-

49th ANNUAL MAIZE GENETICS CONFERENCE

PROGRAM
&
ABSTRACTS

22 - 25 MARCH 2007

PHEASANT RUN
ST. CHARLES, ILLINOIS

This conference received financial support from:

National Science Foundation

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We thank these contributors for their generosity!

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General Information

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 Workshops 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.

Hospitality

After the evening session on Thursday and Friday there will be informal socializing and poster gazing in the Mega Center. Refreshments will be provided each night until 12 AM. On Saturday evening there will be informal socializing in the Mega Center, with music and dancing.
After 12 AM, the Marsalis rooms are 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 your trash and bottles in the party room.

Steering Committee

Please share your suggestions and comments about the meeting with the 2007 Steering Committee:

Anne Sylvester, Chair (annesyl@uwyo.edu)
Tom Brutnell, Co-Chair (tpb8@cornell.edu)
Marja Timmermans (timmerma@cshl.edu)
Richard Schneeberger (rschnee@sbcglobal.net)
Ed Buckler (esb33@cornell.edu)
Erin Irish (erin-irish@uiowa.edu)
Jorge Nieto-Sotelo (jorge@ibt.unam.mx)
Steve Moose (smoose@uiuc.edu)
Mei Guo (mei.guo@pioneer.com)
Peter Rogowsky (Peter.Rogowsky@ens-lyon.fr)
Marty Sachs, local organizer, ex officio (msachs@uiuc.edu)
Karen Cone, treasurer, ex officio (ConeK@missouri.edu)
Mary Schaeffer, abstract coordinator, ex officio (SchaefferM@missouri.edu)
Trent Seigfried, abstract coordinator, ex officio (devolver@iastate.edu)

Acknowledgements

Many thanks go to Trent Seigfried and Mary Schaeffer for their considerable efforts in the assembly and maintenance of the conference website and for the assembly and printing of this program. Thanks also go to Mike McMullen for design and preparation of the poster. The meeting registration was outsourced to the MU Conference Center, and professionally handled by Angela Freemyer and Alice Fisher. Special thanks go to Marty Sachs for his exceptional work at organizing the meeting and to Karen Cone for continued oversight of the meeting budget.

Next Maize Genetics Meeting

The 50th Annual Maize Genetics Conference will be held in Washington DC. Projected dates are February 27– March 2, 2008 (NOTE: one extra day is scheduled to accommodate our landmark 50th anniversary meeting). The tentative location is the Marriott Wardman Park Hotel in DC. Local organizer is Pablo Rabinowicz (pablo@tigr.org), with assistance of Jill Stone, HelmsBrisco, Inc (jstone@HelmsBriscoe.com).

Schedule of Events

Thursday, March 22

3:00 PM – 6:00 PM	**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: Marja Timmermans
7:15 PM	**Blake Meyers, University of Delaware** *Deep Transcriptional Profiling of Plant Small RNAs*
8:05 PM	**Sarah Hake, USDA-ARS, Plant Gene Expression Center** *Dominant Mutants Uncover Regulatory Pathways Controlling Maize Architecture*
9:00 PM – 12:00 AM	**INFORMAL POSTER VIEWING**
12:00 AM – 7:00 AM	**HOSPITALITY**

Friday, March 23

7:00 AM – 8:00 AM	**BREAKFAST**	
8:00 AM – 8:15 AM	**ANNOUNCEMENTS**	
8:15 AM – 10:15 AM	**SESSION 2 – CELL BIOLOGY / CYTOGENETICS**	Chair: Peter Rogowsky
8:15 AM	**Paolo Sabelli, University of Arizona** *Control of Cell Transformation and Endosperm Development in Maize by the Retinoblastoma/E2F Pathway*	
8:35 AM	**Kazuhiro Ohtsu, Iowa State University** *Global Expression Analyses of Genes Involved in Meristem Organization and Leaf Initiation*	
8:55 AM	**Rachel Wang, University of California-Berkeley** *A New View: Meiotic Chromosomes and Homologous Synapsis Revealed by Ultrahigh Resolution Structured Illumination (SI) Microscopy*	
9:15 AM	**David Weber, Illinois State University** *Cytogenetic Confirmation of Transposon-Induced Chromosomal Rearrangements in Maize*	
9:35 AM	**Ina Amarillo, Florida State University** *Construction of a Sorghum BAC-based Cytogenetic Map of Maize Pachytene Chromosome 9*	
9:55 AM	**Elisabeth Esch, Leibniz University** *Quantitative Trait Loci Controlling the Global Recombination Frequency in Maize and Other Species*	
10:15 AM – 10:45 AM	**BREAK W/ BEVERAGES**	
10:45 AM – 12:25 PM	**SESSION 3 – TRANSPOSONS / EPIGENETICS**	Chair: Ed Buckler
10:45 AM	**Jose Gutierrez-Marcos, University of Warwick** *Epigenetic Asymmetry of a Group of Imprinted Genes in Maize Gametes*	
11:05 AM	**Nathan Springer, University of Minnesota** *Epigenetic Variation for Gene Expression Levels in B73 and Mo17*	
11:25 AM	**Susan Wessler, University of Georgia** *Transposition of the Rice MITE mPing in Arabidopsis*	
11:45 AM	**Jay Hollick, University of California-Berkeley** *Heritable Epigenetic Repression at the* pl1 *Locus is Maintained by a Novel* Snf2*-like Protein*	
12:05 PM	**Brent Kronmiller, Iowa State University** *TE Nest: Automated Chronological Annotation and Visualization of Maize Nested Transposable Elements*	
12:30 PM – 1:30 PM	**LUNCH**	

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

Presenters should be at even numbered posters from 1:30 PM to 2:30 PM.
Presenters should be at odd numbered posters from 2:30 PM to 3:30 PM.
Beverages will be available from 3:30 PM to 5:00 PM.

5:00 PM – 6:15 PM **SESSION 4 – TOOLS WORKSHOP** Chair: Tom Brutnell

5:00 PM **Amitabh Mohanty, Cold Spring Harbor Laboratory**
A Molecular Framework for Functional Genomics in Maize Using Fluorescent Protein-Tagged Lines Driven by Native Regulatory Elements

5:10 PM **Agnes Chan, The Institute for Genomic Research**
An Automatic Annotation Pipeline for Maize Genomic Assemblies

5:20 PM **Clifford Weil, Purdue University**
The Maize TILLING Project

5:30 PM **Speaker To Be Announced**
The Maize Full Length cDNA Project

5:40 PM **Speaker To Be Announced**
Topic To Be Announced

5:50 PM **Thomas Brutnell, Boyce Thompson Institute**
A Sequence-Indexed Collection of Ds *Insertion Lines in Maize*

6:00 PM **Community Discussion and Updates**

6:30 PM – 7:45 PM **DINNER**

8:00 PM – 9:00 PM **SESSION 5 – DATABASE TUTORIALS** Chair: Erin Irish

8:00 PM **Carolyn Lawrence, USDA-ARS**
MaizeGDB Made Easy

8:20 PM **Immanuel Yap, Cornell University**
Using Gramene for maize-rice genome comparisons

8:40 PM **Brian Smith-White, NCBI**
Resources at the National Center for Biotechnology Information for Corn Genomics

9:00 PM – 12:00 AM **INFORMAL POSTER VIEWING & HOSPITALITY**

12:00 AM – 7:00 AM **HOSPITALITY**

Saturday, March 24

7:00 AM – 8:15 AM	**BREAKFAST**
8:15 AM – 10:15 AM	**SESSION 6 – PLANT BIOTIC & ABIOTIC INTERACTIONS / BIOCHEMICAL GENETICS** Chair: Steve Moose
8:15 AM	**Joerg Degenhardt, Max Planck Institute** *A Maize (E)-beta-Caryophyllene Synthase Takes Part in Defense Against Herbivores Above and Belowground*
8:35 AM	**Braham Dhillon, Purdue University** crw1 - *A Novel Maize Mutant Exceptionally Susceptible to Western Corn Rootworm*
8:55 AM	**Hartwig H. Geiger, University of Hohenheim** *Estimation and Validation of QTL for Early Vigour of Maize Grown in Chilly Environments*
9:15 AM	**Ervin Nagy, University of Georgia** *Genetic Control of Milo Disease Resistance in Sorghum: An NBS-LRR Gene Corrupted by a Fungal Pathogen*
9:35 AM	**Antje Feller, The Ohio State University** *An ACT-like Domain Participates in the Dimerization of Several Plant Basic-helix-loop-helix Transcription Factors*
9:55 AM	**Gertraud Spielbauer, University of Munchen** *Robustness of Carbohydrate Metabolism in Maize Kernels*
10:15 AM – 10:45 AM	**BREAK W/ BEVERAGES**
10:45 AM – 12:25 PM	**SESSION 7 – GENOMICS / BIOINFORMATICS** Chair: Mei Guo
10:45 AM	**Shiguo Zhou, University of Wisconsin** *Physical Mapping of the Whole Maize Genome via Single Molecule Analysis*
11:05 AM	**Weichang Yu, University of Missouri** *Maize Artificial Chromosomes*
11:25 AM	**Fusheng Wei, University of Arizona** *Genetics, Genomics and Evolution of the Maize B73 Genome*
11:45 AM	**Apurva Narechania, Cold Spring Harbor Laboratory** *Using Mathematically-Defined Repeats to Annotate the Maize Genome*
12:05 PM	**Yan Fu, Donald Danforth Plant Science Center** *An Ultra-Conserved Exon-Skipping Event in Plant Transcription Factor IIIA Gene Suggests the Regulation of 5S rRNA Transcription via Alternative Splicing*
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 2:30 PM.
Presenters should be at even numbered posters from 2:30 PM to 3:30 PM.
Beverages will be available from 3:30 PM to 5:00 PM.

5:00 PM – 6:00 PM **SESSION 8 – COMMUNITY FORUM** Chair: Anne Sylvester

5:00 PM **Sarah Hake, Chair, MGEC**
Torbert Rocheford, University of Illinois
MGEC Report 2006-2007
Allerton Meeting Report

5:10 PM **Nathan Fields, Director of Research & Business Development**
National Corn Growers Association
NCGA, 2006-2007

5:20 PM **Richard McCombie, Cold Spring Harbor Laboratory**
The Maize Genome Sequencing Consortium
Sequencing the Maize (B73) Genome

5:35 PM **Community Discussion**

6:00 PM – 7:00 PM **DINNER**

7:15 PM – 9:00 PM **SESSION 9 – PLENARY TALKS** Chair: Jorge Nieto-Sotelo

7:15 PM **Susan Lindquist, Massachusetts Institute of Technology**
A Protein Chaperone Potentiates the Evolution of New Traits

8:05 PM **Martha James, Iowa State University**
Complex Functional Interactions that Determine Starch Architecture

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

2:00 AM – 7:00 AM **HOSPITALITY**

Sunday, March 25

7:00 AM – 8:30 AM	**BREAKFAST**
8:30 AM – 8:40 AM	**ANNOUNCEMENTS**
8:40 AM – 11:00 AM	**SESSION 10 –** Chair: Richard Schneeberger **DEVELOPMENTAL & PHYSIOLOGICAL GENETICS**
8:40 AM	**Carol Rivin, Oregon State University** *Regulatory Circuits in Maize Embryo Development: ABA, GA and the ROP GTPases*
9:00 AM	**Andrea Eveland, University of Florida** *3' UTR Profiling Resolves Expression Variation of Near-Identical Transcripts in Developing Maize Ovaries*
9:20 AM	**George Chuck, University of California-Berkeley** *The* tasselseed4 *and* Corngrass1 *MicroRNAs of Maize Target Domestication Loci*
9:40 AM	**Virginia Walbot, Stanford University** *Anther Development: Transcriptome Profiling of Fertile and Male-Sterile Anthers*
10:00 AM	**Cristian Forestan, University of Padua** The Role of *ZmPIN1* Genes in Kernel Development
10:20 AM	**Sarah Covshoff, Cornell University** Zmhcf136 *is a Mesophyll Defective Mutant that Lacks a Photosystem II Complex*
10:40 AM	**Matthew Walch, University of Minnesota** *Toward Introducing C4 Photosynthesis to C3 Oat by Use of Oat x Maize Addition Lines*
11:00 AM	**ADJOURNMENT**

Posters

Biochemical Genetics

P1	**Mandeep Sharma** <mxs781@psu.edu>	*Red Aleurone1 of Zea mays is Regulated by Transcription Factors that are Required for Anthocyanin and Phlobaphene Biosynthesis*
P2	**R. Frank Baker** <rfb11@psu.edu>	*Tie-dyed1 Acts and is Expressed in Veins*
P3	**Yi Ma** <yum105@psu.edu>	*Tie-dyed1 and Sucrose export defective1 Function in Different Pathways to Regulate Carbohydrate Partitioning*
P4	**Marie Hasenstein** <mahaas@iastate.edu>	*ZmbZIP1: A Maize Transcription Factor Regulated by Opaque-2*
P5	**Mark Settles** <settles@ufl.edu>	*A Non-Destructive Screen for Maize Kernel Mutants Affecting Seed Composition and Weight*
P6	**Sylvia de Sousa** <smsousa@ufl.edu>	*A Small-Kernel Phenotype for the sorbitol dehydrogenase-1 Mutant*
P7	**Yaqing Du** <yadu@plantbio.uga.edu>	*Biochemical Analysis of Centromere Protein C (CENP-C)*
P8	**Donald McCarty** <drm@ufl.edu>	*Characterization Of A Novel Dominant Defective Kernal Mutant from UniformMu*
P9	**Mingshu Huang** <muh147@psu.edu>	*Characterization of camouflage1, a Maize Mutant in the Chlorophyll Synthesis Pathway, Indicates the Presence of a Functional Homolog*
P10	**Dorothy Tuthill** <dtuthill@uwyo.edu>	*Control of Cell Division and Cell Expansion in Gradients in Developing Maize Leaves*
P11	**Michael Oke** <agriclinkcooperative@yahoo.com>	*Crop Production Situation Report in the Federal Capital Territory - Where Are We and How Did We Get Here?*
P12	**Jeremy DeBarry** <jdebarry@uga.edu>	*Discovery of High Copy Number Genomic Repeats Using the Assisted Automated Assembler of Repeat Families (A.A.A.R.F.) Algorithm*
P13	**Jessica Ponder** <jnp465@truman.edu>	*Elucidation of Loci Influencing Near-Infrared Reflectance Spectra of Maize Grain Using the IBM Population*
P14	**Prem Chourey** <pschourey@ifas.ufl.edu>	*Expression of Several Cytokinin (CK) Genes and Levels of CK Hormones in Developing Seeds of miniature1 (mn1) Seed Mutation Relative to the Mn1 Are Altered*
P15	**Nikolaos Georgelis** <gnick@ufl.edu>	*Genes Encoding the Two Subunits of the ADP-glucose Pyrophosphorylase Evolve at Different Rates in Higher Plants, Yet the Genes Are Equally Sensitive to Activity-altering Missense Mutations When Expressed in E. coli*
P16	**Clifford Weil** <cweil@purdue.edu>	*Genetic Control of Starch Digestion Properties and Starch Granule Architecture*
P17	**Jeffrey Church** <jbchurch@uiuc.edu>	*Genetic Control of the Carbon-Nitrogen Balance in the Leaves of IHP Plants*
P18	**Lifang Zhang** <zhangl@cshl.edu>	*Genome Organization and Structure of Maize miRNAs*
P19	**Brent O'Brien** <bob2373@ufl.edu>	*Identification and Characterization of Selected Maize Cell Wall Mutants Generated in the UniformMu Population*

P20 **Joan Rigau** <rigau@ibmb.csic.es>
Identification of Maize R2R3-MYB Factors Affecting the Lignin Biosynthesis Pathway

P21 **Xiang Yang** <yangx@iastate.edu>
Investigating RAMOSA1 Interacting Proteins via Yeast Two Hybrid Analysis

P22 **David Stern** <ds28@cornell.edu>
Investigating Rubisco Bundle Sheath Chloroplast-Specific Accumulation of Rubisco: What are the Cell Type-Specific Regulatory Steps in rbcL Expression?

P23 **Peter Rogowsky** <peter.rogowsky@ens-lyon.fr>
Molecular and Phenotypic Characterization of a Novel brittle2 Allele in the Maize Kernel

P24 **Farag Ibraheem** <fii100@psu.edu>
Role of a myb Transcription Factor in Induction of 3-deoxyanthocyanidin Phytoalexins and Defense

P25 **Andrew Burt** <aburt@uoguelph.ca>
The High Carotenoid Phenotype of the University of Guelph "Hi-C" Inbred Lines and the Y1 Locus

P26 **Michael Held** <maheld@purdue.edu>
Viral-Induced Gene Silencing of Cellulose Synthase and Cellulose Synthase-like Genes in Barley Reveals a Tightly Controlled Gene Network for Cell Wall Biosynthesis

Bioinformatics

P27 **Chi-Ren Shyu** <shyuc@missouri.edu>
A Computational Approach for Scoring Visually Observed Phenotypic Expression in Maize

P28 **Yan Fu** <yfu@danforthcenter.org>
Ab initio Protein-Coding Gene Finding in Maize and Rice Genomes

P29 **Toni Kazic** <toni@athe.rnet.missouri.edu>
Accelerated Data Collection of Phenotypes and Development Improves Workflow Management

P30 **Diane Janick-Buckner** <djb@truman.edu>
Annotation and Analysis of Global Gene Expression Studies: Creation of a Maize Shoot Apical Meristem Expression Database

P31 **Guy Lima Jr.** <galima@oakland.edu>
Bioinformatics Pipeline for Discovering Helitrons in the Maize Genome Database

P32 **William Spooner** <whs@ebi.ac.uk>
Comparative Genome Analysis in Gramene

P33 **Nick Murphy** <nick@ag.arizona.edu>
Computational Tools from the Chromatin Consortium

P34 **Jeff Glaubitz** <glaubitz@wisc.edu>
Correction for SNP Ascertainment Bias in Population Genetic Analyses of Teosinte

P35 **Gernot Presting** <gernot@hawaii.edu>
Do Centromeric Retroelements Determine Chromosome Size?

P36 **Saranyan Palaniswamy** <Saranyan.Palaniswamy@osumc.edu>
GRASSIUS: A Blueprint for Comparative Regulatory Genomics in the Grasses

P37 **Chengzhi Liang** <liang@cshl.edu>
Gramene, A Comparative Resource of Grass Genomes

P38 **Terry Casstevens** <tmc46@cornell.edu>
Maize Diversity: Accessing Data behind Germplasm, QTL, and Breeding Studies

P39 **Zhiwu Zhang** <zz19@cornell.edu>
Maize Diversity: Associating Genetic Polymorphisms and Phenotypes

P40 **Nick Lauter** <nickl@iastate.edu>
Maize Microarray Platform Translator, a New Tool at PLEXdb to Enhance Capabilities for Meta-Analysis of Gene Expression Profiling Data

P41 **Mary Schaeffer** <SchaefferM@missouri.edu>
MaizeGDB Curation: New Data, Tools and an Invitation to All Cooperators

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MaizeGDB: Four Ways Of Looking At Maps

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Quantitation of Lesion Phenotypes as a Function of Inbred Background

P44 **James Estill** <jestill@uga.edu>
RepMiner: A Graph Theory Based Approach to the Classification and Assembly of the Repetitive Fraction of Sample Sequence Data

P45 **Darwin Campbell** <darwin@iastate.edu>
Submitting Your Data to MaizeGDB to Make it Publicly Available

P46 **Shiran Pasternak** <shiran@cshl.edu>
The Maize Genome Sequence Browser

P47 **Agnes Chan** <achan@tigr.org>
The TIGR Plant Transcript Assemblies Database

P48 **Lisa Harper** <ligule@nature.berkeley.edu>
Three 'Works In Progress': MaizeGDB's Editorial Board, Map Description Pages, and a Project to Integrate Mutant Phenotypes with Existing Data

Cell Biology

P49 **Thomas Slewinski** <tls315@psu.edu>
TIE-DYED1 Localizes to the Endomembrane System

P50 **Christopher Bozza** <cgb25@cornell.edu>
dsyCS and segII Define a Novel Class of Homologous Pairing Mutants

P51 **Arnaud Ronceret** <ar346@cornell.edu>
Analysis of the Molecular Role of PHS1 in Meiotic Chromosome Pairing

P52 **Reuben Tayengwa** <reubent@ufl.edu>
Characterization Of A Novel Brown Midrib Mutant

P53 **Charles Hunter** <jbe@ufl.edu>
Functional Analysis of Cellulose Synthase-Like Genes in Maize

P54 **Matthew Hudson** <mhudson@uiuc.edu>
Light Signal Transduction in Maize

P55 **Kan Wang** <kanwang@iastate.edu>
Nanoparticles Mediated Plant Genetic Transformation

P56 **Lorena Moeller** <lorenam@iastate.edu>
Studying Translocation of Recombinant Proteins in Plants: A Bacterial Antigen (LT-B) as a Model System

Cytogenetics

P57 **Rachel Wang** <rachelcjw@berkeley.edu>
A New View: Meiotic Chromosomes and Homologous Synapsis Revealed by Ultrahigh Resolution Structured Illumination (SI) Microscopy

P58 **Inna Golubovskaya** <innagol@berkeley.edu>
A Novel Meiotic Mutant, mtm00-10, with Aberrant Synapsis Shows a Mixture of Equational and Reductional Chromosome Segregation at Anapahse I

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Comparison of Mitochondrial and Chloroplast DNA Insertions into Nuclear Chromosomes of Maize

P60 **Debbie Figueroa** <figueroa@bio.fsu.edu>
Constructing A Cytogenetic Map Of Maize Core Bin Markers In Oat Addition Lines Using Sorghum BACs As FISH Probes

P61 **Tatiana Danilova** <danilovat@missouri.edu> — *Construction of Maize Somatic Chromosome Cytogenetic Map*

P62 **Fangpu Han** <hanf@missouri.edu> — *Reactivation of Inactive B Centromeres in Maize*

P63 **Moira Sheehan** <mjs224@cornell.edu> — *Using the Maize plural abnormalities of meiosis1 (pam1) Mutant to Dissect the Role of the Telomere Bouquet in Pairing and Recombination*

Developmental Genetics

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P65 **Gregorio Hueros** <gregorio.hueros@uah.es> — *ZmTCI-1, a Myb Related Transcription Factor, is a Key Regulator of the Differentiation and Function of Transfer Cells*

P66 **Diego Fajardo** <diegof@ufl.edu> — *A Putative Role for RNA Splicing in Maize Endosperm-Embryo Developmental Interactions*

P67 **Huai Wang** <wang10@wisc.edu> — *A Single Amino Acid Substitution in TGA1 Liberated Maize Kernels by Affecting Gene Transcription*

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P69 **Beth Thompson** <bethompson@berkeley.edu> — *Analysis of Maize Floral Development Mutants*

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P71 **Candice Hansey** <cnhansey@wisc.edu> — *Axillary Meristem Development in Maize grassy tillers1 Mutants*

P72 **Valeriy Rotarenco** <Valeriy_Rotarenco@hotmail.com> — *Biochemical analysis of kernels with haploid and diploid embryos in maize*

P73 **Brent Buckner** <bbuckner@truman.edu> — *Bioinformatic, Expression and DNA Sequence Diversity Characterization of Two Shoot Apical Meristem Expressed Genes*

P74 **John Woodward** <jbw46@cornell.edu> — *Bladekiller1 is Required for Meristem Maintenance and Leaf Development in Maize*

P75 **Gregorio Hueros** <gregorio.hueros@uah.es> — *Characterisation of an Empty Pericarp Mutant Line, Ep-2312, Impaired in the Development of a Transfer Cell Layer*

P76 **Kimberly Phillips** <kap262@psu.edu> — *Characterization of Maize Gene Developmental disaster1*

P77 **Hector Candela** <hcandela@nature.berkeley.edu> — *Characterization of Maize Mutants with Polarity Defects*

P78 **Solmaz Barazesh** <sxb944@psu.edu> — *Cloning and Characterization of vanishing tassel1 (vt1), A Barren Inflorescence Mutant of Maize*

P79 **Jill Preston** <jcpxt8@studentmail.umsl.edu> — *Complex Patterns of APETALA1/FRUITFULL-like Gene Expression in Grasses: Implications for Spikelet Development*

P80 **Brent Buckner** <bbuckner@truman.edu> — *DNA Sequence Diversity of the Gene Encoding the Rough Sheath2 Interacting KH-Domain Protein Among Inbred Lines and Open-pollinated Landraces*

P81 **Magdalena Segura-Nieto** <msegura@ira.cinvestav.mx> — *Differential Expression of Actin Isovariants During Maize Seed Development*

P82	**Judd Hultquist** <judd.hultquist@mu.edu>	*Differential Gene Expression of Sbp-Box Genes in Zea mays mop1-1 Mutants*
P83	**Elizabeth Takacs** <emt32@cornell.edu>	*Discolored1 (DSC1) Function in Maize Kernel Development*
P84	**Theresa Miller** <theresa.miller@marquette.edu>	*Expression and Functional Characterization of zmco1, a Putative Floral Regulator*
P85	**Jun Huang** <junhuang@waksman.rutgers.edu>	*Genetic and Molecular Characterization of Two Pollen-Specific stk Paralogs*
P86	**Roman Zimmermann** <roman.zimmermann@zmbp.uni-tuebingen.de>	*Identification of Genes Involved in Lateral Root Formation in Zea mays*
P87	**Jorge Nieto-Sotelo** <jorge@ibt.unam.mx>	*Interplay Between Light, High Temperature, and Hsp101 Activity in the Control of the Emergence of Adventitious Roots at the Coleoptilar Node in Maize Seedlings*
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P89	**Gregorio Hueros** <gregorio.hueros@uah.es>	*Is ZmTCRR-1 a Molecular Messenger Connecting Different Seed Compartments?*
P90	**Ryan Douglas** <rnd4@cornell.edu>	*Map-based Cloning of ragged seedling2: A Gene Required for Lateral Leaf Expansion in Maize*
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Plenary Talk Abstracts

Plen1

Deep Transcriptional Profiling of Plant Small RNAs

(presented by Blake Meyers <meyers@dbi.udel.edu>)
Full Author List: Meyers, Blake[1]; Nobuta, Kan[1]; Lu, Cheng[1]; Pillay, Manoj[1]; Kulkarni, Karthik[1]; Hetawal, Amit[1]; Green, Pamela[1]
[1] Delaware Biotechnology Institute & Department of Plant and Soil Sciences, University of Delaware, Newark, Delaware, USA 19711

Small RNAs such as miRNAs and siRNAs are a powerful regulatory force in most eukaryotes because they can function to shut off genes at multiple levels. Deep sequencing of the small RNA component of the transcriptome is an important step toward elucidating the impact of small RNAs on individual genes and the genome as a whole. We have developed and applied small RNA profiling methods based on novel parallel sequencing technologies, including massively parallel signature sequencing (MPSS), 454 Life Science's pyrosequencing technology (454) and Solexa's "sequencing-by-synthesis" (SBS). Using these approaches, we have identified more than 75,000 different small RNA sequences from Arabidopsis (http://mpss.udel.edu/at). By analyzing an rdr2 loss-of-function mutant using MPSS and 454, we characterized the complement of miRNAs expressed in Arabidopsis inflorescence to considerable depth. Nearly all known miRNAs were enriched in this mutant and we identified new miRNAs. More recently, we have been analyzing the small RNA component of grass species, particularly rice (http://mpss.udel.edu/rice) but also in maize. Bioinformatics analyses and experimental approaches are being used to identify novel miRNAs and their targets, as well as to distinguish different classes of small RNAs in these plant species.

Plen2

Dominant Mutants Uncover Regulatory Pathways Controlling Maize Architecture

(presented by Sarah Hake <maizesh@nature.berkeley.edu>)
Full Author List: Hake, Sarah[1]; Neuffer, Gerald[3]; Chuck, George[1]; Moon, Jihyun[2]; Bolduc, Nathalie[2]; Ramirez, Julio[2]; Candela-Anton, Hector[1]; Lunde, China[2]
[1] Plant Gene Expression Center, ARS-USDA, Albany, CA 94710
[2] Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720
[3] University of Missouri, Columbia, MO 65211

Maize has been blessed with a number of dominant mutants that have been informative. I will talk about dominant mutants that have opened different doors of research and the mechanisms, when known, for these dominant mutants. One type of dominant mutations is caused by transposon insertions that turn genes on ectopically. A recent member of this group is Corngrass, due to the insertion of a Stoner element upstream of miR156, which leads to ectopic expression in adult stages of development, Most well studied is Knotted1 (kn1), due to transposon insertions in introns and promoters or tandem duplications. Loss of function alleles indicate a role for kn1 in meristem maintenance. The recessive alleles would not have been identified without a search for loss of the dominant phenotype. Another type of mutation is due to base pair changes in microRNA target sites, thus making the gene immune to regulation by microRNAs. Rolled, which affects abaxialadaxial leaf polarity, is one example (Juarez et al., 2004, Nature 428:84) and Tasselseed6, which affects spikelet meristem fate, is another. We have an additional 20-30 EMS induced dominant mutants waiting for analysis and cloning. Some, like Liguleless narrow (Lgn), were found as half-plant chimeras. Lgn disappears in Mo17, and is very severe in B73. The homozygote in either background lacks internode elongation and makes only small juvenile leaves. The interaction with genes identified by recessive mutations suggests that it plays a role in leaf development. These dominant mutations allow a peak into development and cell biology and should be considered treasures in maize genetics.

