinfrastructure needed to address and solve the team’s grand challenge. Ultimately iPC will address the cyberinfrastructure need of different grand challenges ranging from the molecular, cellular, and developmental to the organismic, ecological, and evolutionary plant sciences.

At its core, iPC is a community-building and educational enterprise. Grand Challenge Teams and iPlant core staff will work together to educate the next generation.

P240

The genome in a garden: maize mutants and public outreach (1932-2007)

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The first time a demonstration garden of maize mutants from each of the ten maize chromosomes was designed and planted for public display, was in 1932, at the World Genetics Congress, held in Ithaca, NY. At that time, the mysteries of the maize genome were beginning to be revealed by the talented team at Cornell University, lead by R.A. Emerson, including Barbara McClintock, Marcus Rhoades, Charles Burnham and George Beadle. Fast forward to the summers of 2005-2007, and we will visit two modern-day maize map gardens, the "Maize-10-Maze", a Florida State University and Florida A&M University collaborative effort at the FAMU research farm in Quincy, Florida, and the scientific descendant of the first maize map garden, planted in the original site in Ithaca, NY. In the true spirit of cooperation that is the hallmark of the maize genetics community, placards created for the Maize-10-Maze were also used in the Ithaca garden, and the primary source of mutant seed for both gardens was the Maize Genetics Cooperation Stock Center. Here, we showcase the beauty of the mutants of maize as a public education resource, and propose the creation of a maize map garden kit for K-12 classrooms.

P241

The maize gene review – a new feature of the Maize Genetics Cooperation Newsletter

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Prior to the existence of on-line databases, the Maize Genetics Cooperation Newsletter (MNL) served a central community role to systematize available mutant stocks, genetic maps, maize publications and addresses for maize geneticists. The very first data entered into MaizeDB, in 1991, came from the MNL compilations supplied by then editor, Ed Coe, and the Maize Genetics Cooperation Stock Center. Text descriptions of genes continued to be supplied by curation of the literature at MaizeDB, the precursor to MaizeGDB, and from the Mutants of Maize (1997), eds MG Neuffer, EH Coe, SR Wessler. Of particular note, this 1997 volume contained short text summaries of individual mutants that were provided by various **experts** in the field, often along with photographs. To support renewed, community submission of similar data, the MNL has initiated a new on-line journal, called the 'maize gene review' (MGR, www.maizegenereview.org). In the MGR, each gene has a page, and an author(s), responsible for supplying a minimal set of information, and updating as new information becomes available. Reviewers familiar with the types of mutants described are requested to provide criticism and updates, which are forwarded to the contributing author, as suggestions on updating their page(s). Reciprocal links are provided to the MaizeGDB for genome and map information and the short summary of each mutant is added to the MaizeGDB locus record. This journal adds to the current tools at MaizeGDB for community curation. The editors, MaizeNewsletter@missouri.edu, welcome your input and contributions. Please contact us if you are interested in providing a page for your favorite mutant.

The MNL is supported by an endowment at the University of Missouri, provided by Maize Cooperators. MaizeGDB is a USDA Agricultural Research Service funded database where the presenting author is a member of the core team that includes: Lisa Harper, Taner Z Sen, Carson M Andorf, Darwin A Campbell, and Carolyn Lawrence (Director).

P242

Tribal College Outreach III: Transitioning to a University

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Tribal Colleges and Universities (TCUs) were created to serve the higher education needs of American Indians, frequently serving geographically isolated populations with limited access to postsecondary education. Little Big Horn College (LBHC), located in Crow Agency, MT, serves the Crow people by providing culturally relevant education, emphasizing intellectual pursuits that support the needs of the tribe. The courses of study offered at LBHC are directly related to the job opportunities and economic development on the Reservation and surrounding communities. The biology curriculum at LBHC focuses on topics related to human health, environmental science and natural resources. To enhance the genetics curriculum at LBHC, we have conducted workshops that focus on classical genetics through study of corn genetics, horse coat color genetics, the human disease genetics such as diabetes, and relevant issues such as transgenic crops. A second workshop held at the University of Wyoming (UW) 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. We expanded our approach to include faculty from LBHC and other MT TCUs. The goal is to bring TCU students to UW to experience a four-year college environment and to encourage lab-based research in corn genetics and molecular methods. In June 2008, we hosted 11 students and faculty from two TCUs, successfully developed PCR-based fingerprinting for water quality testing, thus enabling the students and faculty to perform the molecular aspects of their environmental studies on-site at their TCU. In addition, a student from LBHC, who transferred to UW for completion of a BS degree, will continue in an internship to develop lab-based techniques that can be brought to LBHC for incorporation into their research programs.