Plen3

Complex Functional Interactions that Determine Starch Architecture

(presented by Martha James <mgjames@iastate.edu>)

Plants are unique in their formation of starch as a carbohydrate energy reserve. Knowledge of how starch is formed and mobilized is important for our understanding how plants manage energy for growth and development. The fact that starch has many food and industrial applications also makes understanding starch structural organization interesting from an applied perspective. In particular, the highly branched amylopectin (Ap) component of starch has a complex molecular architecture generated by a number of coordinated and regulated enzymatic activities. Five classes of starch synthase (SS) catalyze reactions that build α-(1→4)-linked linear glucosyl chains, two classes of starch branching enzyme (BE) catalyze the introduction of α-(1→6)-linkages to form branches, and two classes of starch debranching enzyme (DBE) catalyze the hydrolysis of α-(1→6)-linkages, which is believed to render organization to the Ap molecule. Genetic and biochemical analyses of the maize SSs, BEs, and DBEs provide insight into the catalytic function of each isoform. Pleiotropic enzymatic effects of mutations in these starch biosynthetic genes, coupled with direct tests for protein interactions, provide evidence that specific enzymes associate to form functional protein complexes. Of particular interest is ISA1, the product of the maize *sugary1* (*su1*) gene, which exhibits modified enzymatic activity in association with ISA2, and SSIII, the product of the *dull1* (*dul1*) gene, which apparently regulates the activities of SSI, BEIIa, and ISA1 via protein complex assembly. The organization of starch biosynthetic enzymes into functional networks is believed to provide coordinated control of glucan chain elongation and branch linkage placement in Ap, creating a molecular structure that provides for maximal carbohydrate storage. Several transgenic approaches are being used to examine how the interplay of SSIII with other enzymes, and possibly factors such as 14-3-3 proteins, influences the final molecular architecture of Ap, and to provide support for the idea that DBEs provide critical editing functions during starch biosynthesis. Rational design of novel starch forms using combined genetic and transgenic approaches also is being tested.

Short Talk Abstracts

T1

Control of Cell Transformation and Endosperm Development in Maize by the Retinoblastoma/E2F Pathway.

(submitted by Paolo Sabelli <psabelli@ag.arizona.edu>)
Full Author List: Sabelli, Paolo A.[1]; Lizarraga, Lucina[1]; Liu, Yan[1]; Dante, Ricardo R.[1]; Ng, Hong N.[1]; Hoerster, George[2]; Jung, Rudolf[2]; Gordon-Kamm, William J.[2]; Larkins, Brian A.[1]
[1] Dept. of Plant Sciences, University of Arizona; Tucson, AZ 85719
[2] Pioneer Hi-Bred Intl. Inc.; Johnston, IA 50131

Retinoblastoma (RB)-related (RBR) proteins, also known as pocket proteins, play crucial roles in coordinating cell cycle progression with cell differentiation, cell death, and development in all higher eukaryotes. The best characterized function of RBR proteins is regulating the activity of the E2F transcription factors that are required for expression of genes involved in DNA replication and cell cycle progression. The RB/E2F pathway is conserved in plants but we have found important differences among them. Whereas Arabidopsis and most plant species possess a single RBR gene, maize and related cereals have at least two distinct types, RBR1 and RBR3. RBR1 represses the expression of RBR3 by inhibiting E2F activity. Down-regulation of RBR1 in embryogenic callus through expression of wheat dwarf virus RepA stimulates expression of S-phase genes and leads to a dramatic increase in cell proliferation and transformation/regeneration rates. Down-regulation of RBR1 in developing endosperm by RNAi results in up-regulation of E2F targets and stimulation of the endoreduplication cell cycle. However, in both embryogenic callus and developing endosperm down-regulation of RBR1 triggers a dramatic increase in RBR3 expression. This is a new paradigm for plant cell cycle regulation, because it indicates that in maize and related cereals specific regulatory loops within the retinoblastoma family of proteins control the cell cycle in ways that may be analogous to the situation in mammals but remarkably different from what occurs in Arabidopsis and other dicots. Our results suggest that compensatory mechanisms within the retinoblastoma protein family result in a sustained inhibitory activity over the cell cycle, which may help explain the recalcitrance of cereals to genetic transformation. Using a functional genomics approach, we are dissecting the RB/E2F pathway in both regenerating embryogenic callus and developing endosperm. Our results indicate that RBR1 and RBR3 have distinct functions in regulating the cell cycle.

T2

Global Expression Analyses of Genes Involved in Meristem Organization and Leaf Initiation

(submitted by Kazuhiro Ohtsu <kazohtsu@iastate.edu>)
Full Author List: Ohtsu, Kazuhiro[1]; Smith, Marianne B.[1]; Borsuk, Lisa A.[1]; Lu, Pengcheng[1]; Emrich, Scott J.[1]; Zhou, Ruilian[1]; Chen, Tianle[2]; Zhang, Xiaolan[2]; Jin, Hailing[1]; Chen, Hsin D.[1]; Brooks, Lee[5]; Beck, Jon[3]; Buckner, Brent[3]; Janick-Buckner, Diane[3]; Timmermans, Marja C. P.[4]; Scanlon, Michael J.[5]; Nettleton, Dan[1]; Schnable, Patrick S.[1]
[1] Iowa State University, Ames, IA 50011
[2] University of Georgia, Athens, GA, 30602
[3] Truman State University, Kirksville, MO, 63501
[4] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724
[5] Cornell University, Ithaca, NY, 14853

All above ground organs of higher plants are ultimately derived from specialized organogenic structures called shoot apical meristems (SAMs). To identify genes required for meristem function and early leaf development intact maize SAMs were fixed, embedded in paraffin and sectioned. SAM cells were isolated from these sections via laser capture micro-dissection (LCM). RNA was isolated from six biological replications of SAMs and seedlings, amplified and hybridized to three spotted microarrays that together contain 37,660 maize cDNA clones. This experiment identified 2,783 ESTs (7.4% of the total) that are preferentially expressed in the SAM (P 100 fold). To confirm and extend these results cDNA derived from SAMs were sequenced using 454 technology. Among over 260,000 ESTs (454-SAM ESTs) ~14% were retrotransposon-related sequences. Subsequently, a global expression analysis of the L1 (the single cell layer tunica) vs. the L2 (the corpus) of the SAM was also conducted via LCM-microarray. This study identified ~700 ESTs (1.8% of the total) that are preferentially expressed in the L1 or the L2 (P <0.001).

T3

A New View: Meiotic Chromosomes and Homologous Synapsis Revealed by Ultrahigh Resolution Structured Illumination (SI) Microscopy

(submitted by Rachel Wang <rachelcjw@berkeley.edu>)
Full Author List: Wang, Rachel C.[1]; Carlton, Pete[2]; Sedat, John[2]; Cande, W. Zacheus[1]
[1] Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720
[2] Department of Biochemistry and Biophysics, University of California, San Francisco , CA 94143

Homologous chromosome pairing, recombination, and synapsis occur during meiotic prophase and are essential for the reductional division required to ultimately generate haploid gametes. During leptotene, each chromosome develops a linear proteinaceous structure called an axial element (AE). Around the same time, the homology search initiates by the appearance of double strand breaks. In zygotene, the homologues synapse via the polymerization of a central element between the two homologous AEs, forming the synaptonemal complex. At late pachytene, synapsis and recombination are completed. These events are well known cytologicallly, and have been explored with light microscopy and EM. Structured illumination (SI) is an ultrahigh resolution light microscopy developed by John Sedat at UCSF. It overcomes the 250 nm limit of resolution of conventional light microscopy, and is currently able to resolve 2 points that are less than 100 nm apart. We used SI to explore the substructure of pachytene chromosomes, organization of the axial element and formation of the synaptonemal complex by monitoring the distribution of two axial element antibodies, AFD1 and HOP1, and the DNA by DAPI staining. We found that chromomeres of paired chromosomes are bilaterally symetrical and have a left handed helical pitch. Chromosome and axial element organization are different at centromeres. We also found evidence for synaptic adjustment in unsynapsed regions of zygotene chromosomes, as the homologs in the unsynpased regions are very different in length. These regions are associated with interlocks during late zygotene, which imply that the resolution of interlocks between chromosomes may be a rate limiting step to complete synapsis.

T4

Cytogenetic Confirmation of Transposon-Induced Chromosomal Rearrangements in Maize

(submitted by David Weber <dfweber@ilstu.edu>)
Full Author List: Weber, David F.[1]; Pulletikurti, Vinay S.[1]; Chuanhe, Yu[2]; Zhang, Jianbo[2]; Peterson, Thomas[2]
[1] Illinois State University, Normal, IL., USA, 61790-4120
[2] Iowa State University, Ames, IA., USA, 50011-3260

At the maize p1 locus, alleles containing multiple copies of Ac termini are unstable and can give rise to various rearrangements, including deletions, duplications, and inversions. These rearrangements can be easily isolated based on their effect on kernel pericarp pigmentation. We proposed that these rearrangements are caused by alternative transposition reactions involving the termini of different Ac/Ds elements in close proximity. This model also predicts the formation of reciprocal translocations if the transposition target site is on another chromosome and inversions if the target site is on the same chromosome. To test this, we identified candidate translocations and inversion by screening for pollen abortion and female semisterility. These stocks were further characterized using PCR methods (LM-PCR or Ac casting) to isolate the new sequences flanking the junction with Ac. These sequences were used in PCR of DNA from oat-maize addition lines (kindly provided by the Ron Phillips lab, Univ. of Minnesota) to identify the chromosome involved in the putative translocation or the inversion. In this way, we isolated 12 new putative translocations and 3 new putative inversions. Candidate translocations were analyzed cytologically at diakinesis in microsporocytes, and the predicted associations of four chromosomes (rings or chains) were observed, confirming the presence of translocations. Several of the translocations and inversions have also been visualized in pachytene cells. These cytogenetic data, together with the molecular and phenotypic results, provide conclusive evidence that reciprocal translocations and inversions can be generated by alternative transposition reactions. To view an animation of the alternative transposition model, see http://jzhang.public.iastate.edu/Transposition.html.
This research is supported by NSF award 0450243 to T. Peterson and J. Zhang, and 0450215 to D. Weber.

T5

Construction of a Sorghum BAC-based Cytogenetic Map of Maize Pachytene Chromosome 9

(submitted by Ina Amarillo <feamarillo@bio.fsu.edu>)
Full Author List: Amarillo, Ina E[1]; Bass, Hank W[1]
[1] Department of Biological Science, Florida State University, Biology Unit I Chieftan Way,Tallahassee, FL 32306-4370 USA

We have developed a high-density sorghum BAC-based cytogenetic map of maize pachytene chromosome 9. Here we describe the recent findings from our detailed FISH mapping studies in which we have examined a large number of loci spanning chromosome 9. Maize RFLP-selected Sorghum BACs have been chosen for use with the multi-wavelength direct-labeled FISH method described by Koumbaris and Bass (2003, Plant J 35:647). Analysis of 30 FISH-mapped loci revealed colinearity between maize cytogenetic and linkage maps, with localization of the centromere between tda66d and cdo17. Also, this project provides unique data that integrates information from a variety of gene maps of sorghum and maize. Interestingly, we found chromosomal regions that appear to be hotspots of genome expansion (e.g. 9L.55-9L.72). These results will provide valuable new information on current research not only for structural genomics and positional cloning, but also for comparative genomics among the grasses as well.

T6

Quantitative Trait Loci Controlling the Global Recombination Frequency in Maize and Other Species

(submitted by Elisabeth Esch <esch@genetik.uni-hannover.de>)
Full Author List: Esch, Elisabeth[1]; Szymaniak, Jessica M.[2]; Yates, Heather[3]; Pawlowski, Wojciech P.[2]; Buckler, Edward S.[4]
[1] Institute of Plant Genetics, Leibniz Universitat Hannover, Herrenhauser Str. 2, 30419 Hannover, Germany
[2] Department of Plant Breeding and Genetics, Cornell University, 403 Bradfield Hall, Ithaca, New York, 14853, USA
[3] Institute for Genomic Diversity, Cornell University, Ithaca, New York, 14853, USA
[4] Department of Plant Breeding and Genetics, and Institute for Genomic Diversity, Cornell University, Ithaca, NY, 14853, and United States Department of Agriculture-Agricultural Research Service, USA

Recombination is a crucial component of evolution and plant as well as animal breeding, producing new genetic combinations on which selection can act. Rates of recombination vary tremendously, not only between species but also within species and for specific chromosomal segments.

We present a novel approach to identify quantitative trait loci (QTL) influencing global meiotic recombination frequencies. Our method utilizes recombinant inbreed (RI) populations that have been developed for many plants and animals and where segregation of genes influencing the recombination frequency across the entire genome can be observed. We measured the frequency of recombinations accumulated in individual RI lines by counting breakpoints in RI mapping data previously created for maize, wheat, Arabidopsis thaliana, and mouse.

We demonstrate that substantial variation exists for genome-wide crossover rates in both outcrossed and inbred plant and animal species. Subsequently, we used the crossover numbers as a quantitative trait and were able to identify genomic regions that control genome-wide recombination rates in maize as well as the other species.

The new method employed here, in conjunction with marker assisted selection, holds considerable promise for a drastic improvement of breeding techniques, as it enables the creation of hyper-recombinogenic lines, which can help overcome limited recombination that often hampers breeding progress.

T7

Epigenetic Asymmetry of a Group of Imprinted Genes in Maize Gametes

(submitted by Jose Gutierrez-Marcos <J.F.gutierrez-marcos@warwick.ac.uk>)
Full Author List: Gutierrez-Marcos, Jose F.[1]; Costa, Liliana M.[2]; Dal Pra, Mauro[2]; Scholten, Stefan[3]; Kranz, Erhard[3]; Perez, Pascual[4]; Dickinson, Hugh G.[2]
[1] Warwick-HRI, University of Warwick, Wellsbourne, Warwick, CV35 9EF, UK.
[2] Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK.
[3] Biozentrum Klein Flottbek und Botanischer Garten, Entwicklungsbiologie und Biotechnologie, Universitat Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany.
[4] Biogemma, 24 Avenue des Landais, 63170 Aubiere, France.

Plant imprinted genes show parent-of-origin expression in seed endosperm, but little is known about the nature of parental imprints in gametes before fertilization. We have found that single differentially methylated regions (DMRs) correlate with allele-specific expression for a group of imprinted genes in the seed and that one DMR is differentially methylated between gametes. Our data suggest that plants have developed a variety of strategies to epigenetically mark imprinted genes in the endosperm.

T8

Epigenetic Variation for Gene Expression Levels in B73 and Mo17

(submitted by Nathan Springer <springer@umn.edu>)
Full Author List: Makarevitch, Irina M[1]; Haun, William J[1]; Stupar, Robert M[1]; Iniguez, A. Leonardo[2]; Barbazuk, W. Brad[3]; Kaeppler, Shawn M.[2]; Springer, Nathan M.[1]
[1] University of Minnesota, Saint Paul MN 55108
[2] University of Wisconsin, Madison, WI 53706
[3] Donald Danforth Plant Sciences Center, St. Louis, MO 63132

The role of epigenetic alterations as a contributor to intraspecific variation for transcription levels is unclear. We have studied the functional targets of the maize chromomethylase ZMET2 in multiple inbred lines to determine whether the targets for this chromomethylase are conserved or variable within the species. DNA microarrays were interrogated with RNA from the inbred lines B73 and Mo17, and from near-isogenic derivatives containing the loss-of-function allele zmet2-m1. A set of 126 genes that displayed statistically significant differential expression in zmet2 mutants relative to wild type plants in at least one of the two genetic backgrounds were identified. Analysis of the transcript levels in both wild type and mutant individuals revealed that only ten percent of these genes exhibited differential expression in both B73 and Mo17 genetic backgrounds. Further analysis was performed for seven genes that are normally silent in B73 but expressed in B73 zmet2-m1 mutant lines and in wild type Mo17. Mapping experiments confirm that the expression difference in B73 relative to Mo17 is caused by cis-acting regulatory variation. Methylation-sensitive PCR and bisulphite sequencing were used to demonstrate the presence of CpNpG methylation in the B73 allele that is absent in the zmet2-m1 mutant and in wild-type Mo17 for five of these genes. Each of these five genes exist in methylated, transcriptionally silent states in some inbred lines and unmethylated, expressed states in other maize inbred lines, providing evidence for natural variation in epigenetic states for some maize genes.

T9

Transposition of the Rice MITE mPing in Arabidopsis

(submitted by Sue Wessler <sue@plantbio.uga.edu>)
Full Author List: Yang, Guojun[1]; Zhang, Feng[1]; Wessler, Susan R.[1]
[1] University of Georgia, Department of Plant Biology, Athens, Georgia 30602

The first active miniature inverted repeat transposable element (MITE), mPing, was discovered by computer-assisted analysis of rice genome sequence. The mPing element is mobile in rice cell culture and in a few rice strains where it has amplified to over 1000 copies during recent domestication. However, determination of the transposase source and characterization of the mechanism of transposition have been hampered by its high copy number and the presence of several possible autonomous elements in the rice genome. Here we report that mPing is active in Arabidopsis thaliana where its transposition is catalyzed by three sources of transposase from rice, the autonomous Ping and Pong elements and by a cDNA derived from a Ping transcript. Furthermore, we demonstrate that excision of mPing is usually precise and transposition requires the products of two independent element-encoded ORFs. Upon insertion, mPing usually transposes to unlinked sites in the Arabidopsis genome that are preferentially in or near genes. As such, this study reports the development of a valuable system for the dissection of MITE transposition and a potentially powerful new tagging system for gene discovery in plants and possibly other eukaryotes.

T10

Heritable Epigenetic Repression at the *pl1* Locus is Maintained by a Novel *Snf2*-like Protein

(submitted by Jay Hollick <hollick@nature.berkeley.edu>)
Full Author List: Hale, Christopher J.[1]; Stonaker, Jennifer L.[1]; Gross, Stephen M.[1]; Hollick, Jay B.[1]
[1] Department of Plant and Microbial Biology, University of California, Berkeley, CA, 94720-3102

Paramutations represent specific types of epigenetic alterations that persist *trans*-generationally. Genetic screens identify at least 10 *trans*-acting factors necessary for maintaining repressed expression states of the *purple plant 1* (*pl1*) allele, *Pl1-Rhoades*. Here we report that one factor, encoded by the *required to maintain repression 1* (*rmr1*) locus, is a novel Snf2-like protein most similar to a group of predicted proteins that includes Arabidopsis DEFECTIVE IN RNA-DIRECTED DNA METHYLATION1 (DRD1), a protein thought to mediate transcriptional repression through RNA-directed DNA methylation (RdDM). Highlighting an apparent novel role for these molecules, RNA expression analyses suggest RMR1 affects *pl1* RNA stability rather than transcription of *Pl1-Rhoades*. Consistent with a role for RMR1 in RdDM, cytosine methylation patterns upstream of *Pl1-Rhoades* are altered in *rmr1* mutants. Similar alterations exist in plants deficient for a putative RNA-dependent RNA polymerase that may also function in RdDM. These different methylation patterns are not, however, reflective of different *Pl1-Rhoades* expression states. This suggests lack of RMR1, and any associated RdDM pathway, facilitates heritable epigenetic changes in a manner distinct from those mechanisms responsible for paramutation events. Consistent with this interpretation, results of genetic tests show RMR1 is not required to establish repressed paramutant states.

T11

TE Nest: Automated Chronological Annotation and Visualization of Maize Nested Transposable Elements

(submitted by Brent Kronmiller <bak@iastate.edu>)
Full Author List: Kronmiller, Brent A.[1]; Werner, Karin[2]; Wise, Roger[3]
[1] Bioinformatics and Computational Biology, Plant Pathology, Iowa State University, Ames, Iowa, USA, 50011
[2] USDA-ARS Corn Insects and Crop Genetics Research, Plant Pathology, Iowa State University, Ames, Iowa, USA, 50011
[3] Bioinformatics and Computational Biology, USDA-ARS Corn Insects and Crop Genetics Research, Plant Pathology, Iowa State University, Ames, Iowa, USA, 50011

The maize sequencing project is underway and specialized tools are necessary to resolve difficult regions. Seventy percent of the maize genome consists of transposable elements (TEs). Of these, 95% are LTR retrotransposons, which greatly hinder sequence assemblies. A majority of TEs occur in clusters of nested repeats, where a TE inserts within the sequence of an existing element, creating short segments of different types. Mapping of maize TEs is therefore difficult as the resulting fragments are not easily identified, necessitating an accurate nested TE identification tool for complete annotation of the genome. Current repeat software does not address nested TEs, making it especially laborious to resolve even moderately clustered repeat regions. With use of a maize canonical TE database, TE nest identifies and maps repeat incorporations into the original genome sequence while providing chronology of insertion events in Mya based on LTR base pair substitution rate. An insertion graph is produced to give an accurate visual representation of TE integration history showing timeline, location and types of each TE identified, thus creating a framework from which evolutionary comparisons can be made among various regions of the maize genome. We have used TE nest to analyze all 165 finished maize BACs from GenBank along with in-house generated 1.5 Mb rf1-spanning sequence in the centromeric region of chr3, constructed from 19 contiguous BACs. Huck, Opie, and Ji retrotransposons make up 50% of all repeat insertions, showing prolific replication of single TE copies at specific time intervals during maize genome evolution.

T12

A Molecular Framework for Functional Genomics in Maize Using Fluorescent Protein-Tagged Lines Driven by Native Regulatory Elements

(submitted by Amitabh Mohanty <mohanty@cshl.edu>)
Full Author List: Mohanty, Amitabh[1]; Yang, Yan[1]; Lee, Byeong-ha[1]; Gallavotti, Andrea[1]; Ling, Xingyuan[2]; Luo, Anding[2]; Chan, Agnes[3]; Sylvester, Anne[2]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, 1, Bungtown Road, Cold Spring Harbor, NY11724
[2] Department of Molecular Biology, 1000 East University Ave, University of Wyoming, Laramie, WY 82071
[3] The Institute of Genomics Research, 9712 Medical Center Drive, Rockville, MD20850

Maize cells consist of interconnected but discrete compartments that help to maintain cellular function and order. Identifying proteins that localize to these compartments is critical to our understanding of developmental and physiological processes in maize, which in turn provides guiding information for crop improvement. We are using recent advances in genomics to identify proteins that localize to diverse cellular compartments, and generating reporter lines that express proteins tagged with fluorescent proteins. These lines display visual information about when and where the tagged proteins are expressed, and gives clues about their functions. Use of native regulatory sequences also allows us to define maize organ and tissue-specific promoters, which at present are scarcely available. Several biological pathways are being used for gene selection, including the auxin/cytokinin hormone pathways and the RAB-mediated vesicle trafficking pathways. We are including gene-tagging requests from the maize community as well. The outcomes of the project will include a set of stable tagged lines expressing fluorescent protein-derived tags for 100 proteins, representing all known cellular compartments. The tagged gene constructs and lines will be freely available. Sub-cellular localization data is being compiled and is available at http://maize.tigr.org/cellgenomics. The project will therefore, generate resources that bridge cell biology and functional genomics. Localization and expression data for several markers genes such as HistoneH1 (nuclear), Tonoplast intrinsic protein (vacuolar membrane), alpha-Tubulin (microtubules), PIN1 (plasma-membrane), alpha-Zein (protein bodies), Rab2A (vesicles), Rop7 (vesicles), Expansins and Glossy1 (cell wall compartments) are already available. We have also generated transgenic lines harboring several cytokinin-pathway genes, such as histidine kinase receptors, response regulators and cytokinin oxidases (CKOs). It is hoped that these lines will aid in our understanding of cytokinin signaling and leaf development in maize. Analysis of these lines by confocal microscopy will be presented. Funding by the NSF Plant Genome Research Program is gratefully acknowledged.

T13

An Automatic Annotation Pipeline for Maize Genomic Assemblies

(submitted by Agnes Chan <achan@tigr.org>)
Full Author List: Chan, Agnes[1]; Zhao, Qi[1]; Haas, Brian[1]; Amedeo, Paolo[1]; Orvis, Joshua[1]; Wortman, Jennifer[1]; Rabinowicz, Pablo[1]
[1] The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20805

Gene enrichment techniques such as methylation filtration (MF) and high Cot (HC) selection are effective approaches to capture the genic regions of large and highly repetitive plant genomes. The gene-enriched reads are a useful tool for gene discovery in genomes for which a complete sequence is not available. We have released version 5 of the Assembled Zea mays (AZMs) sequences using approximately one million MF and HC reads produced by TIGR and 82,000 MF reads sequenced by CSHL. Approximately 13,000 AZM5 assemblies above 3kb in length have been annotated using an improved automatic TIGR Eukaryotic annotation pipeline, optimized for maize sequences. To collect transcript evidence, maize EST and cDNA sequences were aligned to the AZMs to generate AZM-based EST/cDNA assemblies using the TIGR PASA alignment tool. Additional evidence was generated based on alignment to plant protein sequences collected from public protein databases and cereal transcript assemblies generated from the TIGR Plant Transcript Assemblies (TA) project. Gene predictions were generated using gene finders AUGUSTUS that have been trained for maize and FGENESH. The transposon-related repeat regions of the AZMs were identified based on transposon protein profiles using PSI-blast. Gene modeling was performed using the TIGR Evidence Modeler (EVM), a tool that generates consensus gene models by taking into consideration of weighted gene evidence and avoiding identified repeat regions. Gene evidence used for the AZMs included PASA assemblies, plant protein alignments, plant transcript assembly alignments and gene predictions. Gene models created by EVM were annotated with biological function by selecting a description amongst the best hits from protein and domain databases. The AZM-derived gene models will soon be available via the TIGR MANATEE web pages and Gbrowse at the TIGR Maize Database (http://maize.tigr.org). An overview of the AZM annotation project and results derived from analyses of the gene models will be presented.

T14

A Sequence-Indexed Collection of *Ds* Insertion Lines in Maize

(submitted by Thomas Brutnell <tpb8@cornell.edu>)
Full Author List: Ahern, Kevin[1]; Deewatthanawong, Prasit[1]; Xu, Ling[1]; Kikuchi, Kazuhiro[1]; Conrad, Liza J.[2]; Schares, Justin[3]; Hall, Brad[3]; Dong, Qunfeng[4]; Sabharwal, Mukul[5]; Vollbrecht, Erik[3]; Brendel, Volker[3]; Brutnell, Thomas P.[1]
[1] Boyce Thompson Institute, Cornell University, Ithaca, NY, USA 14853
[2] Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY, USA 14853
[3] Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA, USA 50011
[4] Center for Genomics and Bioinformatics, Indiana University, Bloomington, IN, USA 47405
[5] Iowa State University, Ames, IA, USA 50011

The maize transposable elements Ac/Ds were the first transposons discovered by Barbara McClintock over 50 years ago. Over the years, these elements have been extensively characterized through classical genetic and molecular genetic studies that have elucidated the mechanism of transposition, modes of regulation and utility in gene cloning and characterization. We have exploited this tremendous foundation to develop a two-component Ac/Ds gene tagging platform for maize.Through NSF funding (http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=0501713), members of the Brutnell (http://bti.cornell.edu/Brutnell_lab2/BMGG_home.html) and Vollbrecht (http://www.public.iastate.edu/~vollbrec/index.html) groups are now developing a series of Ds-containing lines for use in both forward and reverse genetic screens. The goal of the project is to create a collection of approximately 10,000 lines, each carrying a unique Ds insertion, with insertions distributed uniformly throughout the maize genome. DNA flanking each Ds insertion will be cloned and sequenced providing a precise physical location for each insertion. We have recently developed high-throughput DNA extraction protocols and methods to rapidly amplify and isolate large genomic fragments (up to 8 kb) flanking Ds insertion sites using inverse PCR. These transposon insertion sites are being integrated into genomic assemblies and displayed graphically through web links at PlantGDB by members of the Brendel group (http://www.plantgdb.org/prj/AcDsTagging/). I will present the genetic scheme used to mobilize Ds insertions throughout the genome and the preliminary characterization of approximately 500 Ds-flanking sequences characterized to date.

T15

Tutorial: MaizeGDB Made Easy

(submitted by Carolyn Lawrence <triffid@iastate.edu>)
Full Author List: Lawrence, Carolyn J.[1]; Schaeffer, Mary L.[2]; Harper, Lisa C.[3]; Campbell, Darwin A.[4]; Seigfried, Trent E.[4]
[1] Corn Insects and Crop Genetics Research Unit, USDA-ARS and Depts. of Agronomy and Genetics, Development, and Cell Biology, 526 Science II, Iowa State University, Ames, IA 50011
[2] Plant Genetics Research Unit, USDA-ARS and Division of Plant Sciences, Curtiss Hall, University of Missouri, Columbia, MO 65211
[3] Plant Gene Expression Center, USDA-ARS, 800 Buchanan St., Albany, CA 94710
[4] Corn Insects and Crop Genetics Research Unit, USDA-ARS, 526 Science II, Iowa State University, Ames, IA 50011

During this presentation, you will learn some simple strategies to improve your abilities to use MaizeGDB (http://www.maizegdb.org). You will find out about: simple search methods, ways to maneuver the website via browsing, how to contribute data, what the MaizeGDB Team will be doing next, and much more. Each MaizeGDB Team member will be introduced at the end of the tutorial so that you can see who to talk to in order to find out more. As always, your feedback and recommendations are solicited and most welcome! Please contact us via feedback form links on the footer of all MaizeGDB pages or by sending email to mgdb@iastate.edu. By communicating your data storage, search, and display needs, you enable us to improve our service to the community.

T16

Using Gramene for Maize-Rice Genome Comparisons

(submitted by Immanuel Yap <ivy1@cornell.edu>)
Full Author List: Yap, Immanuel V[1]; Ni, Junjian[1]; Hebbard, Claire[1]
[1] Cornell University, G-15 Bradfield Hall, Ithaca, NY 14853

Gramene is a curated, open-source, web-accessible data resource for comparative genome analysis in the grasses. Maize researchers and breeders can take advantage of known microsynteny between maize and rice to use maps and genomic sequence from rice as a reference point for gene and marker discovery in maize. The tutorial will highlight how to use Gramene's comparative map viewer (CMap), genome synteny viewer (Ensembl), and ontologies to perform these comparisons between maize and rice.

T17

Resources at the National Center for Biotechnology Information for Corn Genomics

(submitted by Brian Smith-White <smtwhite@ncbi.nlm.nih.gov>)
Full Author List: Smith-White, Brian[1]; Chetvernin, Vyacheslav[1]; Clausen, Cliff[1]; Jang, Wonhee[1]; Kochergin, Andrey[1]; Lopez, John[1]; Maglott, Donna[1]; Meric, Peter[1]; Pruitt, Kim[1]; Raina, Anjana[1]; Resenchuk, Sergey[1]; Rotmistrovsky, Kirill[1]; Church, Deanna[1]; Schuler, Greg[1]; Tatusova, Tatiana[1]
[1] National Center for Biotechnology, U.S. National Library of Medicine, National Institutes of Health, 8600 Rockville Pike, Bethesda, MD 20894

Plant genomics is a simple expansion of the scope of genomics at the National Center for Biotechnology Information (NCBI). In addition to the tools for storage of and analysis of nucleotide sequence such as, respectively, GenBank and BLAST, genomics at NCBI includes databases that enable 1) monitoring the progress of genome sequencing projects (Entrez Genome Projects), 2) datamining of probes (Entrez Probes), 3) datamining of primer sequences (UniSTS), and 4) viewing genome units (MapViewer). These standalone tools are enhanced at NCBI by the capability to move among these and other databases as the data associations dictate. The pan-organism resources are supplemented by plant-specific resources: plant text search, PlantBLAST, and plant-EST BLAST. PlantBLAST provides organism-specific databases composed solely of the accessions associated with mapped loci visible through MapViewer. EST-BLAST provides plant-specific databases composed solely of the ESTs from those plants with more than 50,000 ESTs. The scope of plants with maps - both sequence-based and nonsequence-based - visible in MapViewer has been increased from 10 to 26 organisms. The scope of organisms with mapped probes has increased from 10 to 32. This expanded scope of data will be used in examples of the developing capabilities of the genomic resources for plants at NCBI.

T18

A Maize (E)-beta-Caryophyllene Synthase Takes Part in Defense Against Herbivores Above and Belowground

(submitted by Joerg Degenhardt <degenhardt@ice.mpg.de>)
Full Author List: Degenhardt, Joerg[1]; Koellner, Tobias G.[1]; Held, Matthias[2]; Lenk, Claudia[1]; Hiltpold, Ivan[2]; Turlings, Ted C.J.[2]; Gershenzon, Jonathan[1]
[1] Max Planck Institute for Chemical Ecology, Hans-Knoll-Strasse 8, Jena, Germany D-07745
[2] University of Neuchtel, Institute of Zoology, Neuchtel, Switzerland CH-2007

Maize under attack by arthropod herbivores releases complex blends of volatiles that attract natural enemies of herbivores. This interaction can benefit the plant and was therefore termed "indirect defense". One of the maize volatiles released after herbivore attack, the sesquiterpene (E)-beta-caryophyllene, is emitted by leaves in response to attack by lepidopteran larvae like Spodoptera littoralis and released from roots after damage by larvae of the beetle Diabrotica virgifera virgifera. We demonstrate that (E)-beta-caryophyllene can serve as an attractant for the respective natural enemies of the herbivores. The parasitic wasp Cotesia marginiventris, which lays eggs in lepidopteran larvae feeding on the leaves, can learn to use (E)-beta-caryophyllene to locate plants with larvae and foraging entomophatogenic nematodes in the soil are attracted to (E)-beta-caryophyllene and thus locate and infest the D. v. virgifera larvae damaging maize roots. We identified a maize terpene synthase, TPS23, which produces this (E)-beta-caryophyllene signal both in the leaves and the roots. The expression of TPS23 is controlled on the transcript level and induced by S. littoralis damage aboveground and D. v. virgifera damage belowground. The amino acid sequence of TPS23 is highly conserved among maize lines and species of teosinte, the closest wild relatives of maize. However, most North American maize lines do not express TPS23 and do not release (E)-beta-caryophyllene, whereas European lines and the wild maize ancestor, teosinte, readily do so in response to D. v. virgifera. We argue that the (E)-beta-caryophyllene defense signal was lost during domestication and breeding of the American lines and might reduce the resistance of American maize varieties towards the agronomically important maize pest D. v. virgifera.

T19

crw1 - A Novel Maize Mutant Exceptionally Susceptible to Western Corn Rootworm

(submitted by Braham Dhillon <bdhillon@purdue.edu>)
Full Author List: Dhillon, Braham[1]; Moose, Stephen P.[2]; Johal, Gurmukh S.[1]
[1] Department of Botany & Plant Pathology, Purdue University, West Lafayette, IN, USA 47906
[2] Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA 61801

We have found a unique mutant of maize that exhibits altered responses to insect herbivores. This mutant, which we have named crw1 (for corn root worm susceptibility), was discovered in an Ac-active family as a recessive segregant whose leaves were completely chewed up by an otherwise benign beetle, Diabrotica vergifera vergifera LeConte. Normally this beetle eats only maize pollen and silks, thus posing no threat to the rest of the plant on its own. However its larva - the Western Corn Rootworm (WCR) - is the most devastating pest of maize, causing billions of dollars of damage annually in the form of insecticide costs and yield losses. The phenotype of crw1 is WCR-dependent; not only is the foliage of crw1 at risk when WCR is around, its roots are also severely damaged, causing crw1 mutants to stay small and stunted. In the absence of WCR, crw1 resembles wild-type (WT) siblings in all aspects of growth, development and reproduction. Nevertheless, compared to WT plants, the cell wall of crw1 is altered as inferred from staining with Toluidine Blue O and UV-autofluorescence. There is also an indication that one of the lipoxygenase pathways may have come under aberrant control in crw1. The recessive loss-of-function nature of crw1 suggests that it may be defective in a mechanism(s) that normally deters WCR feeding. Although the nature of this mechanism remains unknown, we have cloned the gene underlying crw1 and it appears to encode a transcription factor unique to plants. Consistent with its role in insect herbivory, Crw1 undergoes a marked increase in expression in a quick and transient fashion following wounding induced either physically or by WCR feeding.

T20

Estimation and Validation of QTL for Early Vigour of Maize Grown in Chilly Environments

(submitted by Hartwig H. Geiger <geigerhh@uni-hohenheim.de>)
Full Author List: Geiger, Hartwig H.[1]; Presterl, Thomas[1]; Wilde, Katinka[1]; Ouzunova, Milena[2]; Moeller, Evelyn M.[1]; Ernst, Karin[3]; Westhoff, Peter[3]
[1] Institute for Plant Breeding, Seed Science, and Population Genetics (350), University of Hohenheim, Stuttgart, Germany 70593
[2] KWS Seed AG, Einbeck, Germany 37555
[3] Department of Development and Molecular Biology, Heinrich-Heine-University Dusseldorf, Dusseldorf, Germany 40225

Maize is particularly sensitive to chilling in the early growth stages. Our aim was to identify and validate QTL controlling variation for early vigour in the juvenile plant stage. Validation consisted of marker-assisted selection, near-isogenic line (NIL) testing, and a candidate-gene approach. A mapping population of 720 doubled-haploid (DH) lines was derived from a cross between two dent inbred lines contrasting in early vigour. The genetic map comprised 188 SSR markers. The DH lines per se and their testcrosses with a flint line were evaluated in field experiments across 11 Central European environments. Plants were harvested in the juvenile stage to assess fresh matter yield as a criterion of early vigour. Composite interval mapping detected seven QTL for line and ten for testcross performance explaining 64% and 49% of the genetic variance. Four QTL for line performance co-localised with QTL for testcross performance. Significant QTL x environment interaction was observed, but no relationship existed between the size of the QTL effects and the mean temperature in the individual environments. For leaf chlorosis, leaf purpling, and frost damage, seven, six, and five QTL were detected, respectively. Some of them co-localised with QTL for fresh matter yield. Marker-based selection was applied to a DH-line population derived from a selected fraction of the mapping population. These new lines were field tested in eight environments. Averaged across six QTL, freshmatter yield increased by 0.46% per positive QTL allele. Three sets of NILs were established for three QTL on chromosomes 4, 5, and 6. BC3 lines were tested in six environments. Results generally confirmed the QTL effects. Various putative candidate genes were detected by SSH or were retrieved from public data bases. In total, 56 genes were mapped in the DH-line population. Thereof, 15 genes are located in the above three major QTL intervals.

T21

Genetic Control of Milo Disease Resistance in Sorghum: An NBS-LRR Gene Corrupted by a Fungal Pathogen

(submitted by Ervin Nagy <dnagye@uga.edu>)
Full Author List: Nagy, Ervin D[1]; Bennetzen, Jeffrey L.[1]
[1] University of Georgia, Genetics Department, Athens, GA, 30602

Milo disease in sorghum is caused by isolates of the soil-borne fungus Periconia circinata that produce PC-toxin. Susceptibility to milo disease is conditioned by a single, semi-dominant gene, termed Pc. The susceptible allele (Pc) converts to a resistant form (pc) spontaneously at a gametic frequency of 10-3 to 10-4. The genomic region including the Pc locus was mapped and cloned. Sequence analysis of this region revealed twelve gene candidates. Several of the predicted genes in the region are homologous to disease resistance loci, including one NBS-LRR resistance gene analogue that is present in multiple tandem copies. Analysis of 13 pc isolines derived from Pc/Pc sorghum revealed unequal recombination and gene conversion events, which removed or truncated one particular member of this NBS-LRR gene family resulting in the loss of susceptibility to the milo disease. The data suggest that the necrotrophic fungus Periconia circinata utilizes the PC-toxin to activate a disease resistance gene, and the resultant hyper-sensitive necrosis allows enhanced fungal infection and proliferation.

T22

An ACT-like Domain Participates in the Dimerization of Several Plant Basic-helix-loop-helix Transcription Factors

(submitted by Antje Feller <feller.11@osu.edu>)
Full Author List: Feller, Antje[1]; Hernandez, Marcela[2]; Grotewold, Erich[3]
[1] Molecular, Cellular, Developmental Biology Program, The Ohio State University
[2] Ohio State Biochemistry Program (currently at Dept. of Veterinary Biosciences), The Ohio State University
[3] Dept. of Plant Cellular and Molecular Biology and Plant Biotechnology Center, The Ohio State University, Columbus, OH 43210

One of the largest transcription factor families in plants is characterized by the presence of a basic-helix-loop-helix (bHLH) domain. The first protein identified containing such a bHLH motif was the maize R gene product, which functions as an essential coactivator of the MYB domain protein C1 for the activation of maize anthocyanin biosynthetic genes. The interaction of these two proteins occurs through the N-terminal region of R and the MYB domain of C1. In addition to the bHLH and the MYB interacting domains, we have identified a homodimerization region in R C-terminal to the bHLH. We show here that this protein-protein interaction domain is necessary for the transcriptional activity of R, and that it is most likely required for stabilizing the binding of C1 to the C1-binding sites in its target promoters.

We also established that this dimerization region has a structural similarity to ACT domains present in several metabolic enzymes. Structure analyses revealed the presence of this domain in several other plant bHLH proteins and we determined by yeast two-hybrid experiments that the presence of an ACT domain correlates with dimerization. The possible participation of this domain in other R-regulated functions will be discussed.

T23

Robustness of Carbohydrate Metabolism in Maize Kernels

(submitted by Gertraud Spielbauer <gspiel@wzw.tum.de>)
Full Author List: Spielbauer, Gertraud[1]; Margl, Lilla[1]; Hannah, L. Curtis[3]; Bacher, Adelbert[2]; Gierl, Alfons[1]; Eisenreich, Wolfgang[2]; Genschel, Ulrich[1]
[1] Lehrstuhl fur Genetik, Technische Universitat Munchen, 85350 Freising, Germany
[2] Lehrstuhl fur Organische Chemie und Biochemie, Technische Universitat Munchen, 85747 Garching, Germany
[3] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611

The central carbohydrate metabolism provides the precursors for the syntheses of various storage products in seeds. While the underlying biochemical map is well established, little is known about the organization and flexibility of carbohydrate metabolic fluxes in the face of changing biosynthetic demands or other perturbations. This question was addressed in developing kernels of maize (Zea mays L.), a model system for the study of starch and sugar metabolism. Developing maize kernels were grown in sterile culture and supplied with either [U-13C6]glucose or [U-13C12]sucrose at specific intervals during kernel development. 13C labeling experiments were carried out with inbred lines, heterotic hybrids, and starch-deficient mutants that were selected to cover a wide range of performances and kernel phenotypes. In total, 46 independent labeling experiments on 19 different genotypes were carried out. Carbohydrate flux distributions were estimated based on glucose isotopologue abundances, which were determined in hydrolysates of starch by using 13C NMR spectroscopy and GC-MS. Similar glucose isotopologue distributions in all samples indicated robustness of carbohydrate fluxes in maize kernels.

T24

Physical Mapping of the Whole Maize Genome via Single Molecule Analysis

(submitted by Shiguo Zhou <szhou@wisc.edu>)
Full Author List: Zhou, Shiguo[1]; Bechner, Mike[1]; Potamousis, Konstantinos[1]; Pape, Louise[1]; Churas, Chris[1]; Lamers, Casey[1]; Runnheim, Rod[1]; Goldstein, Steve[1]; Forrest, Dan[1]; Wing, Rod[1]; Valouev, Anton[1]; Nguyen, John[1]; Waterman, Michael[1]; Schwartz, David[1]
[1] Laboratory for Molecular and Computational Genomics,Department of Chemistry, Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI 53706
[2] Department of Plant Sciences, Arizona Genomics Institute, Unversity of Arizona,Tucson, AZ 85721
[3] Departments of Mathematics, Biology, and Computer Science, University of Southern California, Los Angles, CA 90089

Maize is one of the most important crops in the U. S., and a classical plant genetic and cytogenetic model system. Although genetic and cytogenetic analyses have provided a global view of the genome organization of maize, significant insights will emerge from the complete knowledge and comprehensive annotation of its sequence. As part of this effort, we are constructing a genome-wide restriction map for use as a scaffold for sequence assembly and finishing. The maize genome is notorious for harboring a very complex and extensive panoply of repeats likely to confound traditional sequence assembly approaches. To efficiently tackle such genomic elements, the ordered restriction map we are constructing and other physical or genetic maps will collectively guide difficult sequence assemblies and gap closure operations. Our map construction approach is based on "shotgun optical mapping," which maps hundreds of thousands of individual genomic DNA molecules, and assembles these single molecule maps into contigs that span entire genomes. Although our map assembly approach uses ordered restriction maps created from individual DNA molecules, the map "assembler" algorithm shares many functionalities with software commonly used for shotgun sequence assembly. To date, our mapping progress has been very good: approximately 927.6 Gb of raw genomic DNA single molecule maps have been acquired (371 x genome coverage), and there are now 84 map contigs larger than 4 Mb, spanning 88% of the maize genome. Our current efforts are focused on the completion of this map, the enablement of effective linkages to the maize genetic and FPC maps, and help groups charged with sequencing and assembly. Finally, the rapid establishment of this physical map for maize will provide a new means for studies focusing on the comparative genomics of other maize isolates, or species.

T25

Maize Artificial Chromosomes

(submitted by Weichang Yu <wy593@mizzou.edu>)
Full Author List: Yu, Weichang[1]; Birchler, James A.[1]
[1] Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211

Artificial chromosomes offer opportunities for better genetic engineering of crops than is possible with current plant transformation practices. They can be used as independent platforms for foreign gene expression without random integration into the normal chromosomes. Consequently, genes introduced to the artificial chromosomes do not mutate host genes and avoid the possibility of insertion into inactive regions of the genome. They will also allow unlimited amounts of DNA to be integrated at one site in a sequential manner. Hence, additional genes, multi-gene complexes, or even whole metabolic pathways can be added to the plant genotype. Moreover, artificial chromosomes can be easily removed from a genotype by genetic crosses. We recently developed a technology to produce artificial chromosomes in maize by telomere-mediated chromosomal truncation. Transformation of plasmids with telomere sequences at one end caused the deletion of terminal sequences leaving only the centromeric region in some cases. This method of artificial chromosome production does not rely on cloning centromere sequences and thus is applicable to any plant species. Maize minichromosomes have been developed from the truncations of both A and B chromosomes. Both types of minichromosomes are transmissible from generation to generation at reasonable frequencies. Reporter genes on the minichromosomes are expressed as assayed in various tissues. The transgenes at the terminus of the mini-chromosomes include a Cre/lox site-specific recombination site, and are able to accept additional genetic material using this site-specific recombination system. Thus, additional gene expression cassettes have the potential to be introduced. This artificial chromosome technology should have many applications in plant genetic engineering and agriculture.

T26

Genetics, Genomics and Evolution of the Maize B73 Genome

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

Full Author List: Wei, Fusheng[1]; Coe, Ed[2]; Nelson, William[4]; Bharti, Arvind K.[5]; Engler, Fred[4]; He, Ruifeng[1]; McMullen, Michael[2]; Davis, Georgia L.[2]; Paterson, Andrew H.[6]; Schaeffer, Mary L.[2]; Gardiner, Jack M.[2]; Cone, Karen C.[7]; Messing, Joachim[5]; Soderlund, Carol[4]; Currie, Jennifer[1]; Collura, Kristi[1]; Yu, Yeisoo[1]; The Maize Genome Sequencing Consortium [1,8,9,10]; Wing, Rod A.[1]

[1] Arizona Genomics Institute, Department of Plant Sciences, BIO5 Institute, University of Arizona, Tucson, AZ 85721
[2] Division of Plant Sciences, University of Missouri, Columbia, MO 65211
[3] Plant Genetics Research Unit, Agricultural Research Service, United States Department of Agriculture, Columbia, MO 65211
[4] Arizona Genomics Computational Laboratory, BIO5 Institute, University of Arizona, Tucson, AZ 85721
[5] Plant Genome Initiative at Rutgers, Waksman Institute, Rutgers, The State University of New Jersey, Piscataway, NJ 08854
[6] Plant Genome Mapping Laboratory, Departments of Crop and Soil Science, Plant Biology, and Genetics, University of Georgia, Athens, GA 30602
[7] Division of Biological Sciences, University of Missouri, Columbia, MO 65211
[8] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724
[9] Iowa State University, Ames, IA 50010
[10] Genome Sequencing Center, Washington University School of Medicine, St. Louis, MO 63108

Maize (Zea mays L.) is one of the most important cereal crops and a model for the study of genetics, evolution, and domestication. To better understand maize genome organization and build a framework for genome sequencing, we constructed a sequence ready fingerprinted contig (FPC)-based physical map with 550 contigs. The map covers 93.5% of the genome, of which 86.1% is aligned to the genetic map. The FPC map contains 25,908 genic markers that enabled us to align nearly 73% of the anchored maize genome to the rice genome. The distribution pattern of cDNAs correlates well to that of genetic recombination. In collinear regions, 1 kb in rice corresponds to an average of 3.2 kb in maize, yet maize has a 6-fold genome size expansion. This can be explained by the fact that most rice regions correspond to two regions in maize as a result of its recent polyploid origin. Inversions account for the majority of chromosome structural variations during subsequent maize diploidization. We also find clear evidence of ancient genome duplication predating the divergence of the progenitors of maize and rice. Reconstructing the paleoethnobotany of the maize genome indicates that the progenitors of modern maize contained 10 chromosomes. To sequence the maize genome, we are using the physical map to select a minimum tiling path of BAC clones and are sequencing each clone to approximately a 6-fold coverage. All low copy gene space is then finished. At present we have deposited over 5,800 sequenced BACs in GenBank, and have another 5,000 clones in the pipeline.

T27

Using Mathematically-Defined Repeats to Annotate the Maize Genome

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

Full Author List: Narechania, Apurva[1]; Stein, Joshua[1]; Pasternak, Shiran[1]; Kurtz, Stefan[2]; Ware, Doreen H.[3]; The, Maize Genome Sequencing Consortium[4]

[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[2] Center for Bioinformatics, University of Hamburg, Germany
[3] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; and USDA-ARS NAA Plant, Soil & Nutrition Laboratory Research Unit, Ithaca, NY 14853
[4] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; Arizona Genomics Institute, University of Arizona, Tucson, AZ 85721; Iowa State University, Ames, IA 50011; and Genome Sequencing Center, Washington University, St. Louis, MO 63108

A substantial portion of the maize genome, estimated at 60-70%, is repetitive, comprising mostly retrotransposable elements that inserted within the past 6 million years. Such high repeat content will present challenges to the efficient sequencing, assembly, and annotation of the maize genome. Since there is no guarantee that current libraries of manually-curated repeats are comprehensive, one of the primary challenges will be to identify novel repeats as the project progresses. We are investigating an approach based on mathematically defined repeats that provides user control over desired repeat thresholds and does not require a pre-established library. Using the 0.45 X whole genome shotgun sequence prepared by the DOE Joint Genome Institute as a relatively non-biased representation of the maize genome, we computed the frequency of all constituent k-mers. With this index, we annotated/masked sequenced BAC clones with respect to their repetitive content. We demonstrate that this method is highly effective at identifying sequences harboring known classes of retroelements and distinguishing these from low-copy genic regions regardless of the maize strain assessed. In addition to supporting BAC sequence annotation, the k-mer method can also be used to guide sequence finishing projects by focusing attention on relatively non-repetitive regions, quantifying relative genomic complexity and repetitiveness across the grasses, and evaluating restriction enzymes in maize genome reduction strategies. This study provides a proof-of-concept for the detection of repetitive sequence in the absence of annotated repeat databases, and should therefore prove useful in the future sequencing projects of novel genomes. This work was funded by the NSF/DOE/USDA "Sequencing The Maize Genome" project (NSF #0527192).

T28

An Ultra-Conserved Exon-Skipping Event in Plant Transcription Factor IIIA Gene Suggests the Regulation of 5S rRNA Transcription via Alternative Splicing

(submitted by Yan Fu <yfu@danforthcenter.org>)
Full Author List: Fu, Yan[1]; Chen, Hao[1]; Qi, Zhi[1]; Barbazuk, W. Brad[1]
[1] Donald Danforth Plant Science Center, St. Louis, MO 63132, USA

Using the genome and transcriptome data of Arabidopsis, rice and maize, we performed a genome-wide comparative analysis to identify evolutionarily conserved alternative splicing events that are potentially functionally significant. A conserved exon-skipping event associated with transcription factor IIIA (TFIIIA) for the transcription of 5S rRNA gene was discovered. This event is conserved from moss, perhaps the first multicellular eukaryote, to other higher flowering plants. The most conserved genomic region of TFIIIA is located within the skipped exon. This exon contains a predicted RNA secondary structure, which may play a regulatory role, and inclusion of this exon presumably results in a short truncated protein. The expression of TFIIIA is highly correlated with the expression of components essential to RNA processing and protein synthesis. Taken together, we proposed a regulatory model of 5S rRNA transcription via alternative splicing of TFIIIA in plants. Consistent with this model, the ratio of exon-included isoform to exon-skipped isoform is significantly increased in the seedlings subjected to cycloheximde and ABA treatments that inhibit protein synthesis.

T29

Sequencing the Maize (B73) Genome

(submitted by Richard McCombie <mccombie@cshl.edu>)
Full Author List: The Maize Genome Sequencing Consortium[1,2,3,4]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
[2] Arizona Genomics Institute, University of Arizona, Tucson, AZ 85721
[3] Iowa State University, Ames, IA 50011
[4] Genome Sequencing Center, Washington University School of Medicine, St. Louis, MO 63108

The effort to sequence the Zea mays BT73 genome is underway. The objective of the project is to provide the complete sequence and structures of all maize genes and their locations on both the genetic and physical maps of maize. The gene space will be of finished quality, estimates of gaps between genes and draft representations of the repetitive sequences will be provided when possible, and the sequence will be fully integrated with the genetic and physical maps. Annotation will include gene models, predicted exon/intron structure, incorporation of EST and full-length cDNA data, gene ontology, and relationship with homologs in other organisms, including but not limited to other sequenced plant genomes. These data will be coordinated with existing maize community and comparative databases with the eventual goal of generating complete curation of the genomic sequences to a standard set by established model organism databases. The sequence of the maize genespace and its linkage to the rich genetic and phenotypic data available in that organism will be a valuable asset in understanding the functional properties of the cereal genome and its encoded proteins and biological networks.

T30

Regulatory Circuits in Maize Embryo Development: ABA, GA and the ROP GTPases

(submitted by Carol Rivin <rivinc@science.oregonstate.edu>)
Full Author List: Carroll, Kirstin A.[1]; Kulhanek, Doris[1]; Fowler, John[1]; Rivin, Carol[1]
[1] Oregon State University, Dept. Botany And Plant Pathology, Corvallis, OR 97331-2902

ABA positively regulates maturation phase genes and suppresses vivipary in developing maize embryos. ABA-mutant kernels are viviparous and desiccation-intolerant. However, embryos deficient in both ABA and GA exhibit almost wild-type phenotypes, suggesting that the growth and maturation of the maize embryo, including changes in gene expression, are regulated by ABA-GA antagonism. Type II ROP GTPases may also play a role in maize embryo maturation, as these have been shown to negatively regulate ABA signaling in Arabidopsis. We are using multiply-mutant lines to examine the behavior and gene expression of embryos deficient in two or more of these signal systems. Using microarrays (U. Arizona) and qRT-PCR, we are comparing gene expression in wildtype, ABA-, GA-, and ABA-/GA- mutant embryos at pre-maturation and early maturation phases. These analyses strongly support the hypothesis of ABA/GA antagonism: known maturation genes are much more highly expressed in the ABA-/GA- double mutant than in ABA- mutants. Initial microarray analyses also revealed a variety of genes not known to be ABA or maturation-related that show this same pattern. Overall, 89% of genes found to be differentially expressed between WT and ABA- sibling embryos were also significantly different in ABA-/GA- vs ABA- comparisons. From our initial results, embryo expression of the ROP genes showed unexpected patterns. Of the four rop genes tested, three showed expression increases in both GA- and/or ABA-/GA- samples. This result does not fit the simple model in which GA and ROP both act as negative regulators of ABA signaling. To identify additional components of the hypothesized ABA-GA signaling circuit, we have screened for new EMS mutants that cause vivipary and dessication intolerance in ABA-/GA- seeds (i.e. restoration of the ABA- phenotype), and have identified several candidate lines that appear to segregate for these traits.

T31

3' UTR Profiling Resolves Expression Variation of Near-Identical Transcripts in Developing Maize Ovaries

(submitted by Andrea Eveland <aeveland@ufl.edu>)
Full Author List: Eveland, Andrea L[1]; Kirst, Matias[2]; Latshaw, Susan P.[1]; Avigne, Wayne T.[1]; McCarty, Donald R.[1]; Koch, Karen E.[1]
[1] Horticultural Sciences Department; UF Genetics Institute: University of Florida; Gainesville, FL, 32611
[2] School of Forest Resources and Conservation; UF Genetics Institute; University of Florida; Gainesville, FL, 32611

The specificity and informatic power of 3' UTRs is here harnessed in a novel approach to transcriptome profiling. We describe a modified method of oligo-dT primed cDNA synthesis that biotinylates poly-A tails and thus allows a 3'-anchoring strategy to sequence-based profiles using 454 technology. Specific adaptors, each encoding a unique 4-base recognition key, were ligated to digested cDNAs. This enabled multiplexing of individual samples (up to 48 possible per "run"). By specifically targeting the 3' UTR of expressed sequences, quantitative resolution was achieved for expression variation of Near-Identical Transcripts (NITs). These can include specific alleles, paralogs, or gene-family members. To test this application, we compared immature maize ovary libraries from W22 and an ABA insensitive mutant (W22 background) under drought stress. Multiplexing yielded 228,595 high quality reads including 105,289 and 109,958 reads from wild-type and mutant samples, respectively. Quantitative expression profiles were generated for 14,822 unique 3'-anchored consensus sequences represented by 2 to 2,500 reads (mRNA abundance inferred from read frequency). Of these, 182 showed significant differences ($p<0.0001$) in the number of reads derived from the two libraries. Read frequencies of two closely related consensus sequences (annotated Auxin Repressed Dormancy Associated) indicated opposite responses in wild-type and mutant. BLASTN aligned them in tandem to a single cDNA, however comparison to gene-enriched maize genomic assemblies (MAGIs) revealed that the two ARDA sequences were encoded by paralogous genes. One carried a 14 base indel that created a marker, allowing identification and quantification of differential expression for two ARDA Near-Identical Transcripts. We further investigated the potential for 454-based 3'-UTR profiling in global transcriptome analysis by multiplexing 12 maize ovary samples representing 4 stages of development (from pre-silking to fertilization). Resolution of expression variation between NITs and un-annotated sequences based on this 3'-anchored profiling approach provided an invaluable complement to parallel microarray analyses.