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Career Opportunities at Pioneer Hi-Bred International

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Careers at Dow AgroSciences

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Late Submissions

P245

The world, re-imagined.

(submitted by David Feldman<david.l.feldman@monsanto.com>)
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At Monsanto, we have successfully developed new technologies in our R&D pipeline that have advanced our Breeding platforms. The results have been significant growth in talent needs across the organizations that support corn research and product development. Therefore, we want to provide information about Monsanto and the opportunities available within our Technology organization. More specifically we want to demonstrate our opportunities give scientists the opportunity with grow their careers while contributing cutting edge scientific solutions to support global agricultural productivity and sustainability.

P246

Identification of genes regulated by the transcription factor KNOTTED1

(submitted by Nathalie Bolduc <nath.bolduc@gmail.com>)
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KNOTTED1 (KN1) and other KN1-like homeobox (KNOX) proteins are involved in the establishment and maintenance of shoot meristems in plants. In species with simple leaves such as maize, KNOX proteins are strictly excluded from developing leaves. Dominant mutations such as Kn1-N that allow ectopic kn1 expression in maize leaves induce cellular proliferations and transformations of cell identity, while recessive mutant alleles have reproductive defects. Using chromatin immunoprecipitation, we found that KN1 binds in vivo to an intronic region of ga2ox1, a class of genes whose products are involved in inactivation of gibberellins (GAs). Electrophoretic mobility shift assays demonstrated that the binding occurs through a 15-bp cis-regulatory element comprising two TGAC motifs. In planta, VP16-KN1 activates the transcription of a reporter driven by a chimeric promoter containing multiple copies of the binding site. ga2ox1 is up-regulated in the immature leaves of the dominant Kn1-N mutant and down-regulated in the male and female inflorescences of a null allele (kn1-e1). Furthermore, in situ hybridization experiments showed that kn1 and ga2ox1 mRNA overlap in the vegetative meristem. Taken together, our data support a model where KN1 modulates the accumulation of GAs through the control of ga2ox1 transcription. Combined with the availability of the maize genome, this opens the door for the systematic identification of KN1 targets using massive sequencing of ChIP DNA. Considering the importance of KN1 in the growth of maize ears, identifying its targets will help us to decipher the pathways underlying ears development.

Late Update

P180

Characterizing alcohol soluble proteins in teosinte and tripsacum seeds

(submitted by Anastasia Bodnar <anastasia@geneticmaize.com>)
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Maize inbred lines are deficient in methionine, tryptophan, and lysine. Many attempts have been made to improve levels of these amino acids, including breeding of modern inbred lines with maize landraces and relatives of maize such as teosinte and tripsacum. Because they are the most abundant proteins in the seeds, alcohol soluble seed storage proteins, called zeins, have been a target for improvement. HPLC analysis of alcohol soluble proteins in 27 inbred lines, 17 landraces, and 11 teosintes showed large differences in types and amounts of these proteins, including novel proteins that were not present in the inbred lines (mean number of unique proteins was 22 in inbreds, 25 in landraces, and 36 in teosintes). The α zeins, making up the largest percentage of total zeins, showed the greatest amount of variation (mean number of unique α was 15 in inbreds, 17 in landraces, and 25 in teosintes). Preliminary analysis of tripsacum had similar results, with some significant exceptions: tripsacum was missing some zeins that were present all other samples, and had novel proteins that were not present in any of the other samples.

Late Submissions

P245

The world, re-imagined.

(submitted by David Feldman<david.l.feldman@monsanto.com>)
Full Author List: Feldman, David[1];
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At Monsanto, we have successfully developed new technologies in our R&D pipeline that have advanced our Breeding platforms. The results have been significant growth in talent needs across the organizations that support corn research and product development. Therefore, we want to provide information about Monsanto and the opportunities available within our Technology organization. More specifically we want to demonstrate our opportunities give scientists the opportunity with grow their careers while contributing cutting edge scientific solutions to support global agricultural productivity and sustainability.

P246

Identification of genes regulated by the transcription factor KNOTTED1

(submitted by Nathalie Bolduc <nath.bolduc@gmail.com>)
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Late Update

P180

Characterizing alcohol soluble proteins in teosinte and tripsacum seeds

(submitted by Anastasia Bodnar <anastasia@geneticmaize.com>)
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