T32

The *tasselseed4* and *Corngrass1* MicroRNAs of Maize Target Domestication Loci

(submitted by George Chuck <gchuck@nature.berkeley.edu>)
Full Author List: Chuck, George[1]; Cigan, Mark[2]; Meeley, Bob[2]; Irish, Erin[3]; Sakai, Hajime[4]; Hake, Sarah[1]
[1] Plant Gene Expression Center/U.C. Berkeley, Albany, CA
[2] Pioneer Hi-Bred International, Johnston, IA
[3] University of Iowa, Iowa City, IA
[4] DuPont Crop Genetics, Wilmington, DE

The retention of juvenile traits in the adult reproductive phase characterizes a condition known as neoteny, and abundant speculation exists over whether this has contributed to the evolution of new species. The dominant heterochronic Corngrass1 (Cg1) mutant of maize was considered a neotenic mutation that displays phenotypes that may have been present in the grass-like ancestors of maize. We cloned Cg1 through a combination of positional cloning and activation tagging and found that it encodes a tandem microRNA gene that is over expressed in the meristem and lateral organs. Moreover, one of the targets of Cg1 is a gene known to have played a role in the domestication of maize from its ancestor teosinte.

The recessive tasselseed4 (ts4) mutant of maize displays a change in meristem determinacy as well as a conversion of the normally male flowers in the tassel to female flowers. We cloned the ts4 gene by chromosome walking and found that it also encodes a microRNA. Concurrent with this work, we chromosome walked to a new dominant Tasselseed gene that when mutated is phenotypically similar to ts4. We discovered that this tassel seed is in fact a target of ts4 with an altered microRNA binding site. In other grasses this target gene has been shown to function in the control of inflorescence architecture and flowering time, both critical traits that have been selected for during domestication.

The Corngrass1 and tasselseed4 microRNAs appear to be conversely regulated at different time points during development. We provide evidence that the relative balance between these two microRNAs is important for phase transitions.

T33

Anther Development: Transcriptome Profiling of Fertile and Male-Sterile Anthers

(submitted by Virginia Walbot <walbot@stanford.edu>)
Full Author List: Walbot, Virginia[1]; Ma, Jiong[1]; Morrow, Darren J.[1]; Duncan, David S.[1]; Fernandes, John[1]
[1] Department of Biological Sciences, Stanford University, Stanford CA USA 94305-5020

Intact spikelets containing anthers of less than 0.5 mm plus upper floret anthers at the 1.0 mm (rapid mitotic phase of development) and the 1.5 mm (final mitotic divisions before meiosis starts)were dissected from ms23, msca1, and mac1 for comparison to stage-matched fertile siblings. These materials were in hybrid backgrounds. Transcriptome profiling was performed on a custom 22K Agilent in situ-synthesized microarray. In addition, for the W23 inbred line, these 3 stages were dissected plus 2.0 mm (mid-meiosis), 4 mm (exit from meiosis), 5 mm (maturation phase), and mature pollen for a more comprehensive analysis of the anther transcriptome on a 44K Agilent array. Although the anther contains only 5 cell types in the locules, the 1.0 and 1.5 mm stages express nearly 24,000 distinct transcript types. The progression of transcript changes was analyzed with regard to questions such as: does lineage or late cell interaction determine pre-meiotic fate? are msca1 anthers a homeotic conversion of anther to leaf? are genes expressed during meiosis? how similar is the anther transcriptome before and after meiosis? when do transcripts found in pollen appear in developing anthers? Because the mutants are missing specific cell types, we also analyzed our data to search for putative cell-type specific gene expression patterns. Pilot experiments have also been conducted in collaboration with Zac Cande, Lisa Harper, and Rachel Wang to define the transition into meiosis by profiling the ameiotic1-ref (mitosis rather than meiosis in the microsporocyte) and ameiotic1-pra (early meiotic arrest)alleles. Supported by a grant from the NSF.

T34

The Role of *ZmPIN1* Genes in Kernel Development

(submitted by Cristian Forestan <cristian.forestan@unipd.it>)
Full Author List: Forestan, Cristian[1]; Carraro, Nicola[1]; Canova, Sabrina[1]; Varotto, Serena[1]
[1] Department of Environmental Agronomy and Crop Production - University of Padova; Viale delle Universiti 16, 35020 Legnaro; Padova - Italy

In Angiosperms seed development requires the co-ordinated expression of embryo and endosperm genes and relies on the interaction between the two seed components and between the seed and the maternal tissues. The large reservoir of auxin conjugates deposited into the developing maize endosperm has been suggested to be involved in these interactions, leading to polar embryo development. In Arabidopsis a apical-basal auxin gradient, established by PIN7, triggers the specification of apical embryo structures, whereas, the subsequent PIN1 polar localization, reorganizes the auxin gradient to specify the basal root pole. To verify whether this model also applies for monocots, we investigated on the behavior of *ZmPIN1* genes and auxin accumulation patterns during maize kernel development. We identified a novel putative ortholog of *AtPIN1*, *ZmPIN1c* that encodes a putative protein showing 80% of amino acid identity with *ZmPIN1a* and *ZmPIN1b*. RT-PCR experiments demonstrated that *ZmPIN1* genes show differential expression patterns during kernel formation. *In situ* hybridization assays with *ZmPIN1a* and *ZmPIN1c* specific probes were use to localize *PIN1* transcripts during endosperm development. Immunolocalization assays, using an anti-AtPIN1 antibody, show that in maize the PIN1 proteins are not polarized both in BETL and ESR domains of the endosperm but are polarized in the embryo during embryogenesis. The embryonic SAM is characterized by a central group of cells presenting a polarized PIN1 in a way that suggests auxin fluxes spreading at 360 degrees. In the embryonic root the antibody suggests acropetal auxin fluxes directed towards the RAM. To better describe the auxin fluxes during kernel development we are performing immunolocalization experiments using an anti-IAA antibody. To study the role of auxin polar fluxes in kernel and embryo development we are analyzing also the *defective endosperm-B18* maize mutant, that shows reduced levels of IAA in the endosperm leading to a reduced dry matter accumulation.

T35

Zmhcf136 is a Mesophyll Defective Mutant that Lacks a Photosystem II Complex

(submitted by Sarah Covshoff <sc349@cornell.edu>)
Full Author List: Covshoff, Sarah[1]; Majeran, Wojciech P.[2]; Liu, Peng[3]; Kolkman, Judith[4]; van Wijk, Klaas[2]; Brutnell, Thomas P.[1]
[1] Boyce Thompson Institute for Plant Research; Cornell University; Ithaca, NY 14853
[2] Department of Plant Biology; Cornell University; Ithaca, NY 14853
[3] Department of Statistics; Iowa State University; Ames, IA 50011
[4] Department of Plant Pathology; Cornell University; Ithaca, NY 14853

In maize, C4 photosynthetic activities are partitioned between two morphologically and physiologically distinct cell types, the bundle sheath (B) and the mesophyll (M). To dissect photosynthetic differentiation in maize, we characterized a novel M defective mutant, hcf136. In wildtype plants, RNA gel blots of separated B and M cells show M-specific transcript accumulation of Hcf136, allowing a unique opportunity to define C4 biology in an M perturbed background. Both Hcf136 transcript and protein fail to accumulate in the mutant. Strong protein sequence similarity between ZmHCF136 and its homologues in other species suggests that its function as a photosystem II assembly or stability factor is conserved in maize. Fv/Fm measurements confirm the absence of PSII activity in the mutant, and electron microscopy shows agranal M plastids. 2D-Blue Native-PAGE reveals an absence of the monomeric and oligomeric forms of PSII and rearrangements in oligomeric assemblies of LHCII and LHCI proteins. We utilized oligonucleotide microarrays to globally define transcript profile changes in the B and M cells of Zmhcf136. M cells were isolated by enzymatic digestion of leaf tissues, and B strands were mechanically separated. Six biological replicates of M and B cell isolates from wildtype and hcf136 tissues were compared in a cell-type specific manner. In the mutant M cell, 4181 genes are up-regulated and 3196 genes are down-regulated. In the mutant B cell, 875 genes are up-regulated and 794 are down-regulated. Unexpectedly, 65% of plastid-encoded genes show a change in expression pattern. Interestingly, most gene expression changes in both M and B cells are subtle despite a clear disruption of PSII complex formation and the loss of linear electron transport.

T36

Toward Introducing C4 Photosynthesis to C3 Oat by Use of Oat x Maize Addition Lines

(submitted by Matthew Walch <walch023@umn.edu>)

Full Author List: Kowles, Richard V.[2]; Minnerath, Jeanne M.[2]; Walch, Matthew D.[1]; Bernacchi, Carl J.[3]; Stec, Adrian O.[1]; Rines, Howard W.[4]; Phillips, Ronald L.[1]

[1] Department of Agronomy and Plant Genetics, Microbial and Plant Genomics Institute, University of Minnesota, 1991 Upper Buford Circle, St. Paul, MN 55108

[2] Department of Biology, St. Mary's University, 700 Terrace Heights, Winona, MN 55987

[3] Center for Atmospheric Sciences, Illinois State Water Survey, 2204 Griffith Drive, Champaign, IL 61822

[4] USDA-ARS and Department of Agronomy and Plant Genetics, University of Minnesota, 1991 Upper Buford Circle, St. Paul, MN 55108

Stable Oat x Maize Addition (OMA) lines consisting of a single maize (Zea mays L.) chromosome added to the entire oat (Avena sativa L.) genome have been recovered for each of the ten maize chromosomes. The presence of individual maize chromosomes has been associated with several novel phenotypes in the resulting OMA lines, and the effect on photosynthesis of the C3 oat from the addition of a C4 maize chromosome has been investigated. C4-associated phosphoenolpyruvate carboxylase (PEPc) and pyruvate orthophosphate dikinase (PPDK) were previously mapped to maize chromosomes 9 and 6, respectively. Northern blots showed expression of maize C4-specific PEPc in leaf tissue of OMA 9 plants and PPDK in the leaf tissue of OMA 6 plants. Western immunoblotting using anti-PEPc and anti-PPDK polyclonal antibodies on leaf protein extracts showed the presence of PEPc and PPDK enzymes in OMA 9 and 6 lines. Indirect immunocytological tests using monoclonal antibodies on microtomed leaf tissue showed the presence of PEPc and PPDK in the mesophyll cells of OMA 9 and 6 lines. Enzyme assays of leaf protein extracts showed increased activity of PEPc and PPDK with OMA 9 and 6 lines. Because C4 photosynthesis is a pathway mediated by several enzymes, crosses were made between OMA chromosome 9 and OMA chromosome 6 lines to combine PEPc and PPDK in a single oat plant. Northern blots showed expression of both PEPc and PPDK in the F1. CO2 compensation points of OMA 6 and OMA 9 lines were comparable to normal oat, while the CO2 compensation point of the 6 + 9 combination was significantly lower than normal oat, but still more like C3 photosynthesis than C4 photosynthesis.

Posters

P1

Red Aleurone1 of Zea mays is Regulated by Transcription Factors that are Required for Anthocyanin and Phlobaphene Biosynthesis

(submitted by Mandeep Sharma <mxs781@psu.edu>)
Full Author List: Sharma, Mandeep[1]; Chopra, Surinder[1]
[1] department of crop and soil sciences, ASI bldg, pennsylvania state university, university park, PA, USA 16802

Anthocyanins, flavonones, flavones and phlobaphenes are produced through the flavonoid biosynthesis pathway. Flavonoid 3' hydroxylase (F3'H) is a cytochrome P450 dependent enzyme that plays an important role in generating metabolite diversity by adding a hydroxyl group at 3' position of the B-ring. It was demonstrated earlier that functional red aleurone1 (pr1) locus may encode for F3'H enzyme in maize. Here, we show that f3'h is regulated by transcription factors of anthocyanin as well as phlobaphene biosynthetic pathways in maize. To study the role of f3'h in different branches of flavonoid biosynthesis, a putative f3'h sequence was isolated from maize. Genetic complementation of pr1 using BMS cell line, germinating maize kernels and Arabidopsis thaliana tt7 mutant confirmed that the isolated sequence encodes for a functional F3'H protein. We provide molecular and biochemical evidences that mutation in functional pr1 locus leads to accumulation of the red colored pelargonidin pigment instead of the purple colored cyanidin in aleurone tissue. Additionally, we show that in a segreagating population, light red colored apiferol as compared to dark red luteoforol accumulates in the cob tissue of mutant pr1 maize ears. Expression of c1, r1 and f3'h along with other genes in the anthocyanin biosynthetic pathway was observed in silk, husk and aleurone tissues in plants carrying functional pr1. f3'h transcript was not detected in mutant pr1 plants. Similarly, lines carrying functional p1 gene shows the expression of f3'h while lines carrying recessive p1 alleles did not show f3'h expression. Additional molecular data showing the possible lesion in the pr1 mutant allele under study will be presented.

P2

Tie-dyed1 Acts and is Expressed in Veins

(submitted by R. Frank Baker <rfb11@psu.edu>)
Full Author List: Baker, R. Frank[1]; Braun, David M.[1]
[1] Pennsylvania State University, University Park, PA 16802

The tie-dyed1 (tdy1) mutation causes a yellow and green sectoring pattern in maize leaves. Green sectors display the attributes of wild type leaf tissue, while yellow sectors exhibit excessive carbohydrate levels. tdy1 yellow sectors form within a limited time period when the leaf is emerging from the whorl, and sector boundaries remain fixed once formed. Studies of these sector boundaries showed that lateral veins, the largest class of veins in the maize leaf, frequently coincide with the yellow-green border, implicating a role for veins in limiting sector formation. Results from a clonal mosaic analysis experiment indicated that Tdy1 acts in the innermost leaf layer to regulate the activity of a non-cell-autonomous signal, the accumulation of which results in a region of tissue forming a yellow sector. Based on these studies, we hypothesize that Tdy1 acts specifically in the veins to up-regulate sucrose export in response to high sugar levels, thereby preventing the buildup of excess sugars and transformation of a region of tissue into a yellow sector. The Tdy1 gene has been cloned and encodes a novel protein of unknown function. Consistent with the results from our mosaic analysis, preliminary RNA in situ hybridizations detected Tdy1 mRNA specifically localized to phloem cells. These data support the hypothesis that Tdy1 functions in veins to condition export capacity in emerging leaf tissue.

P3

Tie-dyed1 and *Sucrose export defective1* Function in Different Pathways to Regulate Carbohydrate Partitioning

(submitted by Yi Ma <yum105@psu.edu>)
Full Author List: Ma, Yi[1]; Baker, R. Frank[1]; Braun, David M.[1]
[1] Pennsylvania State University, University Park, PA 16802

tie-dyed1 (tdy1) is a recessive maize mutant developing non-clonal yellow and green sectors in leaf blades. We found that yellow sectors accumulate much more carbohydrates than green sectors or wild type. sucrose export defective1 (sxd1) is another recessive maize mutant with non-clonal leaf sectors containing elevated carbohydrate levels. In sxd1 mutants, callose is deposited over the plasmodesmata (PD) between bundle sheath and vascular parenchyma cells in leaf minor veins, which is proposed to prevent loading sucrose into veins. Sxd1 encodes tocopherol cyclase, an enzyme that functions in vitamin E biosynthesis. Given the phenotypic similarities in leaf sectoring and carbohydrate accumulation between the two mutants, we investigated whether Tdy1 and Sxd1 function in the same genetic pathway. To test this, we generated F2 families segregating both mutants, and we 1) characterized the phenotypes of F2 plants; 2) biochemically quantified their chlorophyll, carbohydrate and vitamin E levels; and 3) microscopically examined callose deposition and PD ultrastructure in the mutants. Double mutant leaves showed reduced green sectors as well as significantly less pigment in yellow tissues, while tdy1 and sxd1 yellow tissues contained similar amounts. These data suggest an additive interaction between the two mutants. However, double mutant yellow tissues did not accumulate more carbohydrate than the single mutants, which might be explained by carbohydrate saturation in these tissues. Upon microscopy investigation, we did not observe callose deposition or any PD alteration in tdy1 plants, suggesting tdy1 accumulates carbohydrates due to a different defect than sxd1. Finally, tdy1 plants synthesize vitamin E indicating Tdy1 does not function in the same biochemical pathway as Sxd1. Our results indicate that tdy1 and sxd1 mutants accumulate carbohydrates by different mechanisms and function in distinct genetic pathways regulating carbon partitioning in leaves.

P4

ZmbZIP1: A Maize Transcription Factor Regulated by *Opaque-2*

(submitted by Marie Hasenstein <mahaas@iastate.edu>)
Full Author List: Hasenstein, Marie A[1]; Jia, Hongwu[2]; Scott, M. Paul[3]
[1] Interdepartmental Genetics; Iowa State University; Ames, IA 50011
[2] Monsanto; Ankeny, IA 50021
[3] USDA-ARS; Ames, IA 50011

The recessive maize mutation opaque-2 (o2) is responsible for decreasing the level of certain zein seed storage proteins which are present in maize endosperm. It has been a valuable mutation in maize ever since it was recognized to increase the level of two essential amino acids, lysine and tryptophan, in maize kernels. However, the opaque-2 mutation introduces several deleterious phenotypes into the maize plant as well, such as an increased susceptibility to pests and an alteration in metabolic capacity. In an effort to identify other components of the Opaque-2 transcriptional regulation system, a transcript profiling microarray was performed comparing B45 and B45o2 endosperm tissue at four developmental stages. From this microarray, a gene we call ZmbZIP1 was identified as being significantly downregulated in B45o2 endosperm. ZmbZIP1 is an Opaque-2 like basic leucine rich zipper (bZIP) protein. This type of protein typically acts as a transcription factor. Interestingly, two Opaque-2 binding sites are present in the promoter region of ZmbZIP1, which may suggest that it and Opaque-2 are part of a larger transcriptional regulatory network. ZmbZIP1 has been shown to be expressed in both mature and immature maize leaves, roots, endosperm, cob and pericarp, while being notably absent in embryonic tissue. We are exploring the possibility that ZmbZIP1 might play the role of a transcriptional regulator in maize tissues.

P5

A Non-Destructive Screen for Maize Kernel Mutants Affecting Seed Composition and Weight

(submitted by Mark Settles <settles@ufl.edu>)
Full Author List: Settles, A. Mark[1]; Armstrong, Paul[2]; Baye, Tesfaye[1]; Casella, George[1]; Pearson, Tom[2]
[1] University of Florida, Gainesville, FL
[2] USDA-ARS, Manhattan, KS

Cereal yield and grain composition are two important targets for improving food security and reducing the environmental impact of agriculture. Seed weight as well as protein, oil, and starch composition have a strong genetic component in maize. We are using single-kernel near infrared reflectance spectroscopy (NIR) to observe genetic segregation of composition mutants. Importantly, single-kernel NIR is non-destructive allowing individual seeds to be phenotyped by NIR and tested for inheritance. We have shown that single-kernel spectra can be used to differentiate between mutant kernels with composition defects and their normal siblings. Moreover, single-kernel NIR in conjunction with individual seed weights can predict kernel composition. We built a single kernel analyzer that allows high-throughput collection of kernel weights and NIR data. We have also developed a novel statistical test to identify families segregating for seed composition mutants. These resources will be used to automate a large-scale screen of the UniformMu transposon-tagging population for dosage-sensitive mutants. Dosage-effect genes show stepwise changes as the number of normal alleles increases instead of the dominant-recessive relationship found for most genes. We hypothesize that these genes will be amenable for engineering cereal crops with desired grain traits.

P6

A Small-Kernel Phenotype for the *sorbitol dehydrogenase-1* Mutant

(submitted by Sylvia de Sousa <smsousa@ufl.edu>)
Full Author List: de Sousa, Sylvia M.[1]; Hunter III, Charles T.[1]; Ankumah, Nana[1]; Avigne, Wayne T.[1]; Koch, Karen E.[1]
[1] Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL, USA, 32611

Sucrose first arriving at the kernel can be cleaved by either invertase or the reversible sucrose synthase reaction. In either case, fructose is a product. Fructose is typically phosphorylated by hexokinase, and then used for respiration or polysaccharide biosynthesis. Alternatively, fructose may follow a less well-known fate in some tissues, where it can be converted to sorbitol by sorbitol dehydrogenase (SDH). This enzyme is highly active in maize endosperm, but since kernels do not store sorbitol, an intermediary role is likely. To help test hypotheses for the significance of sorbitol dehydrogenase in maize, we screened the UniformMu maize population and identified an sdh1mutant (the sole gene for SDH in maize or rice genomes). Since the UniformMu population is highly inbred, the wild type material provides a uniform set of controls for functional analysis of the mutants. The sdh1 mutation reduced maximal SDH activity in developing kernel to less than 6% of that in wild type. Quantification of the small-kernel phenotype as dried-seed weight showed reductions ranging from 11-30%, with a mean of 21% for first-round field analyses (significant to p

P7

Biochemical Analysis of Centromere Protein C (CENP-C)

(submitted by Yaqing Du <yadu@plantbio.uga.edu>)
Full Author List: Du, Yaqing[1]; Shi, Jinghua[1]; Dawe, R. Kelly[2]
[1] Department of Plant Biology, University of Georgia, Athens, GA 30602
[2] Departments of Plant Biology and Genetics, University of Georgia, Athens, GA 30602

Centromeric DNAs are highly repetitive and non-conserved, making it difficult to understand how centromeres maintain their interactions with kinetochore proteins. Currently the best candidate for the protein that mediates this interaction is Centromere Protein C (CENP-C). Our data show that maize CENP-C is a DNA binding protein, and further, that DNA binding activity is localized to a small exon duplication. The same exon duplication region binds to RNA. Our currently model is that RNA facilitates the binding of CENPC to centromeric repeats.

P8

Characterization Of A Novel Dominant Defective Kernal Mutant from UniformMu

(submitted by Donald McCarty <drm@ufl.edu>)
Full Author List: Latshaw, Susan P.[1]; Davidson, Jennifer[1]; McCarty, Donald R.[1]
[1] Horticultural Sciences Department; University of Florida; Gainesville, FL 32611

UniformMu is a large scale inbred and pedigreed Mutator transposon population. We have isolated over 2000 independent seed mutants from this population and have analyzed Mu-flanking sequences from a subset of 130 diverse seed mutants. One line, 01S0473-04 that segregated both a small kernel (smk) and a defective kernel (dek) phenotype was analyzed by backcrossing to the W22 inbred used as a recurrent parent in construction of UniformMu. In the first back-cross generation, apparently dominant embryo lethal (emb) or dek phenotypes were observed in crosses where 01S0473-04 was used a pollen parent; whereas, ears of the reciprocal hybrid cross produced all normal seed. In the segregating ears, ratios of wildtype and mutant kernels ranged from 2:1 to 4:1. Subsequent generations revealed that the factor conditioning the dominant phenotype is transmitted through both the male and the female gametes, however, when transmission occurred through the female, the phenotype was recovered only in pedigrees that included an intermediate self-pollination. We are testing two hypotheses that may explain this unusual pattern of inheritance: 1) an epigenetic phenomenon associated with Mu activity, 2) and/or a mutation that affects imprinting of an essential gene that is activated by transmission through the male. At least two distinct genetic factors have been identified in the line: one yields the dominant phenotype and the other is an emb with Mendelian inheritance. This line was also chosen for MuTAIL cloning and TIR-direct 454 sequencing, as described earlier. Several candidate genes are being traced through multiple generations.

P9

Characterization of *camouflage1*, a Maize Mutant in the Chlorophyll Synthesis Pathway, Indicates the Presence of a Functional Homolog

(submitted by Mingshu Huang <muh147@psu.edu>)
Full Author List: Huang, Mingshu[1]; Braun, David M.[1]
[1] Pennsylvania State University, University Park, PA 16802

camouflage1 (cf1) is a recessive mutant of maize which exhibits a zebra banding pattern with alternating yellow-green and green leaf sectors. This banding pattern is dependent on light/dark cycling; plants grown in continuous light do not develop the cf1 phenotype. Fluorescent light microscopy of cf1 leaf tissues reveals that bundle sheath cells in the yellow-green sector are specifically affected and undergo cell death, while the same cell type in the green sector remains healthy. Interestingly, in double mutant analyses between cf1 and mutants containing leaf tissues with defective chloroplasts such as striate2, bundle sheath cells in albino tissues do not die in the cf1 sectors. This result illustrates that functional chloroplasts are required for the bundle sheath cell-specific death in cf1 mutants. The cf1 gene was cloned via Mutator (Mu) transposon tagging and encodes porphobilinogen deaminase (PBGD), a protein functioning in chlorophyll and heme biosynthesis. In the cf1-m2 mutant allele, the transposon Mu1 is inserted into the 5' UTR, just a few base pairs downstream of the transcriptional start site. No transcript was detected in cf1-m2 mutant leaves, indicating that there should be no functional CF1 protein present in the mutant. However, enzyme activity assays reveal reduced but detectable PBGD activity in mutant plants, which implies the presence of a duplicate gene. We probed a maize B73 BAC library with a cf1 cDNA fragment, and detected hybridization to multiple consecutive clones on chromosome 4 in addition to cf1, which is located on chromosome 5. Further investigations of the duplicate gene and biochemical characterization of the CF1 protein are underway.

P10

Control of Cell Division and Cell Expansion in Gradients in Developing Maize Leaves

(submitted by Dorothy Tuthill <dtuthill@uwyo.edu>)
Full Author List: Tuthill, Dorothy[1]; Fang, Xin[1]; Obuya, James[1]; Sylvester, Anne[1]
[1] Department of Molecular Biology, University of Wyoming, Laramie WY 82071

Cell division and expansion is compartmentalized during maize leaf growth so that rapidly dividing cells are confined to the base of the blade above the ligule. Expansion and differentiation progress in a gradient toward the leaf tip. This distribution of cell growth is reflected in regularly changing patterns of cells, as division slows and expansion increases toward the leaf tip. Gradients in cell cycle control are thus expected. We are identifying sets of genes that co-express at positions in the leaf corresponding to changes in the division/expansion gradient, taking a multi-pronged approach. First, we identified Rab2A1 (warty) that is differentially expressed, along with the homolog Rab1A, in both an apical to basal gradient and in subgradients in growing leaves. These findings are consistent with the function of Rabs, which are highly conserved proteins known to be involved in vesicle trafficking in all eukaryotes and we are testing the hypothesis that Rab2A1 may be transporting wall secretory vesicles. Next, we screened a panel of 20 maize genes that are known or predicted to encode proteins involved in cell cycle processes. Of these, we identified a cdk1, histone1, cyclinA and retinoblastoma that express differentially in the basal to apical gradient. Altered expression of the marker genes in cell division mutants (tangled) and cell expansion mutants (warty) suggests either direct or, more likely, indirect effects of Tan1 and Rab2A1 on cell cycle regulation. Third, microarray experiments are being designed to profile transcription in discrete leaf zones, using the identified markers as controls. Preliminary results of expression patterns in leaves of different developmental stages supports our hypothesis that subgradients within the broader base to tip gradient can be identified.

P11

Crop Production Situation Report in the Federal Capital Territory - Where Are We and How Did We Get Here?

(submitted by Michael Oke <agriclinkcooperative@yahoo.com>)
Full Author List: Adedotun, Michael[1]
[1] Agric-Link Multipurpose Cooperative Society Limited, P.O. Box 11611, Garki Federal Capital Territory Abuja

In the rural areas of the Federal Capital territory namely Gwagalada, Yaba,Kuje, Abaji,Kwali,farmers are greatly noted in the cultivation of different crops such as groundnut,Mellon, Maize,cassava, Beans etc. However there are various problems associated into the crop production to the extent that these affected the citizen and it is such a great problem to the food security in the country.

Another striking findings about the report is that deadly diseases mention Fungi, bacteria witly and as threaten the agricultural livelihood security, reducing the farming productivity, workforce and these alter the agricultural partices in some of the area notable in Kuje area council in Abuja

The paper discuss the farmers participation in the crop production, propagation , problems related to diseases control, caring practices, sanitary environment, feedback on the success stories of some peasant farmers in the Federal Capital Territory and the success stories has provided a useful guide for the setting higher standards,in the farm management helping the farmers in reducing the effect of diseases in the farming communities of the federal capital territory Abuja.

In addition to these a noticeable improvement in the cultivation of crops in the Federal Capital territory,in which international countries can learn in some of these experiences gathered during the report , how they can can contribute in the development of the agricultural sector in Nigeria.

P12

Discovery of High Copy Number Genomic Repeats Using the Assisted Automated Assembler of Repeat Families (A.A.A.R.F.) Algorithm

(submitted by Jeremy DeBarry <jdebarry@uga.edu>)
Full Author List: DeBarry, Jeremy D.[1]; Liu, Renyi[2]; Bennetzen, Jeffrey L.[1]
[1] Department of Genetics; University of Georgia; Athens, GA, USA 30602
[2] Department of Biochemistry and Molecular Biophysics; The University of Arizona; Tucson, AZ, USA 85721

Because of the large numbers of Transposable Elements (TEs) present in many eukaryotic genomes, and the effects that these elements can have on their hosts, there is much interest in learning more about the repeat content of a genome. Traditional methods of TE investigation were limited to chance discovery, or resulted from the identification of repetitive elements that altered the phenotype of the host. Current methods for the investigation of a genome's repeat content require the use of assembled genome sequence data. We present the A.A.A.R.F algorithm for the de novo identification, assembly and classification of high copy number Long Terminal Repeat (LTR) retrotransposon (LRP) families from a set of sample sequences generated for a target organism. The ability to classify the high copy number LRP families using a sample sequencing approach offers the opportunity to investigate a wide array of previously uncharacterized genomes. Further, the characterization of LRPs from many organisms will allow investigation of the commonalities and differences in genome organization that evolved in specific lineages. In this way, important information about the genome organization of many organisms can be obtained in a fast, efficient and cost-effective manner.

P13

Elucidation of Loci Influencing Near-Infrared Reflectance Spectra of Maize Grain Using the IBM Population

(submitted by Jessica Ponder <jnp465@truman.edu>)
Full Author List: Ponder, Jessica N.[1]; Campbell, Mark[2]; Scott, M. Paul[3]; Duvick, Susan A.[4]; Rocheford, Torbert R.[5]; Bonney, Courtney E.[1]
[1] Department of Biology and Chemistry; Truman State University; Kirksville, MO 63501
[2] Department of Agronomy; Truman State University; Kirksville, MO 63501
[3] USDA-ARS; G212 Agron; Ames, IA 50011
[4] Department of Agronomy; Iowa State University; Ames, IA 50011
[5] Department of Crop Sciences; University of Illinois at Urbana-Champaign, Urbana, IL 61801
[6] Department of Physics; Truman State University, Kirksville, MO 63501

Investigation into the specific loci that affect grain chemical composition may lead to useful information needed for improving quality traits in maize grain. Near-infrared (NIR) spectroscopy, a rapid quantitative method commonly used for determining grain components such as protein, starch, and oil, is influenced by many other chemical and physical properties of the grain as well. With this in mind, our objective was to integrate spectral and molecular marker data from the IBM mapping population to identify loci that influence NIR reflectance spectra, which might lead to the identification of novel genes that dictate grain composition. A multivariate discriminate analysis program was implemented to determine whether NIR reflectance spectra of ground seed could be used to consistently predict the presence of alleles at specific loci in the IBM 94 population. Several regions of interest have already been identified. For example, the locus csu696, found on the long arm of chromosome 1, was found to be positively correlated to spectra generated from ground seed produced each year from 2001 through 2004 (r = 0.63, 0.27, 0.32, and 0.65, respectively). Studies are currently underway to investigate the chemical basis for these allele-dependent spectral variations.

P14

Expression of Several Cytokinin (CK) Genes and Levels of CK Hormones in Developing Seeds of *miniature1* (*mn1*) Seed Mutation Relative to the *Mn1* Are Altered

(submitted by Prem Chourey <pschourey@ifas.ufl.edu>)
Full Author List: Rijavec, Tomaz[1]; Kovac, Maja[2]; Dermastia, Marina[3]; Chourey, Prem[4]
[1] University of Ljubljana, Ljubljana, Slovenia
[2] National Institute of Biology, Ljubljana, Slovenia
[3] University of Ljubljana, Ljubljana, Slovenia and National Institute of Biology, Ljubljana, Slovenia
[4] USDA-ARS & University of Florida, Gainesville, FL, USA

Developing seeds are a rich source of two major hormones, auxin and CK. Unlike auxins, CKs constitute a family of diverse compounds with little or no knowledge of their specific physiological functions in the plant. Zeatin - the first CK to be discovered was isolated from maize, notably from developing kernels that have the highest levels of it of all plant tissues. CKs are critical to normal cell divisions, and are also implicated in endoreduplication and in regulating sink strength. We obtained a comparative developmental transcript profile by q-PCR of nearly a dozen CK genes, and preliminary data on levels of several major CKs (specifically, Zeatin, Zeatin-Riboside, Z-9-Glucoside and isopentenyladenosine, iPR)), by immunochromatography and HPLC to better understand possible role of sugar status in CK homeostasis in developing seeds. The mn1 mutant is of interest because it shows pleiotropic changes both at cellular and metabolic levels due to a loss of the Mn1-encoded cell wall invertase that controls the flux of sucrose in developing endosperm. Major qualitative and quantitative changes were detectable in Z and ZR levels in a spatial fashion at 12 and 16 DAP; and, higher levels of iPR persisted in the mn1 relative to the Mn1 at 20 DAP. As for the CK gene transcripts, most notable changes occurred during 8 to 12 DAP stages that coincide with degeneration of maternal nucellar tissue and rapid cell divisions in endosperm. Gene transcripts that showed the most dramatic changes include, (all Zm) N-Gt1 (N- glucosyltransferase and C-Zog (cis zeatin-specific-O-glucosyltransferase) that catalyze, respectively, irreversible N- and reversible O-specific glucosylations of CK, Ckx1 (cytokinin oxidase: degrades bioactive CK), IPT1 (isopentenyltransferase: catalyzes a rate limiting step in CK biosynthesis) and Hik1 (histidine kinase: a CK-receptor). Additional data from other developmental stages and possible significance of these results in sugar-hormone signaling will be discussed.

P15

Genes Encoding the Two Subunits of the ADP-glucose Pyrophosphorylase Evolve at Different Rates in Higher Plants, Yet the Genes Are Equally Sensitive to Activity-altering Missense Mutations When Expressed in E. coli

(submitted by Nikolaos Georgelis <gnick@ufl.edu>)
Full Author List: Georgelis, Nikolaos[1]; Braun, Edward[2]; Shaw, Janine[1]; Hannah, L. Curtis[1]
[1] University of Florida; Program in Plant Molecular and Cellular Biology and Horticultural Sciences; Gainesville, FL, 32611
[2] University of Florida; Department of Zoology; Gainesville, FL, 32611

The rate of protein evolution is generally thought to reflect, at least in part, the proportion of amino acids within the protein that are needed for function. In the case of ADP-glucose pyrophosphorylase (AGPase) this premise led to the hypothesis that AGPase small subunit is more conserved compared to the large subunit because a higher proportion of amino acids in the small subunit are required for enzyme activity compared to the large subunit.

Two approaches were used to test this hypothesis. Using a bacterial expression system, the probability that a random non-synonymous mutation altered AGPase activity was calculated for each subunit of the maize endosperm isoform. Both subunits exhibited the same probability. Second, functional large and small subunit genes from mutagenized populations were sequenced and the ratio of non-synonymous to synonymous mutations was calculated. This ratio was the same for both subunits. Results indicate that the small and the large subunit AGPase genes are equally predisposed to enzyme activity-altering non-synonymous mutations when expressed in one environment with a single complementary subunit.

As alternative hypotheses, we suggest that the small subunit exhibits more evolutionary constraints in planta than does the large subunit because it is less tissue specific, less redundant and must form functional enzyme complexes with different large subunits. Independent approaches provide data consistent with these alternative hypotheses.

P16

Genetic Control of Starch Digestion Properties and Starch Granule Architecture

(submitted by Clifford Weil <cweil@purdue.edu>)
Full Author List: Groth, Deborah[1]; Chernyshova, Alona[1]; Harper, Carla[1]; Widya, Yenny[2]; BeMiller, James[2]; Weil, Clifford F.[1]
[1] Agronomy Dept., Purdue University, West Lafayette, IN 47907 USA
[2] Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, IN 47907 USA

Carbohydrate research increasingly is focused on changing the biochemical nature of starch to create more efficient substrates for biofuel production, healthier foods for human consumption and more efficient livestock feed. A key factor in these processes is the rate at which starch is digested by amylases. Starch digestibility is influenced heavily by genetically controlled factors including starch granular and molecular structure and composition. We have identified maize mutant varieties with altered starch digestibility among segregating families of EMS-mutagenized maize, using our miniaturized, high-throughput single kernel preparation and starch digestion assay. In a preliminary screen of 465 families, we have identified 53 segregating mutants with slower rates of digestion compared to wild type, and 61 others with faster rates of digestion, suggesting there are several hundred genes overall genes overall that impact rates of starch digestion. We have also begun to characterize genes involved in controlling formation of protein-lined channels connecting the starch granule surface to the central cavity of the granule where digestion is initiated. In addition to starch biosynthetic proteins, these channels contain actin and tubulin, suggesting they are formed by invaginations of the amyloplast created by cytoskeletal elements as the amyloplast expands during starch granule formation. Characterizing the differences between B73 and Mo17 in relative degree of channelization using the IBM population suggests two major loci, on Chromosomes 2 and 3, and a number of minor loci that contribute to channelization. We have defined more dramatic differences among other inbred lines and will be using the NAM lines to map the loci involved and isolate the relevant genes.

P17

Genetic Control of the Carbon-Nitrogen Balance in the Leaves of IHP Plants

(submitted by Jeffrey Church <jbchurch@uiuc.edu>)
Full Author List: Church, Jeffrey B.[1]; Seebauer, Juliann R.[1]; Schneerman, Martha C.[1]; Below, Fredrick E.[1]; Moose, Stephen P.[1]
[1] Department of Crop Sciences; University of Illinois, Urbana-Champaign; Urbana, IL 61801-3873

Primary assimilation of nitrogen in higher plants is a complex process that often competes with other metabolic systems for resources derived from photosynthesis. In maize, optimal control of these reactions is especially important in the vegetative source tissues, which supply crucial resources to the developing kernels and are important regulators of high yield. We examined the effects of high (200 ppm) and low (14 ppm) N supply on the leaves of B73 and Illinois High Protein (IHP), a genotype that accumulates high levels of total plant N and the highest known concentrations of seed protein. To control N responses, we grew IHP and B73 plants to the 8-leaf stage in a previously developed hydroponics system. Initial characterizations confirmed changes in chlorophyll concentration, total photosynthesis, whole-plant N content, and total biomass, where IHP accumulated significantly more root and shoot N than B73, although B73 exhibited greater biomass at both N rates. Leaf samples from these plants are currently being characterized by microarray analyses, qPCR expression validations, enzyme assays, western blots and metabolite profiles. Specific qPCR characterizations correlated the observed physical responses with the altered transcription of genes related to the synthesis and catabolism of key amino acids and photosynthetic intermediates. Additional qPCR information on related genes will be presented, as well the diurnal responses and allelic polymorphisms of these genes, and the relevant gene lists generated from microarray analyses. Insights gained from these studies will be discussed in the context of current experiments examining metabolite profiles and enzyme activities, as well as similar responses of hybrids and inbreds grown in the field.

P18

Genome Organization and Structure of Maize miRNAs

(submitted by Lifang Zhang <zhangl@cshl.edu>)
Full Author List: Zhang, Lifang[1]; Maher, Christopher[1]; Musket, Theresa[2]; Davis, Georgia L.[2]; Ware, Doreen H.[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[2] Dept of Agronomy, Univ of Missouri, Columbia, MO 65211
[3] USDA ARS

microRNAs are small noncoding RNAs that regulating gene expression of genes involved in critical processes. To meet the increasing demand of cereals from a growing world population, a great emphasis has been placed on the agricultural community to maximize production. Over the last few decades a great deal of work has been placed on identifying protein-coding genes that control key agronomic traits. We are interested in studying the miRNA component of the genome and their role in improving crops yields, nutritional value, and resistance to insects and abiotic stresses. In order to accomplish these objectives we are focusing on the genome organization and structure of maize miRNAs.

Regions of conserved protein-coding gene order between cereals are believed to represent functionally important regions with potentially conserved phenotypes. Since many highly conserved miRNA gene families have been demonstrated to have similar functional roles across cereals we believe that miRNA are also likely to reside within regions of conserved gene order. In order to analyze the genome organization of miRNA genes, we physically mapped maize miRNA genes and looked to see if they reside in syntenic spans with rice. Overall, we identified 10 maize miRNAs that had a corresponding syntenic rice miRNA. The fact that these miRNAs maintained conserved physical locations following the divergence between rice and maize suggests their conserved, and critical, regulatory role.

Recent studies have shown that miRNA genes are transcribed by RNA polymerase-II (pol-II). Therefore, we are interested in characterizing the primary-miRNA (pri-miRNA) gene structure, transcription start sites (TSS), alternatively spliced transcripts, and polycistronic miRNA genes using 5' and 3' RACE. These results will enable promoter classification for computational prediction of cis-regulatory elements dictating spatiotemporal-specific expression. This work serves as a theoretical basis for germplasm enhancement through the manipulation of miRNA expression and regulation of their targets in cereals.

This work was funded by USDA

P19

Identification and Characterization of Selected Maize Cell Wall Mutants Generated in the UniformMu Population

(submitted by Brent O'Brien <bob2373@ufl.edu>)
Full Author List: O'Brien, Brent A.[1]; Koch, Karen E.[1]; Avigne, Wayne T.[1]; McCarty, Donald R.[1]; Carpita, Nicholas C.[3]; Latshaw, Susan P.[2]; Vermerris, Wilfred E.[1]; Settles, A. Mark[1]; Hannah, L. Curtis[1]
[1] Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL, 32611
[2] Horticultural Sciences Department, University of Florida, Gainesville, FL, 32611
[3] Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, 47907

The plant cell wall is an intricate composite of numerous carbohydrate-based compounds. Understanding how cell wall components are synthesized and integrated is essential to both plant biology, and efforts to utilize this renewable energy source in an efficient manner. This is especially true for the distinctive Type-II cell wall of maize and other grasses, which differs markedly from that of Arabidopsis and dicot species. For maize, the transposon-mutagenic Uniform Mu population allows new mutations to be identified, localized to a specific gene, and studied in a uniform, inbred background. Here, a database of randomly-sequenced Mu-flanks was used to identify maize lines carrying mutations in cell wall genes. In addition, plants with visible phenotypes were used to construct Mu-flank libraries for sequence-based identification of potentially causal Mu-inserts. Co-segregational analyses are being used to determine which lines produce a phenotype that could be attributed to a Mu insert in a cell wall gene. Although a number of these tests are in progress, we have thus far developed homozygous mutant lines for a cellulose synthase (CES) gene, a putative cytochrome-C synthase, and a polygalacturonase. These have yet to display phenotypes under field conditions, but more thorough appraisals are in progress. We have also characterized a unique "shredded" mutant with leaves that rip vertically along regions of apparently-brittle cell walls. It is especially interesting that some putative cell wall mutations also display an abnormal kernel phenotype. Understanding which cell wall genes are required for normal seed development will prove vital to future efforts to develop crops that maintain normal seed production while producing biomass that can be used efficiently

P20

Identification of Maize R2R3-MYB Factors Affecting the Lignin Biosynthesis Pathway

(submitted by Joan Rigau <rigau@ibmb.csic.es>)
Full Author List: Caparris-Ruiz, David[1]; Fornala, Silvia[1]; Capellades, Montserrat[1]; Puigdominech, Pere[1]; Rigau, Joan[1]
[1] Laboratori de Genetica Molecular Vegetal, Consorci CSIC-IRTA, Jordi Girona 18-26, 08034 Barcelona, Spain.

Lignification is a complex process common to all vascular plants. The accumulation of lignins in plant cell walls increases the strength and stiffness of fibres, improves the efficiency of water transport through the vascular system, and protects plants from pathogen attack.

Over the last few years, the regulation of some genes of the lignin biosynthetic pathway has been unraveled by the isolation of R2R3-MYB factors. At present a clear role in the repression of lignin biosynthesis has only been attributed to R2R3-MYB factors belonging to the subgroup 4.

In maize virtually nothing is known concerning the molecular mechanisms involved in the regulation of lignin genes. We showed that the maize COMT gene promoter drives the expression of GFP with the same specificity in Arabidopsis and maize, suggesting that the molecular mechanisms governing the COMT gene expression are at least partially conserved between such two evolutionary distant species. Therefore, we used the maize COMT as a target gene to identify factors acting as repressors of maize lignification. The examination of the maize COMT gene promoter revealed a putative ACIII box, suggesting the involvement of MYB factors in its gene expression regulation. Using degenerated primers from well known subgroup 4 R2R3-MYB factors we isolated five new maize R2R3-MYBs and generated A. thaliana lines over-expressing these factors to study their possible involvement in the regulation of COMT gene. We observed that only the ZmMYB31 and ZmMYB42 subgroup 4 factors down-regulate both the A. thaliana and the maize COMT genes. We showed that ZmMYB31 and ZmMYB42 regulate other genes of the lignin pathway and affect the lignin content of the A. thaliana transgenic plants. In addition, ZmMYB42 also represses other branches of the phenylpropanoid pathway such as the flavonoid and sinapate esters biosynthesis.

P21

Investigating *RAMOSA1* Interacting Proteins via Yeast Two Hybrid Analysis

(submitted by Xiang Yang <yangx@iastate.edu>)
Full Author List: Yang, Xiang[1]; Bortiri, Esteban[2]; Hake, Sarah[2]; Vollbrecht, Erik[1]
[1] Dept. of Genetics, Development and Cell Biology, Iowa State University, Ames, IA. 50010
[2] University of California, Berkeley, 800 Buchanan Street, Albany, CA. 94710

RAMOSA 1 (RA1) is a plant specific EPF protein with a Cys2-His2 zinc finger DNA binding domain and an EAR repression domain. In ra1-R strong mutants, both the tassel and the ear become more branched. In ra1 weak mutants, tassel branching is slightly increased and ears produce disordered rows. Besides ra1, two other ramosa genes (ra2 and ra3) have been identified to perform similar functions, and a ramosa pathway has been described to regulate meristem determinacy and therefore inflorescence architecture. Genetic and molecular experiments place ra1 downstream of both ra2, which encodes a LOB-domain protein, and ra3, which encodes a trehalose 6-phosphate phosphatase. To further elucidate the ramosa pathway and the mechanism of RA1 action, yeast two hybrid (Y2H) analysis was used to investigate the relationships between three RAMOSA proteins and to screen for RA1 interacting proteins from cDNA expression libraries. Several putative RA1-interacting proteins, including transcription factors, are being verified by bait-prey swap experiments in yeast and by in vitro pull-down experiments.

P22

Investigating Rubisco Bundle Sheath Chloroplast-Specific Accumulation of Rubisco: What are the Cell Type-Specific Regulatory Steps in *rbcL* Expression?

(submitted by David Stern <ds28@cornell.edu>)
Full Author List: Wostrikoff, Katia L.[1]; Clemente, Tom[2]; Stern, David B.[1]
[1] Boyce Thompson Inst., Cornell Univ., Tower Rd., Ithaca NY 14853
[2] Tower Rd.
[3] Plant Science Initiative, Dept. of Agronomy & Horticulture, N308 Beadle Center, Lincoln, NE 68588

Rubisco catalyzes CO_2 fixation in chloroplasts, which is the first step of the Benson-Calvin cycle. The Rubisco complex comprises two types of subunits: a large subunit (LS, 55 kDa), encoded by the chloroplast *rbcL* gene, and a small subunit (SS, 15 kDa), encoded by the nuclear *RBCS* multigene family. Being encoded in two genetic compartments, Rubisco subunits' expression and proper accumulation into an L_8S_8 stoichiometry require tight regulation. In tobacco and in the green alga *Chlamydomonas reinhardtii*, Rubisco stoichiometry relies both on proteolytic degradation of unassembled SS subunits and on the regulation of LS translation by the availability of its assembly partner, a phenomenon known as CES (Control by Epistasy of Synthesis).

In C4 plants, Rubisco accumulation is restricted to bundle sheath chloroplasts, leading to an increased efficiency of photosynthesis. Several regulatory pathways that could be involved in this differential accumulation have been previously characterized in maize. On the one hand, for example, *RBCS* gene expression is restricted to bundle sheath cells at the level of transcription. On the other hand, *rbcL* mRNA accumulation and translation are decreased in mesophyll cells. We have investigated whether Rubisco may be restricted to bundle sheath cells in maize due to differentially regulated assembly, either due to the absence of the SS itself, or due to the absence of an assembly factor. We have characterized transgenic lines expressing the *RBCS* transcript in both cell types and/or a nucleus-encoded, chloroplast-targeted, LS. Results suggest that Rubisco assembly itself is a differentially regulated step between bundle sheath and mesophyll chloroplasts.

P23

Molecular and Phenotypic Characterization of a Novel *brittle2* Allele in the Maize Kernel

(submitted by Peter Rogowsky <peter.rogowsky@ens-lyon.fr>)
Full Author List: Cossegal, Magalie[1]; Cassagnet, Hervanne[1]; Chambrier, Pierre[1]; Guyon, Virginie[2]; Perez, Pascual[2]; Balzergue, Sandrine[3]; Moing, Annick[4]; Rogowsky, Peter M[1]
[1] RDP, UMR879 INRA-CNRS-ENSL-UCBL, 46 allee d'Italie, F-69364 Lyon Cedex 07, France
[2] Biogemma SAS, 24 ave des Landais, F-63170 Aubiere, France
[3] URGV, UMR1165 INRA-CNRS-UEVE, 2 rue Gaston Cremieux, F-91057 Evry Cedex, France
[4] PBV, UMR619 INRA-UB1-UB2, 71 ave Edouard Bourlaux, F-33883 Villenave d'Ornon, France

In an effort to clone mutations responsible for defective kernel phenotypes in a Mutator induced mutant collection, we performed systematic AIMS reactions on bulked F2 and F4 segregants of 36 monogenic recessive mutants. Consistent differential bands between mutant and wildtype were detected in 13 mutants, and in mutant H2328 co-segregation was found between the Mutator insertion and the kernel phenotype. However, further genetic mapping and allelism tests demonstrated that the insertion revealed by the AIMS technique was not responsible of the phenotype, which was actually the result of another, closely linked Mutator insertion in exon 6 of the Brittle2 gene. Bt2 is one of three genes in the maize genome coding for the small subunit of ADP-glucose pyrophosphorylase (AGPase), a key enzyme in starch synthesis. RT-PCR experiments with gene-specific primers confirmed a predominant expression of Bt2 in endosperm, of Agpsemzm in embryo and of Agpslzm in leaf, but also revealed considerable additional expression in various tissues for all three genes. The phenotypic analysis showed that at 30 days after pollination (DAP) mutant kernels were plumper than wildtype ones, that the onset of kernel collapse took place between 31 and 35 DAP and that the number of starch grains was greatly reduced in the mutant endosperm but not the mutant embryo. To provide further insight into the impact of the bt2-H2328 mutation, the transcriptome and metabolome of wildtype and mutant kernels were assessed at mid-development (35 DAP). Hybridization of the 16 K Affymetrix array led the conclusion that not only the hexose but also the amino acid metabolism was strongly affected in bt2-H2328 kernels. Principal component analysis (PCA) of the NMR results confirmed a strong increase of sucrose in mutant kernels and revealed that the bt2-H2328 mutation did not affect exclusively the endosperm but also the embryo at the metabolic level.

P24

Role of a myb Transcription Factor in Induction of 3-deoxyanthocyanidin Phytoalexins and Defense

(submitted by Farag Ibraheem <fii100@psu.edu>)
Full Author List: Ibraheem, Farag[1]; Chopra, Surinder[1]
[1] Department of crop and soil sciences, The Pennsylvania State University, University Park, State college, PA, 16802

Phytoalexins are low molecular weight antimicrobial compounds that significantly contribute to plant's defense against pathogens. The ability of these compounds to contain fungal growth and development in plant tissues has generated considerable interests to engineer them in economically important crops. In response to pathogenic (Colletotrichum sublineolum) and non pathogenic fungi (Cochliobolus heterostrophus), sorghum plants produce phytoalexins of 3-deoxyanthocyanidin flavonoid type. These compounds are induced at the site of infection and include luteolinidin, apigeninidin, and their derivatives. The biosynthesis of these compounds is partially understood. These phytoalexins have structural similarities to flavan-4-ols which are precursors to the brick red phlobaphene pigments that accumulate in sorghum and maize seed pericarp and other tissues. We have previously cloned a sorghum myb transcription factor yellow seed1 (y1) which is orthologus to the maize pericarp color1 (p1). y1 and p1 control pholbaphene biosynthesis in sorghum and maize respectively and have a high degree of similarity in their coding regions but their regulatory regions are highly divergent. Unlike p1, y1 is expressed in leaf tissues and is induced by fungal infection. We have used tansposon-mediated mutagenesis to develop sorghum lines with mutations in y1 locus. In the current work, we have used these lines to understand biosynthesis of 3-deoxyanthocyanidin phytoalexins and phlobaphene in sorghum. Sorghum line expressing a functional y1 allele (Y1-rr-3; red pericarp, red glume) accumulated significantly higher amounts of sorghum phytoalexins and showed enhanced resistance against anthracnose fungus than the one that carried a null y1 allele. The signaling cascade that leads to, or is co-induced with, phytoalexin biosynthesis was further investigated by measuring time course accumulation of a number of global signaling compounds such as SA, JA, IAA, and ABA after fungal treatment. Further, the heterologus expression of sorghum y1 is being tested in maize and results indicate that y1 is active in maize leaf and other tissues as well. Our results suggest that y1 might be able to drive biosynthesis of 3-deoxyanthocyanidin phytoalexins and other phenolic compounds in maize leaves.

P25

The High Carotenoid Phenotype of the University of Guelph "Hi-C" Inbred Lines and the Y1 Locus

(submitted by Andrew Burt <aburt@uoguelph.ca>)
Full Author List: Burt, Andrew J.[1]; Smid, Matthew P.[1]; Gardner, Carrie-Anne M.[1]; Shelp, Barry J.[1]; Lee, Elizabeth A.[1]
[1] Department of Plant Agriculture, University of Guelph; Guelph, ON, Canada N1G 2W1

The University of Guelph "Hi-C" inbred lines, which result from an original set of crosses between exotic open-pollinated populations and adapted germplasm, exhibit a deep orange endosperm and high carotenoid accumulation in the seed. The thirty-four Hi-C lines exhibited a total carotenoid concentration of 35.4 to 88.3 microg(g dry wt)-1 in 2005 and 40.9 to 140.8 microg(g dry wt)-1 in 2006and carotenoid profiles that are best described as either high-lutein or high-zeaxanthin. Currently, the genetic bases for high carotenoid accumulation and the two discrete profile types are being investigated. Here, we report on the high carotenoid phenotype.

The Y1 locus is the region coding for the maize seed-specific phytoene synthase, the first dedicated step in carotenoid biosynthesis. Molecular fingerprinting of this region indicated that the Hi-C lines have a fixed exotic allele at the Y1 locus, although phylogenetic analysis revealed that the Hi-C lines cluster to their adapted, rather than exotic lineages. The high carotenoid phenotype is therefore associated with a presumably highly-functional exotic allele at the Y1 locus. However, crosses of Hi-C lines to yellow dent inbred lines demonstrated that the high carotenoid phenotype is recessive in some backgrounds; one possible explanation for thsi is that the high carotenoid phenotype is attributable to a non-functional allele downstream of zeaxanthin in the biosynthetic pathway. Other loci in the carotenoid pathway are being investigated in order to reconcile the genetic basis of the phenotype with its recessive inheritance and to determine if the exotic Y1 allele is solely responsible for the high carotenoid phenotype or one of multiple contributing loci.

P26

Viral-Induced Gene Silencing of Cellulose Synthase and Cellulose Synthase-like Genes in Barley Reveals a Tightly Controlled Gene Network for Cell Wall Biosynthesis

(submitted by Michael Held <maheld@purdue.edu>)
Full Author List: Held, Michael A[1]; Kessans, Sarah A[1]; Brandt, Amanda S.[2]; Yong, Weidong[1]; Scofield, Steven R.[2]; Carpita, Nicholas C.[1]
[1] Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907
[2] USDA-ARS Crop Production and Pest Control Research Unit, West Lafayette, IN 47907

Viral-induced gene silencing of members of the CesA/Csl gene superfamily in Hordeum vulgare cv. Blackhulless was performed using the barley stripe mosaic virus. Targets were designed for silencing the CesA, CslH, and CslF gene families, as well as a single CesA gene. Barley seedlings inoculated with engineered and control viruses were harvested at the onset of third leaf emergence. As a spatial and temporal marker of gene silencing, plants were co-inoculated with viruses targeting the phytoene desaturase gene family. Compared to controls, plants silenced for CesA genes were reduced in the ability to incorporate D-14C-Glc de novo into cellulose as well as mixed-linkage (1->3),(1->4)-beta-D-glucan. Expression of CesA genes were attenuated in these plants, as well as numerous other non-target cell wall glycosyltransferases, including members of the Csl and GT-8 families. Plants silenced for the CslF and CslH gene families were also reduced in the ability to incorporate D-14C-Glc into mixed-linkage (1->3),(1->4)-beta-D-glucan as well as cellulose. The data presented here demonstrate that the CesA genes of barley encode cellulose synthases and that silencing particular cell wall biosynthetic enzymes can have a pronounced affect on the expression of other related genes. Further, our data support the existence of a tightly-linked cell wall gene network, in which the expression of individual members share common control points.

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P27

A Computational Approach for Scoring Visually Observed Phenotypic Expression in Maize

(submitted by Chi-Ren Shyu <shyuc@missouri.edu>)
Full Author List: Green, Jason[1]; Shyu, Chi-Ren[1]
[1] Department of Computer Science, University of Missouri, Columbia, MO, USA 65211

Understanding the manifestation of a phenotype from an organism's genotype has long been a goal of scientists. While there has been some success in linking genotype to phenotypic expression, this success has generally been limited to measurable phenotypes, those for which expression can be measured explicitly using a ruler, scale, etc. There exist, however, many phenotypes for which direct measurement is not possible, e.g. disease resistance, but expression quantification is still desired, e.g. for QTL mapping. The typical method for overcoming this issue is to design a scoring rubric that maps scalar values to textual descriptions of phenotypic expression. Domain experts can then assign scores by matching overall expression to these textual descriptions. The accuracy of this approach suffers, however, as it is heavily dependent upon the consistency and objectiveness of the scorer as well as the granularity of the scoring rubric.

We propose a computational method that can provide objective and consistent expression scores from phenotypic images using such a baseline rubric. Our approach relies on a small set of ground-truth labeled expression images which are manually labeled using the rubric. The images to be scored are processed using a variety of image processing and computer vision algorithms with digital signatures outputted. To automatically score a phenotype image, the most visually similar labeled images are found, and the scores for these images are used in conjunction with their similarity measures to the unscored image to assign an expression score. The granularity of the scoring rubric is no longer a concern, as the scores generated may fall in between the predefined rubric scores. With more consistent and finer-grained scoring, it is thought that the number and accuracy of loci found from QTL mapping using these scores will be increased.

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P28

Ab initio Protein-Coding Gene Finding in Maize and Rice Genomes

(submitted by Yan Fu <yfu@danforthcenter.org>)
Full Author List: Fu, Yan[1]; Zimmermann, Robert[2]; Zhang, Chenhong[1]; Brent, Michael R.[2]; Barbazuk, W. Brad[1]
[1] Donald Danforth Plant Science Center, St. Louis, MO 63132, USA
[2] Washington University, St. Louis, MO 63130, USA

Maize genome sequence is the knowledge infrastructure for the next generation of plant molecular genetics and comparative genomics that will provide the foundation for improving maize and other cereal crops. The rice genome has been nearly completed and a large-scale effort to sequence the maize genome has commenced in 2006. To better annotate both monocot genomes it is essential to develop high-throughput computational tools to accurately predict protein-coding genes. A significant improvement in gene prediction accuracy has come from dual-genome prediction programs, such as TWINSCAN, which integrate traditional probability models like those underlying GENSCAN and FGENESH with information obtained from the alignments between two genomes. Here we describe an NSF funded project to improve ab initio protein-coding gene prediction in maize genome by optimizing TWINSCAN via the identification of a comprehensive “training set” of complete and annotated maize gene models. A similar strategy has been applied to rice gene model collection and gene prediction. We demonstrate here that the gene prediction performance by TWINSCAN for both maize and rice genes have been significantly improved.

P29

Accelerated Data Collection of Phenotypes and Development Improves Workflow Management

(submitted by Toni Kazic <toni@athe.rnet.missouri.edu>)
Full Author List: Melia-Hancock, Susan[1]; Kazic, Toni[2]
[1] University of Missouri; Division of Plant Sciences; Columbia, Missouri, USA 65211
[2] University of Missouri; Dept. of Computer Science; Columbia, Missouri, USA 65211

Collection of data in the field is inevitably constrained by time and the urgency of pollinations. Thus, automating data collection can increase the quality and volume of data one can obtain, and help manage tasks such as mutant scoring and pollinations. We barcode plants and use a simple Palm-type device (Symbol Technologies' SPT 1800) with a built-in scanner to collect a variety of observations. These include mutant scoring, current tassel staging, shoot and tassel bagging, and cutbacks. The data are then transferred to a spreadsheet for examination and planning the next day's work. Because each one includes the date and time of the observation, such records are particularly valuable for dynamic phenomena, such as the progress of shedding and tracking the kinetics of phenotype development. We illustrate the usefulness of such data with two examples: determining the most urgent pollinations and tracking the appearance of mutant phenotypes of Les genes. To facilitate this work, several Perl scripts that generate barcoded plant tags, labels, and harvest tags have been written. They take a comma-separated values file (.csv) from a spreadsheet or database, generate a barcode using the investigator's numerical genotype, and lay out the text in a PostScript file for printing as is or conversion to PDF prior to printing. These scripts rely on GNU barcode 0.98 and LaTeX, both freely available, can be easily tailored by individual investigators to suit their requirements, and are freely available.

P30

Annotation and Analysis of Global Gene Expression Studies: Creation of a Maize Shoot Apical Meristem Expression Database

(submitted by Diane Janick-Buckner <djb@truman.edu>)
Full Author List: Janick-Buckner, Diane[1]; Browning, Kate[1]; Fritz, Ashleigh[1]; Hoxha, Eneda[1]; Kamvar, Zhian[1]; Zhang, Xiaolan[2]; Ohtsu, Kazuhiro[3]; Schnable, Patrick S.[3]; Scanlon, Michael J.[4]; Beck, Jon[1]; Buckner, Brent[1]
[1] Truman State University, Kirksville, MO, 63501
[2] University of Georgia, Athens, GA, 30602
[3] Iowa State University, Ames, IA, 50011
[4] Cornell University, Ithaca, NY, 14850

As part of a larger project aimed at understanding the genes involved in shoot apical meristem function and leaf primordia formation, we have performed functional annotations of over 5,400 maize genes. These genes were identified as being differentially regulated in one of several microarray hybridization studies. We have created a database with a web-based interface to facilitate the functional annotation process, as well as to securely store our results (http://sam.truman.edu). Annotation of each differentially regulated gene includes the presentation and evaluation of BLASTn, BLASTx and InterProScan searches. Assigning a gene to a functional category was done following an evaluation of the scientific literature, as well as additional information found in a variety of databases including the Kyoto Encyclopedia of Genes and Genomes, European Bioinformatics Institute, The Arabidopsis Information Resource, and others. Information from these sources is stored in our annotator's notes section. The SAM database is searchable by gene name, accession, and keywords in the notes, which facilitate the analysis and interpretation of the microarray experiments. Evaluation and interpretation of these data is ongoing and representative examples will be presented.

P31

Bioinformatics Pipeline for Discovering Helitrons in the Maize Genome Database

(submitted by Guy Lima Jr. <galima@oakland.edu>)
Full Author List: Lima Jr., Guy A.[1]; Lal, Shailesh K.[2]; Singh, Gautam B.[1]
[1] Department of Computer Science and Engineering; Oakland University; Rochester, MI, USA 48309
[2] Department of Biological Sciences; Oakland University; Rochester, MI, USA 48309

Helitrons are believed to be highly abundant within the Maize genome. Since the current methods for Helitron discovery are time consuming, relatively few have been identified and mapped onto the maize genome. Existing methods necessitate using manual input, inspection, and verification of data via web-based tools. This in turn requires storage and manual pre-processing of intermediate results. These inefficiencies often make it impractical for discovering Maize Helitrons on a larger scale. A bioinformatics analysis pipeline for automating the discovery of previously unknown putative Helitrons in the Maize genome is implemented.

The analysis pipeline automates the Helitron discovery by utilizing a database to store the intermediate results in a format independent of the tools utilized. The processing begins by locating sites of the characteristic 3' and 5' termini of Helitrons by using the Basic Local Alignment and Search Tool (BLAST). The target databases containing the maize genome sequences are independently queried using a 20 bp termini at the 5'-end and a 30 bp termini at the 3'-end derived from the previously known Helitrons. Relevant hits are stored in a database as either potential 5' or 3' termini of a putative Helitron. A Helitron is hypothesized to exist when both a 3' and 5' termini are found in the proper orientation as reported by a model validation subsystem integrated within the analysis pipeline. Furthermore, upon identifying an instance of a putative Helitron the system ensures its uniqueness from all previously discovered Helitrons.

After the discovery of a unique Helitron is thus validated, the system catalogs the sequence and its associated data. It is then assigned a new name in accordance with the naming scheme adopted for Maize Helitrons. The newly discovered Helitron sequence and associated data is made publicly available at http://genomecluster.secs.oakland.edu/helitrons.

P32

Comparative Genome Analysis in Gramene

(submitted by William Spooner <whs@ebi.ac.uk>)
Full Author List: Spooner, William[1]; Wei, Sharon[1]; Zhao, Wei[1]; Hurwitz, Bonnie[1]; Liang, Chengzhi[1]; Stein, Lincoln D.[1]; Ware, Doreen H.[2]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, USA, NY11724
[2] USDA-ARS NAA Plant, Soil & Nutrition Laboratory Research Unit, USA

Gramene (http://www.gramene.org) is a curated, open-source, web-accessible data resource for genome analysis in the grasses.

With the sequenced genomes of rice, Arabidopsis and poplar in the public domain, the Sorghum assembly in preparation, and the sequencing of maize well underway, the multi-species comparative analysis of plant genomes has recently become feasible at both protein and genomic DNA levels. The number of plant species covered by whole-genome FingerPrint Contig (FPC) maps is also increasing; through mapping of marker and clone-end sequences to the sequenced genomes these maps form an important additional resource for analysis of genomic synteny.

Gramene uses 'Compara' (developed by the Ensembl project, http://www.ensembl.org) as its framework for comparative genome analysis. This consists of database schemas, data analysis pipelines, and visualisation tools. 'Compara' products include; gene trees (with associated orthologs/paralogs), whole-genome DNA alignmnents, and syntenic blocks.

Gene trees between rice, maize and Arabidopsis, and syntenic blocks between rice, maize and several wild rice species are currently available from the Gramene Genome Browser and BioMart query interfaces. Compara data for several further species will be added over the coming months.

The Gramene project is supported by National Science Foundation grant No. 0321685 and USDA ARS.

P33

Computational Tools from the Chromatin Consortium

(submitted by Nick Murphy <nick@ag.arizona.edu>)
Full Author List: Murphy, Nicholas J.[1]; McGinnis, Karen M.[1]; Chandler, Vicki L.[2]
[1] Plant Sciences; University of Arizona; Tucson, AZ 85721
[2] BIO5 Institute, Plant Sciences; University of Arizona; Tucson, AZ 85721

A number of computational tools have been developed to meet the needs of the Functional Genomics of Maize Chromatin Consortium. CCDB is the internal database and "electronic fieldbook" of the project, though its design is general enough to serve as a model for how to collect and organize data generated by any large-scale reverse genetics project. Features include support for data and users from multiple geographic locations, fine-grained access control lists for users, pedigree management and recursive genotype inference, extensive reporting capabilities such as a visualization of constructs propagating through descendant lines, seed inventory management, and integration with the Chromatin Consortium Web site. Its interface was implemented using Ruby on Rails, and the data is stored in a MySQL database. Other tools include Dice-o-matic, a Perl script to analyze properties of a given sequence found to be correlative of RNAi efficacy, and MATT, a package of functions in R to summarize and present the results of microarray experiments. The source code to these programs will be released under the open-source MIT license. A summary of the capabilities of each tool, as well as some example output data, will be presented.

P34

Correction for SNP Ascertainment Bias in Population Genetic Analyses of Teosinte

(submitted by Jeff Glaubitz <glaubitz@wisc.edu>)
Full Author List: Glaubitz, Jeff[1]; Briggs, William[1]; Sanchez-Gonzalez, Jesus[2]; Nielsen, Rasmus[3]; Gaut, Brandon[4]; Doebley, John F.[1]
[1] Genetics Department, University of Wisconsin, Madison, WI, USA 53706
[2] Centro Universitario de Ciencias Biologicas y Agropecuarias, Universidad de Guadalajara, Guadalajara, JA, Mexico
[3] Department of Biology, University of Copenhagen, Copenhagen, Denmark DK-2100
[4] Department of Ecology and Evolutionary Biology, University of California-Irvine, CA, USA 92697

As the wild progenitor of maize, conservation of the genetic resources of teosinte (Zea mays ssp. parviglumis) is of considerable interest. Wild populations of teosinte serve as the ultimate reservoir of genetic resources that could prove useful for deployment in commercial lines or land races of maize. The first step in gene conservation of any species is to characterize the distribution of genetic diversity within and between natural populations. We have obtained DNA samples from 20 plants from each of 12 widespread populations of teosinte and have assayed these at nearly 1000 single nucleotide polymorphisms (SNPs) from 600 genes. A key difficulty in the analysis of these data is accounting for the pervasive influence of ascertainment bias. Ascertainment bias refers to the bias that results when determining which nucleotides are polymorphic (i.e., which are SNPs) based upon a small panel of sequenced individuals. As a result of this bias, rare polymorphisms tend to be overlooked, and hence are not assayed. This bias against rare polymorphisms results in distorted population genetic inferences. Unfortunately, correcting for this bias is not a straightforward matter: methods published to date generally make several oversimplifying assumptions, such as that only one or two populations are studied, each in Hardy-Weinberg equilibrium. However, it should be possible, in theory, to use coalescent-based simulations of the assumed evolutionary history of the sampled populations, combined with simulation of the SNP ascertainment process, to correct for ascertainment bias in more complex situations such as in this study. This is an iterative, approximate Bayesian approach, in which the assumed evolutionary history is modified until the simulated results fit the observed data. Parameter values for the evolutionary models that fit the observed data provide estimates of the actual population genetic parameters. We will illustrate this approach and present our results to date.

P35

Do Centromeric Retroelements Determine Chromosome Size?

(submitted by Gernot Presting <gernot@hawaii.edu>)
Full Author List: Presting, Gernot[1]; Sharma, Anupma[1]; Wolfgruber, Thomas[1]
[1] University of Hawaii; Molecular Biosciences and Bioengineering; Honolulu, HI, USA 96822

Centromeric retroelements (CR) are located almost exclusively near the centromeres of plant chromosomes and appear to play a role in centromere function. Analysis of the emerging maize genome sequence has revealed new insights into the evolution of cereal CRs. Most notable is the discovery that maize contains a much larger number of CRs than rice, which can be attributed primarily to a significant expansion of the CRM1 subfamily in maize. No homolog of the CRM1 subfamily could be identified in the Oryza sativa genome, although one incomplete copy of this element was found in Oryza officinalis. In addition, autonomous copies of an element related to the non-autonomous maize CR CentA, as well as a novel CR subfamily of maize and rice (CRM4/CRR4) were discovered. The phylogenetic relationships of the various CRs from maize and rice were determined using the gag domain. The emerging picture of CRs in plants indicates that the abundance of this group of chromodomain-containing retrotransposons in a genome is correlated with genome size. Indeed, it is possible that the genome expansion of maize that has resulted in single maize chromosomes approaching the size of the entire rice genome, has been enabled by a concomitant expansion of the CRM1 subfamily. Similarly, the genome reduction in Arabidopsis may be the result of the apparent lack of functional autonomous CR elements in this species, which may act to limit chromosome size.

P36

GRASSIUS: A Blueprint for Comparative Regulatory Genomics in the Grasses

(submitted by Saranyan Palaniswamy <Saranyan.Palaniswamy@osumc.edu>)
Full Author List: Palaniswamy, Saranyan K.[1]; Gray, John[2]; Davuluri, Ramana V.[1]; Grotewold, Erich[3]
[1] Dept. of Mol Virology, Immunology & Medical Genetics, The Ohio State University, Columbus, Ohio 43210
[2] Dept. of Biology, University of Toledo, Toledo, Ohio 43606
[3] Dept. of Plant cellular & Molecular Biology, The Ohio State University, Columbus, Ohio 43210

An emerging premise in regulation of gene expression is to identify the regulatory networks in which Transcription Factors (TFs) participate to categorize the temporal and spatial expression of all genes in an organism. This fact underscores that it is vital to establish the architecture of plant promoters, TFs, cis-elements and its direct targets to understand the functionalities. The increasing amount and availability of genomic data from maize and other grasses has provided the necessity for an integrated, comparative regulatory genomics based resources and tools that contribute to identification of gene regulation. We present a platform GRASSIUS, the Grass Regulatory Information Server (*http://www.grassius.org/*) that integrates data from experimental results, literature and other publicly available repositories and resources with capabilities to visualize and annotate gene expression information. Currently, GRASSIUS houses three databases, GRASSTFDB (Grass Transcription Factor Database), GRASSPROMDB (Grass Promoter Database) and GRASSREGNET (Grass Regulatory Network Database). GRASSTFDB provides a comprehensive collection of transcription factors from maize, sugarcane, sorghum and rice. Other grasses will be included as sequence information becomes available. Transcription factors, defined here specifically as proteins containing domains that suggest sequence-specific DNA-binding activities, are classified based on the presence of 50+ conserved domains. Links to resources that provide information on mutants available, map positions or putative functions for these transcription factors are provided. Transcription factors are being named using a standard nomenclature that will simplify references to them as well as comparative analyses between the grasses. Transcription factors from these grasses are currently being grouped into 43 families. GRASSPROMDB will provide a collection of sequences for promoters from maize, sugarcane, sorghum and rice genes. In these promoters, predicted and experimentally verified cis-regulatory elements, presumably recognized by transcription factors, are indicated. GRASSREGNET will provide the integration of experimentally demonstrated interactions between transcription factors and specific gene promoters into regulatory motifs. GRASSIUS is expected to significantly benefit from community input, for example through voluntary curation contributions. GRASSIUS can be accessed at http://www.grassius.org.

P37

Gramene, A Comparative Resource of Grass Genomes

(submitted by Chengzhi Liang <liang@cshl.edu>)
Full Author List: Liang, Chengzhi[1]; Hebbard, Claire[2]; Jaiswal, Pankaj[2]; Ni, Junjian[2]; Spooner, William[1]; Yap, Immanuel[2]; Youens-Clark, Ken[1]; Ren, Liya[1]; Wei, Sharon[1]; Tecle, Isaak Y.[2]; Casstevens, Terry M.[3]; Ravenscroft, Dean[2]; Avraham, Shulamit[1]; Zhao, Wei[1]; Canaran, Payan[1]; Tung, Chih-Wei[2]; Thomason, Jim[1]; Hurwitz, Bonnie[1]; Ware, Doreen H.[4]; Buckler, Edward S.[4]; McCouch, Susan R.[2]; Stein, Lincoln D.[1]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Rd, Cold Spring Harbor, NY, USA, 11724
[2] Department of Plant Breeding and Genetics, 240 Emerson Hall, Cornell University, Ithaca, NY, USA, 14853
[3] Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA, 14853
[4] USDA-ARS NAA Plant, Soil & Nutrition Laboratory Research Unit, Cornell University, Ithaca, NY, USA, 14853

Gramene (www.gramene.org) is a curated, open-source, web-accessible comparative data resource for plant (mainly grass) genomes. The database provides agricultural researchers and plant breeders with genomic and genetic information on rice and other grasses. Gramene's comparative data includes large scale genome comparisons, orthologous gene sets, cross species sequence mappings, genetic diversities within species or between closely related species. The comparative data is made on top of abundant genetic and physical maps, genome sequences, genes, proteins, pathways, genetic markers, QTLs, and variations, which are invaluable to users by themselves. Controlled vocabularies are used to associate different types of data together. A species module gives each species a summary for all data available in Gramene.

Gramene holds a quarterly release schedule with both updated data and software tools, and is accessed by researchers in over 100 countries around the world. Online tutorials and help documents provide users with an overview of how to conduct a search within each database. Workshops are held to train users in using the database. This presentation summarizes the overall data organization in Gramene with a focus on the comparative aspect of recent updates and discusses some future directions.

The project is supported by National Science Foundation grant No. 0321685 and USDA ARS.

P38

Maize Diversity: Accessing Data behind Germplasm, QTL, and Breeding Studies

(submitted by Terry Casstevens <tmc46@cornell.edu>)
Full Author List: Casstevens, Terry M.[1]; Bradbury, Peter J.[3]; Canaran, Payan[4]; Youens-Clark, Ken[4]; Doebley, John F.[5]; Glaubitz, Jeff[5]; Kroon, Dallas E.[1]; McMullen, Michael[6]; Sanchez-Villeda, Hector[6]; Schroeder, Steve[6]; Sun, Qi[3]; Ware, Doreen H.[4]; Zhang, Zhiwu[1]; Zhao, Wei[4]; Buckler, Edward S.[1]
[1] Institute of Genomic Diversity, Cornell University, Ithaca, NY, USA
[2] USDA-ARS
[3] Cornell Theory Center, Cornell University, Ithaca, NY, USA
[4] Cold Spring Harbor Laboratory, NY, USA
[5] Department of Genetics, University of Wisconsin, Madison, WI, USA
[6] Division of Plant Sciences, Univerisity of Missouri, Columbia, MO, USA

Maize diversity is the basis of feeding a large proportion of the world. Unlocking the complex associations between the maize genome and observable traits require vast amounts of human and computational resources for data collection and analysis. A major challenge of this task involves storing large genotypic and phenotypic data sets and providing access to the data. We developed Panzea, a public website dedicated to making maize diversity data available to the community. The infrastructure includes a database schema (GDPDM; www.maizegenetics.net/gdpdm, sourceforge.net/projects/gdpdm), a Java middleware and advanced search (GDPC; www.maizegenetics.net/gdpc, sourceforge.net/projects/gdpc), and web-based tools (including: a sequence alignment-SNP viewer, Germplasm Search, Molecular Diversity Search, etc.) (www.panzea.org and www.gramene.org/db/diversity/diversity_view). Anyone can implement the diversity database schema (GDPDM) to organize their collections of genetic and phenotypic data. The middleware provides a highly-flexible advanced search tool to retrieve, integrate, and display customized data sets. A genetic association and diversity analysis tool (TASSEL; www.maizegenetics.net/tassel, sourceforge.net/projects/tassel) also uses the middleware to acquire data for analysis. We also provide web based tools for making data available on our website by utilizing existing tools when applicable and developing generic stand-alone tools. These include various interactive search pages and visualization tools. All these components are free open-source tools and can be used by other crop species (i.e. wheat, rice). We are also working (1) to incorporate more data from diversity projects, (2) to develop community upload tools, and (3) to create enhanced query, display and analysis tools. We encourage community input and collaboration on this effort, so that the largest possible community can access and productively use diversity data.

P39

Maize Diversity: Associating Genetic Polymorphisms and Phenotypes

(submitted by Zhiwu Zhang <zz19@cornell.edu>)
Full Author List: Zhang, Zhiwu[1]; Bradbury, Peter J.[2]; Kroon, Dallas E.[1]; Casstevens, Terry M.[1]; Buckler, Edward S.[3]
[1] Cornell University, 175 Biotechnology Bldg, Ithaca, NY 14853
[2] USDA-ARS Cornell University, 741 Rhodes Hall, Ithaca, NY 14853
[3] USDA-ARS Cornell University, 159 Biotechnology Bldg, Ithaca, NY 14853

Maize is a very diverse species with high level of DNA sequence polymorphism and a broad range of phenotypic variation. These facts together with a fast decay of linkage disequilibrium (LD) combine to make association analysis in maize both useful and challenging. One of the key challenges in such a diverse population is eliminating false positives due to population substructure and family relationships. TASSEL implements general linear model and mixed linear model approaches to incorporate population and family structure to reduce false positive and increase statistical power simultaneously. Other features of TASSEL, such as data transformations, missing data imputation and principal component analysis, provide tools to enhance the analyses and the integration of multiple traits. An LD analysis tool shows the LD structure among tested markers, which may help in understanding association results. Linkage disequilibrium is estimated by the standardized disequilibrium coefficient, D', as well as r2 and P-values. Diversity analysis can be performed by giving diversity estimates including average pair-wise divergence (pi), segregating sites and theta. Most of analysis results and input data can be visualized graphically to aid interpretation, such as phylogenies trees in normal or circular layout. The major feature of next release is to have function units which can be executed interactively or assembled as a pipeline to benefit a long running analysis with multiple steps. TASSEL was written in Java and can be run in multiple operating systems. The software package including help document and tutorial can be downloaded at www.maizegenetics.net/tassel. The open source code is available at http://sourceforge.net/projects/tassel.

P40

Maize Microarray Platform Translator, a New Tool at PLEXdb to Enhance Capabilities for Meta-Analysis of Gene Expression Profiling Data

(submitted by Nick Lauter <nickl@iastate.edu>)
Full Author List: Cannon, Ethalinda[1]; Lauter, Nick[2]; Nadkarni, Yogesh[1]; Moscou, Matt[1]; Dickerson, Julie[1]; Wise, Roger[2]
[1] Iowa State University, Ames IA, 50011
[2] USDA-ARS, Corn Insects and Crop Genetics Research and Iowa State University, Ames, IA, 50011

PLEXdb (Plant Expression Database, http://www.plexdb.org) is a public resource that enables access to and analysis of gene expression data for plants and plant pathogens. Maize currently has 6 publicly available gene expression profiling platforms. In order to facilitate meta-analyses among these platforms, we have developed the alpha version of Maize Microarray Platform Translator, a web-based tool that allows a user to input a genelist from one platform and receive the corresponding gene list for any of the other five platforms. For genes on such a list that exist in the query platform but not in the subject platform, a message of "no match found" is returned. The connections between platforms are made via blastn of the platforms' design sequences to the current NCBI UniGene set. We will update the blast results as the UniGene set is updated, which will allow more matches to be found as singleton fragments become part of existing contigs. The e-value criterion and selection of top hits to be included can be controlled by the user, allowing stringent or relaxed translations to be made. This feature will be more important as we extend the platform translation capabilities across species, a process we have begun for platforms in the grass and legume families. These tools will promote maximal use of future and existing data within our community by expanding our capabilities to develop and test hypotheses without performing benchwork.

P41

MaizeGDB Curation: New Data, Tools and an Invitation to All Cooperators

(submitted by Mary Schaeffer <SchaefferM@missouri.edu>)
Full Author List: Schaeffer, Mary L.[1]; Harper, Lisa C.[2]; Baran, Sanford[3]; Campbell, Darwin A.[3]; Seigfried, Trent E.[3]; Lawrence, Carolyn J.[4]
[1] Plant Genetics Research Unit, USDA-ARS and Division of Plant Sciences, University of Missouri, Columbia, MO USA 65211
[2] Plant Gene Expression Center, USDA-ARS, 800 Buchanan St., Albany, CA 94710
[3] Corn Insects and Crop Genetics Research Unit, USDA-ARS, 526 Science II, Iowa State University, Ames, IA 50011
[4] Corn Insects and Crop Genetics Research Unit, USDA-ARS and Depts. of Agronomy and Genetics, Development, and Cell Biology, 526 Science II, Iowa State University, Ames, IA 50011

Curation activity at MaizeGDB includes managing (1) data integration into the basic tables of the databases, (2) updates of composite map products, such as the neighbors maps, and (3) updates of standard and structured vocabularies such as the plant anatomy, growth and development ontologies and a trait ontology, created for maize in 1995. The current focus of our work is on genetic maps, their documentation, annotation and data such as sequence accessions that help integrate with the B73 genome sequencing project at Washington University. We would greatly appreciate your help in this endeavor, especially your expertise! We extend an invitation to any cooperator, be they graduate student or well established researcher, to enter new data at MaizeGDB. In particular, new mutants that are not yet described in MaizeGDB and updates of mutants already in the database are welcome. Both short and detailed text descriptions, such as found in resources like Wikipedia, may be entered directly using the Annotation Tool developed at MaizeGDB for this purpose. To create new phenotype/mutant records, a set of Community Curation Tools is available. A brief 'how-to' for both data contribution methods will be presented. We are especially interested in sequence, map, and gene product information that will be helpful for annotation of the B73 genome sequence.

P42

MaizeGDB: Four Ways Of Looking At Maps

(submitted by Trent Seigfried <devolver@iastate.edu>)
Full Author List: Seigfried, Trent E.[1]; Campbell, Darwin A.[1]; Schaeffer, Mary L.[2]; Harper, Lisa C.[4]; Lawrence, Carolyn J.[3]
[1] Corn Insects and Crop Genetics Research Unit, USDA-ARS, 526 Science II, Iowa State University, Ames, IA 50011
[2] Plant Genetics Research Unit, USDA-ARS and Division of Plant Sciences, Curtiss Hall, University of Missouri, Columbia, MO 65211
[3] Corn Insects and Crop Genetics Research Unit, USDA-ARS and Depts. of Agronomy and Genetics, Development, and Cell Biology, 526 Science II, Iowa State University, Ames, IA 50011
[4] Plant Gene Expression Center, USDA-ARS, 800 Buchanan St., Albany, CA 94710

MaizeGDB (Maize Genetics and Genomics Database) is the research database for the maize community. The site features a wealth of resources and data facilitating the scientific study of maize. This poster will highlight four distinct easy-to-access map displays for retrieving detailed multi-dimensional information on genetic maps, providing data sets that are useful for genomics researchers and plant breeders alike.

P43

Quantitation of Lesion Phenotypes as a Function of Inbred Background

(submitted by Toni Kazic <toni@athe.rnet.missouri.edu>)
Full Author List: Kazic, Toni[1]; Green, Jason[1]; Harnsomburana, Jaturon[1]; Shyu, Chi-Ren[1]
[1] University of Missouri; Dept. of Computer Science; Columbia, Missouri, USA 65211

Complex phenotypes have components that fall on multiple, classificatory axes. Although such phenotypes can be very distinct to the eye, they are difficult to describe and even harder to quantitate. We are computationally characterizing a number of lesion mutants in Mo20W and W23 backgrounds with respect to size, color, and average density. Leaves were quantitatively photographed on plants in the field in late July -- early August, 2006 at the Genetics Farm in Columbia, using a Gretagmacbeth mini color checker as a color and size standard. Images were first segmented into lesions and then the distributions of multiple features were computed. We observe marked differences along these axes as a function of lesion mutant and inbred background. For example, M. G. Neuffer describes Les10 expression as higher in W23 than in Mo20W, and to the naked eye our preliminary photographs are consistent with this description. However, image analysis shows that Les10 in Mo20W shows more total lesions, more large lesions, and more small lesions than the same mutation in W23. The average density of lesions in Mo20W is greater, with many small satellite lesions around the larger lesions. This clustering of lesions is probably responsible for the qualitative impression that expression is higher in W23. This technique should be useful in characterizing other components of lesion phenotypes and other visible, complex phenotypes. This work is partially supported by NSF grant DBI-0447794.

P44

RepMiner: A Graph Theory Based Approach to the Classification and Assembly of the Repetitive Fraction of Sample Sequence Data

(submitted by James Estill <jestill@uga.edu>)
Full Author List: Estill, James C.[1]; Bennetzen, Jeffrey L.[2]
[1] Department of Plant Biology, University of Georgia, Athens, GA, 30605
[2] Department of Genetics, University of Georgia, Athens, GA, 30605

Understanding the phylogenetic distribution and abundance of transposable elements (TEs) will help us to further understand the evolution of genome size and the influence of TEs on the evolution of the protein coding regions of the genome. A comprehensive study of TEs would benefit from the ability to incorporate low-coverage shotgun DNA sequence datasets from previously unstudied genomes. Our PERL program RepMiner seeks to utilize these data by taking a graph theory approach to the classification and assembly of the repetitive fraction of low-coverage sequence data. We validated the efficacy of the RepMiner graph-based classification scheme using a database of known maize TEs. The application of the RepMiner approach to the assembly of low-coverage sequence libraries is illustrated using maize WGS data as well as a simulated low-coverage maize BAC library. Our current work explores the influence of sample size, sequence read length, and the choice of clustering algorithm on the performance of the RepMiner process.

P45

Submitting Your Data to MaizeGDB to Make it Publicly Available

(submitted by Darwin Campbell <darwin@iastate.edu>)
Full Author List: Campbell, Darwin A.[1]; Schaeffer, Mary L.[2]; Harper, Lisa C.[3]; Seigfried, Trent E.[1]; Lawrence, Carolyn J.[4]
[1] Corn Insects and Crop Genetics Research Unit, USDA-ARS, 526 Science II, Iowa State University, Ames, IA 50011
[2] Plant Genetics Research Unit, USDA-ARS and Division of Plant Sciences, Curtiss Hall, University of Missouri, Columbia, MO 65211
[3] Plant Gene Expression Center, USDA-ARS, 800 Buchanan St., Albany, CA 94710
[4] Corn Insects and Crop Genetics Research Unit, USDA-ARS and Depts. of Agronomy and Genetics, Development, and Cell Biology, 526 Science II, Iowa State University, Ames, IA 50011

The Maize Genetics and Genomics Database (MaizeGDB) is the community resource for maize data and can be accessed online at http://www.maizegdb.org. Researchers in the maize community create many useful datasets that have broader impact to the larger community and frequently researchers want to share their data and may be puzzled by the steps taken to integrate their data into MaizeGDB. Here, we outline an example of the process used to include the Maize Tilling data type into the database using common software.

P46

The Maize Genome Sequence Browser

(submitted by Shiran Pasternak <shiran@cshl.edu>)
Full Author List: Pasternak, Shiran[1]; Narechania, Apurva[1]; Stein, Joshua[1]; McMahan, Linda[1]; Spooner, William[1]; Liang, Chengzhi[1]; Wei, Sharon[1]; Faga, Ben[1]; Stein, Lincoln D.[1]; Ware, Doreen H.[2]; Maize Genome Sequencing Consortium, The[3]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; and USDA-ARS NAA Plant, Soil & Nutrition Laboratory Research Unit, Ithaca, NY 14853
[3] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; Arizona Genomics Institute, University of Arizona, Tucson, AZ 85721; Iowa State University, Ames, IA 50011; and Genome Sequencing Center, Washington University, St. Louis, MO 63108

The Maize Genome Sequencing Project, a collaboration among the Washington Uni-versity Genome Sequencing Center, the Arizona Genomics Institute, Iowa State Univer-sity, and Cold Spring Harbor Laboratory, aims to sequence the maize genespace to fin-ished quality using a BAC-based approach. Given the complex and highly repetitive to-pology of the genome, ongoing analysis and annotation is crucial to guiding the se-quencing effort. A multifaceted informatics framework has been built at CSHL for ana-lyzing the maize genome sequence. An annotation pipeline accesses Genbank for maize sequence updates on a weekly basis and automatically analyzes BACs that reach an improved phase. The pipeline further performs ab initio gene prediction using Fgenesh, alignment to known sequence and marker data sets, and repeat analysis. As the project matures, whole-genome alignment to rice will be added. Annotations and analysis results leverage the project's mapping and finishing efforts by incorporating new knowledge about the structure of the maize genome. The data are made immedi-ately available on a public browser, at http://www.maizesequence.org. The browser pro-vides high-level views of maize physical maps, with sequenced BACs visually anchored, including detailed BAC-oriented views of fine-grained annotations. The interface, built on top of Ensembl, provides reciprocal links to other useful maize community resources, notably Gramene and MaizeGDB, and encourages user feedback. Externally annotated data sets will be incorporated as needed to serve the maize community. This work was funded by the NSF/DOE/USDA "Sequencing The Maize Genome" project (NSF #0527192).

P47

The TIGR Plant Transcript Assemblies Database

(submitted by Agnes Chan <achan@tigr.org>)
Full Author List: Childs, Kevin[1]; Liu, Jia[1]; Rabinowicz, Pablo[1]; Town, Chris[1]; Buell, C. Robin[1]; Chan, Agnes[1]
[1] The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20805

With the goal of building a comprehensive resource for assembly and annotation of gene transcripts from Expressed Sequence Tag (EST) and experimentally-derived cDNA sequences, we have developed the TIGR Plant Transcript Assemblies (TA) database (http://plantta.tigr.org). In order to facilitate comprehensive cross-species comparative studies, the TA database includes all plant species for which more than 1,000 EST or cDNA sequences are available in GenBank. The current release includes 233 TAs representing 232 plant species. EST and cDNAs sequences are downloaded and assembled using TGICL. The TGICL tool is a pipeline which first clusters the input sequences from individual plant species based on an all-versus-all pairwise comparison using Megablast, and subsequently creates the final assemblies using CAP3. Functional annotation and orientation of the TAs are determined using the UniProt Reference Clusters (UniRef100) protein database as the reference resource. The TA database allows users to perform keyword-based text search or sequence-based searches using the TA blast server. A taxonomy tree-view enables searching TAs amongst individual or multiple species, grouped by genus or higher order taxonomic classification. The TAs and their annotation are also available through web interfaces and FTP downloads. The TA database is regularly updated to include new EST and cDNA submissions to the GenBank Nucleotide database.

We are currently working on expanding the functionality of the TA database to provide TA expression profile and level based on EST library classification and frequency, Gene Ontology annotation, improved history tracking and orthologous family classification from selected plant species.

Exclusively for maize, in addition to the species-specific maize TAs built using all publicly available 1,000,000 maize EST/cDNAs, an inbred-specific maize TA has been built using 650,000 EST/cDNAs derived from the B73 inbred, the genome of which is currently being sequenced. The B73-specific TA will be a useful tool for inbred-specific transcript analysis and genome annotation.

P48

Three 'Works In Progress': MaizeGDB's Editorial Board, Map Description Pages, and a Project to Integrate Mutant Phenotypes with Existing Data

(submitted by Lisa Harper <ligule@nature.berkeley.edu>)
Full Author List: Harper, Lisa C.[1]; Schaeffer, Mary L.[2]; Seigfried, Trent E.[3]; Campbell, Darwin A.[3]; Lawrence, Carolyn J.[4]
[1] Plant Gene Expression Center, USDA-ARS, 800 Buchanan St., Albany, CA 94710
[2] Plant Genetics Research Unit, USDA-ARS and Division of Plant Sciences, Curtiss Hall, University of Missouri, Columbia, MO 65211
[3] Corn Insects and Crop Genetics Research Unit, USDA-ARS, 526 Science II, Iowa State University, Ames, IA 50011
[4] Corn Insects and Crop Genetics Research Unit, USDA-ARS and Depts. of Agronomy and Genetics, Development, and Cell Biology, 526 Science II, Iowa State University, Ames, IA 50011

Many endeavors at MaizeGDB are ongoing. These range from curating data from current literature to creating pages to explain what is meant by certain terms, descriptors, and data representations. Described here are three such tasks that are currently underway: Editorial Board coordination, map description page creation, and details on a new project aimed at integration of RescueMu and EMS mutant/phenotype data from the Maize Inflorescence Architecture and Maize Gene Discovery Projects with other mutant/phenotype records and query mechanisms. In addition, we solicit suggestions from cooperators for items in MaizeGBD that would benefit from further scientific descriptions or tutorials.

P49

TIE-DYED1 Localizes to the Endomembrane System

(submitted by Thomas Slewinski <tls315@psu.edu>)
Full Author List: Slewinski, Thomas L.[1]; Ma, Yi[1]; Braun, David M.[1]
[1] 616 Mueller Laboratory, University Park, PA 16802

The Tiedyed1 (Tdy1) gene encodes a novel transmembrane protein that we hypothesize plays an important role in carbon partitioning and sugar transport in developing maize leaves. As one approach to characterize the gene's function we are determining its expression at the tissue, cellular and subcellular levels. By RT-PCR we determined that the gene is expressed in all tissues analyzed. RNA in situ hybridizations localize the transcript to developing phloem cells. As a first step to determine the protein's location within cells we used various computer programs to predict potential targeting information. However, bioinformatic approaches yielded widely different results and thus proved to be ineffective in predicting TDY1's subcellular location. Therefore to determine where within cells TDY1 resides we produced translational fusions of TDY1 to the fluorescent reporter proteins GFP, YFP and mCherry. These constructs were transiently expressed in onion epidermal cells and localize the protein to the endomembrane system. By co-expressing the TDY1 reporter fusion constructs with other known fluorescent marker proteins, preliminary data suggest that TDY1 localizes to the endoplasmic reticulum (ER). To confirm these results, we are producing stable transformations of the TDY1 fluorescent protein fusions expressed by the native promoter in Arabidopsis and maize.

P50

dsyCS and *segII* Define a Novel Class of Homologous Pairing Mutants

(submitted by Christopher Bozza <cgb25@cornell.edu>)
Full Author List: Bozza, Christopher G[1]; Pawlowski, Wojciech P.[1]
[1] Plant Biology, Cornell University; Ithaca, NY USA 14853

Pairing of homologous chromosomes during prophase of meiosis is essential for accurate segregation of genetic material and successful gamete production. While many other mechanisms of meiosis, e.g. recombination, are well explored, homologous pairing remains the most mysterious meiotic activity.

We are using a forward genetic approach to find genes affecting homologous pairing and dissect their roles in the genetic network regulating pairing. Screening through the collection of meiotic mutants in maize, we identified two novel homologous pairing-defective mutants, *dsyCS* and *segII*. Both these mutants were known to show presence of univalent chromosomes at metaphase I, which implies a lack of crossovers. We found that both mutants are also deficient in the installment of the recombination protein RAD51 on meiotic chromosomes in zygotene and pachytene, showing numbers of chromosomal RAD51 foci equal to ~2% of the wild-type number. Continuing recombination analysis, the formation of double strand breaks was assayed. Preliminary data suggests the presence of double strand breaks at the appropriate time in meiosis for both *segII* and *dsyCS*.

segII and *dsyCS* were subsequently examined by florescent in situ hybridization (FiSH) for pairing at the 5S rRNA locus and found to have unique and significant pairing defects. Pachytene chromosomes in *segII* display 60% associations with non-homologous partners instead of their proper homologs. In *dsyCS*, all chromosome associations are between non-homologous partners.

The phenotypes of the *dsyCS* and *segII* mutants are similar to the phenotype of the previously characterized *phsI* mutant, although they are not identical. We hypothesize that *segII* and *dsyCS* along with phs1, define a novel class of meiotic genes that play key roles in the genetic network in regulating homologous pairing. Both mutants came from *Mutator* populations and their cloning is underway.

P51

Analysis of the Molecular Role of *PHS1* in Meiotic Chromosome Pairing

(submitted by Arnaud Ronceret <ar346@cornell.edu>)
Full Author List: Ronceret, Arnaud[1]; Pawlowski, Wojciech P.[1]
[1] Cornell university, Plant Breeding and Genetics; 418 Bradfield Hall; Ithaca, NY14850, USA

While much is known about meiotic recombination and synapsis, chromosome pairing is the least understood process of meiosis. We aim to understand chromosome pairing at the mechanistic level by elucidating the molecular components that are involved in the process. Maize has historically been a species of choice for genetic studies of plant meiosis and offers numerous features critical for this research, notably the synchronization of meiocyte development in anthers that allows for collecting large quantities of material for proteomic analyses.

The identification of the maize poor homologous synapsis1 (phs1) mutant and cloning the phs1 gene were the starting points for this research. phs1 plays a role in ensuring that pairing occurs exclusively between homologous chromosomes. The phs1 gene encodes a novel protein with several short conserved domains but with a low overall level of evolutionary conservation. We hypothesize that the PHS1 protein is part of a complex that facilitates a key step in chromosome pairing. In order to elucidate how the PHS1 protein directs chromosome pairing in meiosis, we are pursuing two complementary approaches:
(i) characterization of the PHS1 protein and its behavior during meiosis
(ii) identification of proteins that interact with PHS1.

Localization of PHS1 will provide insight into when and where the protein acts during meiosis. We developed an antibody against PHS1 and are using it to localize the protein in meiocytes with 3-dimensional deconvolution microscopy.

To identify proteins interacting with PHS1, we developed the proteomic strategy of TAP-tagging the maize PHS1 and purify the putative PHS1-containg protein complex using the TAP-tag. Transgenic maize plants expressing ZmPHS1-CTAP (the TAP tag fused to the C-terminus of the PHS1 protein) and NTAP-ZmPHS1 (N-terminal fusion) have been generated and are currently being analyzed. Anthers enriched in zygotene meiocytes will be used to purify proteins that show affinity to PHS1.

P52

Characterization Of A Novel Brown Midrib Mutant

(submitted by Reuben Tayengwa <reubent@ufl.edu>)
Full Author List: Tayengwa, Reuben[1]; Koch, Karen E.[2]; McCarty, Donald R.[2]; Carpita, Nicholas C.[3]; Vermerris, Wilfred E.[1]
[1] University of Florida, Agronomy Department, Box 103610 Gainesville, FL 32610.
[2] University of Florida, Department of Horticultural Sciences and Plant Molecular and Cellular Biology, Box 110690 Fifield Hall, Gainesville, FL 32610.
[3] Purdue University, Botany and Plant Pathology, Lilly Hall, 915 West State Street, West Lafayette, IN 47907

As part of the NSF Plant Genome project "Identification and characterization of cell wall mutants in maize and Arabidopsis using novel spectroscopies," 2,200 families of Mu-tagged maize lines from the UniformMu population developed at the University of Florida were planted at Purdue University in the summer of 2002-2004. Among the mutants that were identified was a novel brown midrib (bm) mutant showing a subtle orange-brown midrib that was not allelic to any of the four known bm mutants in maize. The mutants, bm1, bm2, bm3, and bm4 are Mendelian recessives and are recognized by reddish-brown vascular tissue in the leaves and stems resulting from changes in lignin content and/or composition. The pigmentation to which this character is due has been found in the stem, the root, the leaves, the tassel and the cob of the plant. The objective of my research is to characterize this novel brown midrib mutant and to identify the genetic basis of the mutation. Since the bm mutant was isolated from a transposon-active mutagenic population, a Mutator (Mu) element is probably the cause of the mutation. This insertion will enable isolation of the Brown midrib gene using Mu-TAIL (Thermal Assymetric InterLaced) PCR. In order to investigate effects of the bm mutation on the plants, we have characterized the bm plants in terms of growth and development, lignin content and composition, biomass conversion efficiency via enzymatic saccharification. While the four existing bm mutants have a clear cell wall phenotype, this novel bm mutant does not show changes in lignin content, lignin subunit composition or biomass conversion efficiency. The flowering time is delayed by four days. On average the bm plants are shorter than the wild-type by 26 cm. We are now investigating the chemical nature of the orangish-brown pigmentation.

P53

Functional Analysis of Cellulose Synthase-Like Genes in Maize

(submitted by Charles Hunter <ibe@ufl.edu>)
Full Author List: Hunter III, Charles T.[1]; Avigne, Wayne T.[1]; Vermerris, Wilfred E.[1]; McCarty, Donald R.[1]; Carpita, Nicholas C.[2]; Koch, Karen E.[1]
[1] University of Florida; Plant Molecular and Cellular Biology Program; Gainesville, FL, 32611
[2] Purdue University; Botany and Plant Pathology; West Lafayette, IN, 47907

Cellulose Synthase-Like (CSL) genes are candidates for encoding enzymes that synthesize the backbones of the diverse hemicelluloses found in plant cell walls. This has recently been demonstrated for members of the CSLA and CSLF subfamilies, but functions of other CSL subfamilies remain unclear. The UniformMu maize population is being screened by reverse genetics to identify Mu transposon insertions in specific CSL genes. Analysis of maize plants carrying transposon-induced "knockout" alleles of specific CSL genes can help define their roles in the distinctive type-II cell walls of grasses. Thus far, Mu insertions have been identified in CSLA6, CSLA7, and CSLD1. Each has been demonstrated to be germinally heritable. Phenotypic screens and cell wall compositional analyses of mutant versus wildtype plants are being conducted in highly-uniform backgrounds resulting from continuous introgression of the population into a W22 inbred. Side-by-side comparisons of whole-plant, organ-specific, and microscopic features are being performed. No significant differences have been observed in field grown plants with insertions in CSLA6 or CSLA7, but further analysis is currently underway. A putative phenotype involving root hair elongation has been observed in homozygous mutants of CSLD1. This phenotype appears to be analogous to the Arabidopsis "kojak" mutant in which root hairs appear to initiate but fail to elongate normally. Confirmation of this phenotype through additional testing and by identification of additional alleles is in progress.

P54

Light Signal Transduction in Maize

(submitted by Matthew Hudson <mhudson@uiuc.edu>)
Full Author List: Hudson, Matthew E[1]; Swaminathan, Kankshita[1]; Kumar, Indrajit[1]; Li, Ying[1]; Bellendir, Stephanie[1]; Win, Hlaing[1]; Fliege, Christina[1]
[1] Department of Crop Sciences, University of Illinois, Urbana, IL 61801

Despite the agronomic importance of photomorphogenic responses, our understanding of the molecular events by which these are mediated is limited, especially in crop plants. In Arabidopsis and related eudicot species, studies of photomorphogenesis have recently defined a number of genes and plant hormone pathways that play a central role in light responses. However, photomorphogensis is still poorly characterized in Arabidopsis at the level of the contrasting molecular development of different tissues. We have undertaken to translate existing knowledge of photomorphogenesis in Arabidopsis into a better understanding of the mechanism of phytochrome responses, particularly shade-avoidance, in maize, and to use maize as a tool to investigate the developmentally determined differences between photomorphogenic responses within individual tissues. Comparison of proteins between key light signaling components identified in maize indicates that there is extensive conservation of both key functional domains and protein-protein interactions between Arabidopsis and maize.

P55

Nanaparticles Mediated Plant Genetic Transformation

(submitted by Kan Wang <kanwang@iastate.edu>)
Full Author List: Torney, Francois[1]; Trewyn, Brian[2]; Lin, Victor[2]; Wang, Kan[1]
[1] Plant Transformation Facility, Plant Science Institute, Iowa State University, Ames, Iowa 50011-1010
[2] Department of Chemistry, Iowa State University, Ames, Iowa 50011

Plant genetic engineering relies mostly on biolistic and Agrobacterium-mediated transformation technologies. Both techniques allow DNA delivery into plant cells and subsequent integration into the genome. Recently, the development of nanomaterials such as mesoporous silicate nanoparticles (MSN) was shown to deliver marker genes into animal cells (Radu et al., J. Am. Chem. Soc. 126, 13216-13217, 2004). The distinct feature of this nanoparticle is that it can both deliver DNA as well as chemicals encapsulated in the particles. Controlled release of the filling substance is also possible using this material (Gruenhagen et al., Appl. Spectrosc. 59, 424-431, 2005). Here we show that this material can be used for transforming tobacco mesophyll protoplasts, intact leaves and immature maize embryos. Transgene expression was observed both transiently and stably. We can induce gene expression in transgenic plants using MSN to deliver a transgene-inducing chemical under controlled-release conditions. In addition, we can deliver two different biogenic species into plant cells and release the encapsulated chemical in a controlled manner to trigger the expression of co-delivered transgene in the cell. We envision that further development of this system offers new possibilities to plant biotechnology.

P56

Studying Translocation of Recombinant Proteins in Plants: A Bacterial Antigen (LT-B) as a Model System

(submitted by Lorena Moeller <lorenam@iastate.edu>)
Full Author List: Moeller, Lorena[1]; Torney, Francois[1]; Wang, Kan[1]
[1] Department of Agronomy, Iowa State University, Ames, Iowa 50011-1010

The B subunit of Escherichia coli heat labile enterotoxin (LT-B) was produced in maize as a potential vaccine or vaccine component for humans or livestock (Chikwamba et al, 2002). Detection of recombinant LT-B by immunogold labeling electron microscopy showed that it accumulates inside the starch granules of transgenic maize kernels when carrying its native bacterial signal peptide or a 27kD gamma-zein signal peptide (Chikwamba et al, 2003). Interest in the mechanism of protein translocation into starch granules arises from the advantages of expressing recombinant proteins in starch fractions of cereals, which include ease of purification and reduced native protein content. We have generated translational fusions of LT-B with the green fluorescent protein (GFP) to track the movement of the recombinant LT-B in plant cells. To establish whether the LT-B protein is translocated into other plastid species we are testing LT-B::GFP fusions under the 35S cauliflower mosaic virus promoter in Arabidopsis thaliana mesophyll and root culture protoplasts. We are also establishing a system to study translocation of the LT-B::GFP fusions under the 27kD gamma-zein promoter in maize endosperm tissue using developing maize endosperm (B73) and trying to establish a HiII endosperm callus culture. This study will help establish the translocation route for the recombinant LT-B and will serve as a model to exemplify how recombinant proteins utilize the cellular machinery to reach their final sub-cellular destination. This information may be useful in future design of strategies for expression of recombinant proteins in plants.

P57

A New View: Meiotic Chromosomes and Homologous Synapsis Revealed by Ultrahigh Resolution Structured Illumination (SI) Microscopy

(submitted by Rachel Wang <rachelcjw@berkeley.edu>)
Full Author List: Wang, Rachel C.[1]; Carlton, Pete[2]; Sedat, John[2]; Cande, W. Zacheus[1]
[1] Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720
[2] Department of Biochemistry and Biophysics, University of California, San Francisco , CA 94143

Homologous chromosome pairing, recombination, and synapsis occur during meiotic prophase and are essential for the reductional division required to ultimately generate haploid gametes. During leptotene, each chromosome develops a linear proteinaceous structure called an axial element (AE). Around the same time, the homology search initiates by the appearance of double strand breaks. In zygotene, the homologues synapse via the polymerization of a central element between the two homologous AEs, forming the synaptonemal complex. At late pachytene, synapsis and recombination are completed. These events are well known cytologicallly, and have been explored with light microscopy and EM. Structured illumination (SI) is an ultrahigh resolution light microscopy developed by John Sedat at UCSF. It overcomes the 250 nm limit of resolution of conventional light microscopy, and is currently able to resolve 2 points that are less than 100 nm apart. We used SI to explore the substructure of pachytene chromosomes, organization of the axial element and formation of the synaptonemal complex by monitoring the distribution of two axial element antibodies, AFD1 and HOP1, and the DNA by DAPI staining. We found that chromomeres of paired chromosomes are bilaterally symetrical and have a left handed helical pitch. Chromosome and axial element organization are different at centromeres. We also found evidence for synaptic adjustment in unsynapsed regions of zygotene chromosomes, as the homologs in the unsynpased regions are very different in length. These regions are associated with interlocks during late zygotene, which imply that the resolution of interlocks between chromosomes may be a rate limiting step to complete synapsis.

P58

A Novel Meiotic Mutant, *mtm00-10*, with Aberrant Synapsis Shows a Mixture of Equational and Reductional Chromosome Segregation at Anapahse I

(submitted by Inna Golubovskaya <innagol@berkeley.edu>)
Full Author List: Golubovskaya, Inna N.[1]; Wang, Rachel C.[1]; Harper, Lisa C.[2]; Cande, W. Zacheus[1]
[1] University of California at Berkeley, Department of Molecular and Cell Biology, Berkeley, CA 94720
[2] Plant Gene Expression Center, USDA-ARS, 800 Buchanan St., Albany, CA 94710

In a novel maize meiotic mutant mtm00-10, homologous chromosome synapsis and recombination are reduced nearly 50% in male meiosis, and the number of univalents ranges from 6 to 20. Fluorescence in situ hybridization (FISH) demonstrates that a normal telomere bouquet is formed, but homologous pairing of a 5S rDNA locus is only 10%. Chromosome synapsis is aberrant as exemplified by non-homologously synapsed chromosomes (mostly foldbacks), and unresolved interlocks during pachytene. In the mutant, there are a normal number of RAD51 foci at pachytene, but some foci persist longer, especially in the foldback regions. Immunolocalizations of ASY1/HOP1, AFD1 and SGO1 show that they have a similar timing and distribution to wt. A novel and extremely interesting phenotype is the presence of both reductionally and equationally segregating chromosomes in anaphase 1. This novel feature of mtm00-10 distinguishes this mutant from all others in our collections.

P59

Comparison of Mitochondrial and Chloroplast DNA Insertions into Nuclear Chromosomes of Maize

(submitted by Leah Westgate <westgatel@missouri.edu>)
Full Author List: Westgate, Leah[1]; Lough, Ashley[1]; Hui, Alice[1]; Donnelly, Laura[1]; Wolf, Mark[1]; Birchler, James A.[1]; Newton, Kathy[1]
[1] University of Missouri; Columbia, MO, 65211

We have examined mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) insertions into the nuclear chromosomes of B73 using fluorescence in situ hybridization (FISH). Nineteen cosmids, representing over 95% of the 570 kb NB mitochondrial genome, were fluorescently labeled and hybridized to metaphase root tip chromosomes. Twelve regions, representing over 90% of the 140 kb chloroplast genome, were labeled and hybridized in the same manner. The mtDNA and cpDNA insertion sites into chromosomes were compared in the B73 inbred line. The majority of the mtDNA and cpDNA insertions were not shared. In addition, sublines (different sources) of B73 and also W23 were examined for further variation in mtDNA insertion sites. Differences were observed between the different sources of B73 and W23, raising the possibility that mtDNA is being transferred and incorporated into nuclear DNA continually. These results reinforce the idea that organelle DNA insertions are significant contributors to genome diversity within maize.

P60

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

(submitted by Debbie Figueroa <figueroa@bio.fsu.edu>)
Full Author List: Figueroa, Debbie M.[1]; Amarillo, Ina E.[1]; Strobel, Cornelia E.[1]; Lawrence, Carolyn J.[2]; Bass, Hank W.[1]
[1] Department of Biological Science Florida State University Biology Unit I, Chieftan Way Tallahassee, FL 32306-4370 USA
[2] USDA-ARS & MaizeGDB Iowa State University 526 Science II Ames, IA 50011 USA

We are developing a pachytene cytogenetic FISH map of the maize (Zea mays L.) genome using maize marker-selected sorghum BACs, corresponding to the 90 maize Core Bin Marker (CBM) loci from the UMC98 maize linkage map, as described by Koumbaris & Bass (2003, Plant J. 35:647). Current cytogenetic mapping of the CBM loci is focused on chromosomes 1, 3, 4, 5, 6, and 8. Fourty-seven of the 90 CBM probes have been subjected to sorghum BAC library screening. Analysis of chromosome addition lines 4 and 6 revealed maintenance of previously published arm ratios (Long/Short = 1.6+/-0.29 for 4, 2.7+/-0.38 for 6). We are also continuing the RFLP full-length insert sequencing (FLIS) project to enable *in silico* screening for suitable sorghum BACs. So far, at least 55 RFLPs have been annotated and deposited into GenBank. The completion of a cytogenetic map will provide a means for integrating the physical, genetic, and cytological maps of maize with an added dimension of genome-wide cross-anchoring to the maps of sorghum. The project is described at http://www.cytomaize.org/ and mapping and image data are released through MaizeGDB. This project will provide insights into the structure of the maize genome, while creating new technologies and reagents for chromosome research.

P61

Construction of Maize Somatic Chromosome Cytogenetic Map

(submitted by Tatiana Danilova <danilovat@missouri.edu>)
Full Author List: Danilova, Tatiana V.[1]; Birchler, James A.[1]
[1] University of Missouri Columbia, 117 Tucker Hall, Columbia, MO USA 65211

Maize somatic chromosomes are excellent for cytological studies because they are available independent of the season and easy to identify. Sequences equally distributed along the chromosome 9 DNA FPC (Finger Printed Contigs) map were selected to place on maize mitotic chromosomes using FISH (Fluorescent in situ Hybridization). FISH probes were produced as pooled PCR products based on sequences of the genetic markers or repeat-free parts of mapped BAC clones. Most probes were visualized on chromosome 9. Some of the probes hybridized to an unexpected position on chromosome 9 or to other chromosomes in two or more positions. The correlation of the sequence positions on the FPC map and cytogenetic maps of pachytene and somatic maize chromosomes and their resolutions were compared. The probe collection developed can be used as FISH landmarks or chromosome "banding paint" for physical mapping sequences including transgenes and BAC clones and for studying chromosomal rearrangements.

P62

Reactivation of Inactive B Centromeres in Maize

(submitted by Fangpu Han <hanf@missouri.edu>)
Full Author List: Han, Fangpu[1]; Gao, Zhi[1]; Lamb, Jonathan[1]; Yu, Weichang[1]; Birchler, James A.[1]
[1] Division of Biological Sciences, 117 Tucker Hall, University of Missouri-Columbia, MO, 65211-7400

Recently, we discovered a B chromosome centromere, containing all of the normal DNA elements, translocated to the short arm of chromosome 9 but which was inactivated for centromeric function, a state that was epigenetically inherited over many generations. When normal B chromosomes were added to this genotype, thus supplying the long arm tip that is required for nondisjunction, the inactive centromere regained the property of nondisjunction causing chromosome 9 to be differentially distributed to the two sperm at high frequency or causing chromosome breakage and producing new translocations. B centromere fragments were found in outcrosses using pollen from the plants containing 9-Bic-1 and normal B chromosomes. The B centromere with 9S chromatin has been found in the progeny of these crosses with centromere specific sequences CentC and CRM only detected in the B centromere region, together with the 9S specific DNA marker bz. CenH3 antibody results indicated that the B centromere has signal, indicating the inactive centromere has regained activity. Furthermore, we found a different type of reactivation phenomenon in the progeny of a new dicentric chromosome with one inactive centromere (stable for several generations) which came from the breakage-fusion-bridge cycle of a B-9Dp-9 chromosome. Meiotic analysis of this dicentric chromosome revealed that there is 23.48% bridge formation at AII, but no bridges occurred at AI. Reactivation of this inactive centromere was only found in meiosis II. In some cases, when the dicentric chromosome entered metaphase II and started anaphase II, only one inactive sister centromere recovered function. This created a true dicentric and bridge formation whereas the other sister chromatid centromere remained inactive. These findings indicate an epigenetic component to centromere specification that is reversible.

P63

Using the Maize *plural abnormalities of meiosis1* (*pam1*) Mutant to Dissect the Role of the Telomere Bouquet in Pairing and Recombination

(submitted by Moira Sheehan <mjs224@cornell.edu>)
Full Author List: Sheehan, Moira J.[1]; Golubovskaya, Inna N.[2]; Pawlowski, Wojciech P.[1]
[1] Department of Plant Breeding and Genetics; Cornell University; Ithaca, NY, USA 14853
[2] Department of Molecular and Cell Biology; University of California Berkeley; Berkeley, CA, USA 94720

The telomere bouquet is a unique chromosome arrangement that occurs during prophase I of meiosis and is found in most species examined to date. The term describes the attachment of telomeres to the nuclear envelope (NE) followed by their clustering at a single location on the NE, generally juxtaposed to the microtubule organizing region. In maize, the bouquet forms at the end of leptotene, continues through zygotene, and dissolves in early pachytene. The coincidental timing of telomere clustering with chromosome pairing, recombination and synapsis suggests a role of the bouquet in these processes. Attachment of telomeres and confinement of chromosomes to a small area may assist the sequence-dependent homology search by limiting the physical volume for the search and facilitating constructive chromosome movements. Furthermore, since synapsis initiates at the telomeres, the bouquet may help catalyze pairing and synapsis.

The maize *plural abnormalities of meiosis1* (*pam1*) mutant is the best-characterized bouquet mutant in higher eukaryotes. In *pam1* plants, telomeres attach to the nuclear envelope but cluster slowly, if at all. This causes asynchrony starting at zygotene that will persist to the pollen stage, producing multiple-stage overlaps, and variably sized pollen and microspores. Homozygous plants are male and female sterile and have a distinct tassel phenotype. To understand the role of the bouquet in meiotic prophase events, we will examine early pairing interactions using a number of probes including chromosome paints and single loci probes in FISH, combined with the superb resolution of 3-dimensional deconvolution microscopy. We will investigate the timing of pairing and bouquet function with a time-course study spanning leptotene through pachytene in wild-type and *pam1* plants. We will also look for ectopic interactions of homeologous chromosome regions when nonhomologous pairing occurs in the *pam1* mutant using duplicated loci in syntenic regions of the genome.

P64

BIF2 and *BA1* Interact in Maize Axillary Meristem Development

(submitted by Andrea Skirpan <als152@psu.edu>)
Full Author List: Skirpan, Andrea[1]; Wu, Xianting[1]; McSteen, Paula[1]
[1] The Pennsylvania State University, Department of Biology, University Park, Pa 16802

Axillary meristems produce the vegetative branches and inflorescence structures of a plant. Proper axillary meristem development is essential to the growth and reproductive success of most plants. The "barren inflorescence" class of maize mutants has defects in axillary meristem initiation and/or development and is characterized by a reduced number of branches and spikelets in the inflorescence and floral defects.

barren inflorescence2, bif2, encodes a protein kinase co-orthologous to PINOID. To understand how BIF2 functions in axillary meristem development, we sought to identify and characterize components of its signaling pathway. BIF2 was used to screen yeast two-hybrid tassel and ear libraries, resulting in the identification of several classes of interacting proteins. Here, we report the physical interaction of BIF2 and BARRENSTALK1 (BA1), show that BA1 is phosphorylated by BIF2 and present a novel nuclear localization of BIF2. The BIF2;BA1 interaction is supported in vivo by double mutant analysis. This is the first report of a downstream signaling component in the PID/BIF2 signaling pathway.

P65

ZmTCI-1, a Myb Related Transcription Factor, is a Key Regulator of the Differentiation and Function of Transfer Cells

(submitted by Gregorio Hueros <gregorio.hueros@uah.es>)
Full Author List: Hueros, Gregorio[1]; Gomez, Elisa[1]; Perez, Pascual[2]; Sellam, Olivier[2]; Gerentes, Denise[2]; Paul, Wyatt[2]; Sanz, Yolanda[1]; Royo, Joaquin[1]
[1] Dpto. Biologia Celular y Genetica. Universidad de Alcal. Alcal de Henares, 28871 (Madrid). Spain.
[2] Cereal Functional Analysis Group. Biogemma SAS. 8, Rue des Freres Lumiere 63028 Clermont-Ferrand. Cedex 2. France.

The endosperm is a major component of the monocot seed, it stores nutrients that will be used by the embryo upon germination. Nuclei at the periphery in the syncitial endosperm will give rise to the aleurone in all the endosperm surface, except for the zone in contact with the placento-chalaza region. In this area, the "epidermal" cells develop into transfer cells, specialized in the transport of nutrients and developing seed defense.

ZmTCI-1 (Zm Transfer Cell Inducer-1; syn. ZmMRP-1) is a maize endosperm transfer cell specific gene, it encodes a transcription factor of the single-Myb domain family and it is expressed at the basal region of the syncitium since the earliest developmental stages. In transient expression experiments, ZmTCI-1 trans-activates the promoter sequences of transfer cell specific genes, some of which posses anti-microbial peptides features.

Based on these results, we proposed the implication of ZmTCI-1 in one of the following processes:
A. The regulation of defense mechanisms in the endosperm transfer cells
B. The control of the differentiation of the cells at the base of the endosperm towards transfer cells
C. The establishment of the endosperm apical-basal axis of development

In order to prove-disprove these hypothesis, we have tried to follow a reverse-genetics approach, ie manipulation of the expression of ZmTCI-1 followed by the observation of the resulting phenotype. We have searched for KO mutants in insertion collections in maize, and have tried antisense approaches in maize and ectopic expression approaches in tobacco, Arabidopsis and maize, in all cases and for different reasons unsuccessfully.

Finally, we have successfully expressed ectopically ZmTCI-1, obtaining unexpected evidences on the biological function of ZmTCI-1 in maize. This communication will discuss those evidences and their impact in our current understanding of transfer cell biology.

P66

A Putative Role for RNA Splicing in Maize Endosperm-Embryo Developmental Interactions

(submitted by Diego Fajardo <diegof@ufl.edu>)
Full Author List: Fajardo, Diego[1]; Gomez, Elisa[2]; Royo, Joaquin[2]; Tseung, Chi-Wah[1]; Hueros, Gregorio[2]; Settles, A. Mark[1]
[1] University of Florida, Gainesville-Florida 32611
[2] Universidad Alcala de Henares, Madrid, Spain

Endosperm-embryo interactions are an important but poorly understood aspect of seed development. The Rough endosperm3 (Rgh3) locus is involved in these interactions at a developmental level. rgh3 seed mosaics marked with the pr1 anthocyanin gene indicate that Rgh3 is required in the endosperm for the normal development of the embryo. Rgh3 also has an autonomous function in the embryo and is required for seedling viability. We identified a tightly-linked transposon-tag from the rgh3-70 allele. This transposon insertion disrupts a predicted splicing factor with a U2 snRNP auxiliary factor homology motif (UHM). UHMs are RNA recognition motif (RRM)-like domains that function in protein-protein interactions. Analysis of maize ESTs indicated that Rgh3 is expressed in endosperm tissue. Consistent with this expression pattern, the rgh3 phenotype is characterized by an overproliferation of the aleurone cells and aberrant development of the basal endosperm transfer cell layer. An analysis with endosperm cell type specific markers in mutant rgh3 seeds suggests that the basal endosperm transfer cell defect occurs after cell type specification. Rgh3 maps to the long arm of chromosome 5. Complementation tests with other seed mutants mapped to the same region indicated that Rgh3 is a novel locus. Our data suggest that RNA splicing may have an important role in endosperm-embryo developmental interactions.

P67

A Single Amino Acid Substitution in *TGA1* Liberated Maize Kernels by Affecting Gene Transcription

(submitted by Huai Wang <wang10@wisc.edu>)
Full Author List: Wang, Huai[1]; Zhao, Qiong[1]; Nguyen, Bao Kim[1]; Iniguez, A. Leonardo[2]; Nussbaum-Wagler, Tina[1]; Studer, Anthony J.[1]; Kaeppler, Shawn[2]; Doebley, John F.[1]
[1] Laboratory of Genetics, University of Wisconsin, Madison, WI 53706
[2] Department of Agronomy, University of Wisconsin, Madison, WI 53706

Teosinte glume architecture1 (Tga1), a member of sbp-box gene family encoding transcriptional regulators, controlled the liberation of the kernel from the stony fruitcase of teosinte during maize domestication. Genetic and evolutionary analyses have shown that there is only one fixed nucleotide difference between teosinte and maize in Tga1, which causes a Lys to Asp substitution at the 6th amino acid of the protein. Furthermore, there are no discernable differences in Tga1 mRNA expression between teosinte and maize alleles. To test if the Lys to Asp substitution controls a functional difference between teosinte and maize TGA1, we employed a protoplast transient assay system. We show that the Lys to Asp substitution transforms TGA1 into a transcriptional repressor, which likely underlies the differences in teosinte and maize glume architecture. We also compared the transcriptional profiles between immature ears from maize isogenic lines containing either Tga1 maize or teosinte alleles. The microarray data revealed that a large number of genes were affected significantly in gene expression. Our results demonstrate that a single molecular mutation in a key regulator can cause dramatic changes in both the transcriptome and phenotype.

P68

Analysis of *ramosa1*-Related Genes in Maize

(submitted by Becky Weeks <rlmauton@iastate.edu>)
Full Author List: Weeks, Becky[1]; Kessler, Sharon[2]; Vollbrecht, Erik[1]
[1] Department of Genetics, Developmental, and Cellular Biology; Iowa State University; Ames, IA 50011
[2] Institute for Plant Biology; University of Zurich; Zurich, Switzerland

The EPF family is composed of Cys2-His2 zinc finger proteins that are putative transcription factors. Several intronless, single-finger EPF genes, including the inflorescence branch regulator ramosa1, are preferentially expressed in or near meristems. Learning the specific functions of these genes may be useful in elucidating mechanisms of meristem function. One particular such gene, tentatively named epf261, has a possible role in tassel development and we have begun to characterize this gene. ESTs for epf261 have been recovered only from young tassel primordia. We mapped the gene to bin 2.04, using the IBM mapping population. To determine this gene's function, we obtained a potential knockout mutant using the maize-targeted mutagenesis (MTM) system. Two MTM lines appear to have the same MuDR transposon inserted 175 bp upstream of the most 5-prime end contained in EST sequences. Based on marker phenotypes in kernels of these families, MuDR, the autonomous element, has been inactivated, thus preventing excision of the transposon from the location of interest. We will also present initial analysis of epf261 transcription in various tissues by RT-PCR, and observations of greenhouse-grown plants that contain the Mu insertion. In addition to epf261, we are characterizing the new allele ramosa1-N408E, which we obtained from the maize genetics co-op stock center, by PCR and DNA gel blot analysis. Lastly, we present a couple images of anaphase spreads of general interest to the maize community, showing a chromosome double-bridge resulting from a four strand double crossover in an inversion heterozygote. These images were produced in an NSF-sponsored cytogenetics course at ISU taught by Tom Peterson and Dave Weber.

P69

Analysis of Maize Floral Development Mutants

(submitted by Beth Thompson <bethompson@berkeley.edu>)
Full Author List: Thompson, Beth E.[1]; Bartling, Linnea[1]; Hake, Sarah[1]
[1] Plant Gene Expression Center; Albany, CA 94710; Department of Plant and Microbial Biology, University of California, Berkeley, 94720

Maize produces two types of inflorescences, the tassel and the ear. The tassel produces staminate (male) flowers, and the ear produces produces pistillate (female) flowers. The floral meristem is determinate, and initiates a limited number of floral organs before being consumed by organogenesis. Flowers are initially bisexual, and sex determination occurs through the selective abortion of inappropriate sex organs. We are interested in understanding the genetic and molecular regulation of floral development and sex determination in maize. To this end, we are taking a genetic approach and are characterizing mutants involved in these processes. Here we report progress on the identification and characterization of three genes required for floral development and sex determination in the maize inflorescence. bearded-ear (bde) is required floral meristem determinacy, organ identity and carpel abortion in the tassel. We have recently cloned bde and found it encodes a MADS-box transcription factor. We are currently examining the expression pattern of bde in wild-type and mutants using RNA in situ hyrbidization. In addition, we are mapping two other regulators of inflorescence development. Ts*1374 is a dominant EMS-induced tassel seed allele that maps to the long arm of chromosome 1. fuzzy tassel (fzt) is a novel pleiotropic regulator of maize floral development that we are mapping using bulk segregant analysis.

P70

Analysis of the Maize Mutant Suppressor of *Sessile Spikelets1* (*Sos1*)

(submitted by Xianting Wu <xzw104@psu.edu>)
Full Author List: Wu, Xianting[1]; McSteen, Paula[1]
[1] Department of Biology, The Pennsylvania State University, 208 Mueller Lab, University Park, Pennsylvania 16802 USA

Suppressor of Sessile Spikelets1 (Sos1) is a semi-dominant locus (Doebley et al., 1995), mapped on the short arm of chromosome 4. Plants homozygous for Sos1 have a reduced number of tassel branches and single instead of paired spikelets. SEM analysis shows that the sessile spikelets are absent while the pedicellate spikelets are normal. Plants heterozygous of Sos1 have a weaker phenotype than homozygous plants, producing occasional paired spikelets. Also, tassel branch number in Sos1/+ plants is intermediate between Sos1/Sos1 plants and wild type. This indicates that Sos1 may function in branch and spikelet meristem initiation.

Other genes have been identified to play a role in axillary meristem initiation, such as barren inflorescence 2 (bif2), a gene regulating auxin transport and barren stalk 1 (ba1), a bHLH transcription factor. To study Sos1 interaction with these inflorescence genes, we generated double mutants. Genetic and chi-square analysis shows that bif2 and ba1 are epistatic to Sos1 indicating that Sos1 might be involved in auxin transport or response.

To clone Sos1, we used chromosome walking. Sos1 in the B73 background was crossed to Mo17 to generate our mapping population. SSR and IDP markers near Sos1 on chromosome 4 were used to screen for polymorphisms between the two genetic backgrounds. So far we have narrowed down the region containing Sos1 to between two closely linked markers within two FPC BAC contigs.

P71

Axillary Meristem Development in Maize *grassy tillers1* Mutants

(submitted by Candice Hansey <cnhansey@wisc.edu>)
Full Author List: Hansey, Candice N[1]; de Leon, Natalia[1]
[1] Department of Agronomy, Plant Breeding and Plant Genetics; University of Wisconsin; Madison, WI 53706

Biomass derived from plant material is economically important for the future of the biofuel industry in the USA. One important aspect of increasing plant biomass, primarily in the grasses, involves lateral branch (tiller) formation. Both genetic and environmental factors such as light quality and quantity affect axillary meristem activation and subsequently lateral branch formation in plants. The grassy tillers1 (gt1) gene plays a role in axillary branch development in maize. Isogenic lines W64A and W64Agt1/gt1 were used to evaluate the genome modulation effect of gt1 mutant alleles and their interaction with light conditions using long oligonucleotide spotted microarrays. Analysis of the transcriptional profiles in these conditions will be presented. Fluorescent microscopy imaging with a rhodamine and mixed filter was performed to visualize axlliary meristem activation and development. Images of gt1 mutant and wild-type plants ranging from ten to twenty days after germination will be presented.

P72

Biochemical analysis of kernels with haploid and diploid embryos in maize

(submitted by Valeriy Rotarenco <Valeriy_Rotarenco@hotmail.com>)
Full Author List: Rotarenco, Valeriy A[1]; Kirtoca, Ilea H[1]; Jacota, Anatol G.[1]
[1] Institute of Genetics and Physiology, Padurii 20, Chisinau, Moldova, MD-2002

Using haploid plants in breeding work and for genetic researches becomes more and more intensive. However, due to the inhibition of the R1-nj-gene expression (marker gene allows haploids to be identified), there could be problems during haploid-seed screening. Thus, there is a need to find an alternative way for the identification of haploids at the level of dry seeds.

It was assumed that kernels with haploid and diploid embryos differ by their biochemical structure, especially by oil content.

Haploid and diploid kernels (hybrids with the MHI haploid inducer (Chalyk, 1999)) of eight genotypes (4 inbred lines and 4 hybrids) have been used for a biochemical analysis.

First, it was revealed a significant excess of the oil content in the diploids over the haploids. The results of this test have been sent to Maize Genetics Cooperation Newsletter, volume 81.

In further work, protein content was estimated and no significant difference between haploids and diploids was found. Then, the biochemical structure was estimated in endosperms, separately. It was revealed that the endosperms of the haploids and the diploids did not differ by their biochemical structure (oil and protein).

Based on the obtained results the following conclusions were made: kernels with haploid and diploid embryos significantly differ by oil content; this difference is caused by the oil concentrated in embryos; ploidy of embryos does not influence on the biochemical structure of endosperms.

For practical purposes an oil test (on the basis of spectral analysis) can be applied as a marker to identify haploid kernels.

P73

Bioinformatic, Expression and DNA Sequence Diversity Characterization of Two Shoot Apical Meristem Expressed Genes

(submitted by Brent Buckner <bbuckner@truman.edu>)
Full Author List: Swaggart, Kayleigh[1]; Wong, Cheryl[1]; Smith, Heath[1]; Aurand, Kelsey[1]; Schnable, Patrick S.[2]; Janick-Buckner, Diane[1]; Buckner, Brent[1]
[1] Truman State University, Kirksville, MO 63501
[2] Iowa State University, Ames, IA 50011

The shoot apical meristem (SAM) is the source of all above-ground plant tissues. In an ongoing collaboration we have used cDNA microarray technology to identify genes that are differentially expressed in the maize SAM when compared to whole seedling. Two such shoot apical meristem expressed genes (SEGs) were selected for further bioinformatic, expression and nucleotide diversity analyses. Bioinformatic resources including BLASTn, BLASTx, RepeatMasker, InterProScan and Protcomp were used to further evaluate these SEGs. These analyses revealed that SEG1 exhibited no similarity to any known protein or functional motif, while InterProScan of SEG2 demonstrated a region with weak similarity to a cyclin F-box domain. RT-PCR for both SEG1 and SEG2 was performed on mRNA isolated from thirteen different maize tissues. This study indicated that SEG1 and SEG2 were expressed in the majority of these tissues. Approximately 650 bp regions of both SEG1 and SEG2 containing both exonic and intronic sequence were amplified and sequenced from seven North American inbreds, five New Mexican open-pollinated landraces, five Mexican open-pollinated landraces and nine accessions of teosinte. The nucleotide diversity among the maize and teosinte lines is consistent with neutral evolution at both of these loci.

P74

Bladekiller1 is Required for Meristem Maintenance and Leaf Development in Maize

(submitted by John Woodward <jbw46@cornell.edu>)
Full Author List: Woodward, John B.[1]; Freeling, Michael[2]; Scanlon, Michael J.[1]
[1] Cornell University; Ithaca, NY 14853
[2] University of California-Berkley; Berkeley, CA 94704

BLADEKILLER1 (BLK1) is required for maintenance of shoot and axillary meristems and affects development of the leaf blade. The phenotype of mature blk1 mutants shows a successive reduction in leaf blade width and length that is first apparent in the upper juvenile leaves and becomes more severe in adult leaves. Leaves from upper adult nodes are often bladeless; however, all ligules, auricles, and sheaths are unaffected. Plants with the blk1 phenotype also fail to develop functional reproductive organs, although reduced tassels and ears may form in less severe mutant plants. SEM analysis has revealed that blk1 shoot apical meristems (SAMs) are often smaller in size than their wild-type siblings, suggesting a deficiency in meristem maintenance. Additionally, immature blk1 tassels have a reduced number and irregular pattern of spikelet meristems. Expression analysis using qRT-PCR has revealed that several genes implicated in meristem development, including TD1, ABPHL1, and KN1, have reduced transcript accumulation in mutant axillary meristems. Recently, the blk1 locus has been mapped to a distal region of chromosome 3L between the SSR markers umc2152 and umc1717. An immediate goal of this project is to identify the mutated locus and eventually characterize BLK1 function at a molecular level. Characterizing BLK1 will provide a better understanding of events controlling the fate of meristem populations in maize and may identify key connections between leaf and meristem development.

P75

Characterisation of an Empty Pericarp Mutant Line, Ep-2312, Impaired in the Development of a Transfer Cell Layer

(submitted by Gregorio Hueros <gregorio.hueros@uah.es>)
Full Author List: Paniagua, Carlos[1]; Lopez, Maribel[1]; Peacock, Stephanie O.[2]; Maruhnich, Estephanie[2]; O'Brien, Brent A.[2]; Koch, Karen E.[2]; Hueros, Gregorio[1]
[1] Dpto. Biologia Celular y Genetica. Universidad de Alcale. Alcale de Henares, 28871 (Madrid). Spain.
[2] Plant Molecular and Cellular Biology Program, Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, USA.

The endosperm outer cell layer differentiates into the aleurone, which encloses the embryo-endosperm tissues almost completely. At the base of the maize endosperm, however, cells switch to a different differentiation program leading to the formation of a transfer cell layer. Transfer cells (TC) contain numerous cell wall ingrowths and are specialized in the transport of nutrients from the pedicel into the growing endosperm.

We have approached the study of the regulatory pathways controlling transfer cell development through the characterization of TC-specific regulatory genes (see poster "ZmTCI-1, A myb-Related Transcription...") and Mu-induced mutant lines affected in TC development.

Here we describe the characterisation of a mutant line screened out from the Uniform-Mu collection. This line belongs to the empty pericarp class of mutants, which at maturity show only residual remnants of endosperm tissues. Morphological studies showed, however, that the development of the mutant endosperm is approximately normal up to 8-10DAP. Latter on, when the grain filling phase starts, the mutant endosperms clearly depart from the developmental pathway followed by their wild type siblings and eventually abort. Molecular analyses of this line using TC and aleurone specific probes and antibodies show that the basal cell layer of the Ep-2312 endosperms acquire aleurone, rather than TC, identity. Our results suggest that the lack of a functional transfer cell layer might be a primary cause of the failure to accomplish the grain filling phase observed in the mutant endosperms.

P76

Characterization of Maize Gene *Developmental disaster1*

(submitted by Kimberly Phillips <kap262@psu.edu>)
Full Author List: Phillips, Kimberly[1]; Skirpan, Andrea[1]; Kaplinsky, Nick[2]; McSteen, Paula[1]
[1] The Pennsylvania State University; Department of Biology; University Park, PA, 16802
[2] Swarthmore College; Department of Biology; Swarthmore, PA, 19081

Plant hormones play key roles in various aspects of growth and development. Auxin is a particularly important plant hormone that is known to control such factors as shoot elongation, inflorescence development, and root development. A large number of mutations have been observed which affect the regulation and function of auxin.

The semi-dominant maize mutant Developmental disaster1 (Dvd1) has been identified as a potential disruptor of auxin signaling. Dvd1 mutant plants exhibit developmental abnormalities in shoots and roots, including shortened internodes, twisted stems, and underdeveloped roots, as well as a barren inflorescence phenotype in the tassel. Homozygous mutants incur the most severe defects, even limiting survival in some cases to no more than five weeks. In addition, Dvd1 mutants have revealed different levels of severity in B73 and Mo17 genetic backgrounds. Heterozygotes in the B73 background resemble wild-type plants and show few developmental irregularities beyond reduced height and fewer tassel branches. Conversely, Mo17 may contain a background modifier of the gene and clearly exhibits severe mutant developmental phenotypes in both Dvd1 hetero- and homozygous plants.

Preliminary quantitative analyses for Dvd1 phenotypes have been performed using data for plant height, leaf, and ear counts. In addition, Scanning Electron Microscopy has been used to analyze the barren inflorescence phenotype in developing meristems. Double mutant analyses are also in progress to further characterize the role of Developmental disaster1 in different maize signaling pathways. Finally, the region of the Dvd1 gene has been narrowed to 3 contigs on chromosome 5 using simple sequence repeat primers and a mapping population of about 1400 individuals.

P77

Characterization of Maize Mutants with Polarity Defects

(submitted by Hector Candela <hcandela@nature.berkeley.edu>)
Full Author List: Candela, Hector[1]; Gerhold, Abby[1]; Stevenson, Scott[1]; Zlotnicki, Monica[1]; Hake, Sarah[1]
[1] Plant Gene Expression Center; USDA and University of California at Berkeley; Albany, CA, 94710

We have recently cloned *milkweed pod* (*mwp*), a gene that is required for abaxial identity in normal leaves. In addition to the *mwp-R* reference allele, which carries a retrotransposon insertion, we have isolated four additional alleles in targeted tagging screens. We will report the analysis of these new alleles and the characterization of the expression pattern of *mwp* by in situ hybridization and RT-PCR. Similar to *mwp*, the dominant *Rough sheath4* (*Rs4*) mutation causes extended cell proliferation of abaxial mesophyll layers in the sheath of maize leaves. A more severe phenotype is observed after five generations of backcrossing *Rs4* in the Mo17 inbred background, but not in B73. To identify the modifiers responsible for the observed phenotypic differences, we have begun crossing B73- and Mo17-introgressed *Rs4* mutants with the recombinant inbred lines of the IBM population. We plan to take advantage of the dominance of *Rs4* and the genotypic scores available for the IBM lines to map QTL that affect cell proliferation in the F1 progeny of these crosses.

Wavy auricle in blade1 (*Wab1*) is a dominant mutation that disrupts the proximal-distal pattern of maize leaves. A positional cloning approach has led us to place the gene in a relatively small candidate interval. Our progress towards the molecular identification of *Wab1* among the existing candidate genes will also be presented.

P78

Cloning and Characterization of *vanishing tassel1* (*vt1*), A Barren Inflorescence Mutant of Maize

(submitted by Solmaz Barazesh <sxb944@psu.edu>)
Full Author List: Barazesh, Solmaz[1]; Skirpan, Andrea[1]; McSteen, Paula[1]
[1] The Pennsylvania State University, University Park, PA 16802

vanishing tassel1 (vt1) mutants have a barren inflorescence phenotype with reduced numbers of tassel branches, spikelets and floral organs, caused by a failure to initiate or maintain inflorescence axillary meristems.

Previously studied barren inflorescence mutants barren inflorescence2 (bif2) and barrenstalk1 (ba1) have been shown to have defects in auxin transport or signaling. However, vt1 has additional phenotypes, such as defects in the apical meristem and feminization of the tassel, which are not observed in the other barren inflorescence mutants. Studies of double mutants between vt1 and either bif2 or ba1 suggest that vt1 acts in a separate pathway.

vt1 has been cloned using a transposon tagging approach, and appears to encode a novel protein. Work is underway to further elucidate the function of VT1 in inflorescence and axillary meristems.

Phenotypic characterization, double mutant analyses and preliminary data on cloning vt1 will be presented.

P79

Complex Patterns of APETALA1/FRUITFULL-like Gene Expression in Grasses: Implications for Spikelet Development

(submitted by Jill Preston <jcpxt8@studentmail.umsl.edu>)
Full Author List: Preston, Jill C[1]; Kellogg, Elizabeth A.[1]
[1] University of Missouri - Saint Louis, Department of Biology - R223, One University Blvd, Saint Louis, MO 63121

APETALA1/FRUITFULL (AP1/FUL) show distinct but overlapping patterns of expression in maize (Zea mays) and rice (Oryza sativa) suggesting discrete functional roles in the morphological transition to flowering, specification of spikelet meristem identity, and specification of floral organ identity. In this study, we analyzed the expression of recently duplicated genes FUL1 and FUL2 across phylogenetically disparate grasses to test hypotheses of gene function. In combination with other studies, our data support a conserved and redundant role for both genes in floral meristem identity, a general role for FUL1 in floral organ identity, and a more specific role for FUL2 in outer floral whorl identity. In contrast to Arabidopsis AP1/FUL genes, expression of FUL1 and FUL2 is consistent with an early role in the morphological transition to flowering. In general FUL1 has a wider expression pattern in all spikelet organs than FUL2, but both genes are expressed throughout spikelet organs of some cereals. We hypothesize that FUL1 and FUL2 have multiple redundant functions in early inflorescence development, and implicate sub-functionalization of FUL2 in the modification of outer floral organ identity.

P80

DNA Sequence Diversity of the Gene Encoding the *Rough Sheath2* Interacting KH-Domain Protein Among Inbred Lines and Open-pollinated Landraces

(submitted by Brent Buckner <bbuckner@truman.edu>)
Full Author List: Wong, Cheryl[1]; Swaggart, Kayleigh[1]; Smith, Heath[1]; Aurand, Kelsey[1]; Schnable, Patrick S.[2]; Janick-Buckner, Diane[1]; Buckner, Brent[1]
[1] Truman State University, Kirksville, MO 63501
[2] Iowa State University, Ames, IA 50011

The shoot apical meristem (SAM) is the source of all above-ground plant tissues. Previously, Rough Sheath2 Interacting KH-domain Protein (RIK) was implicated in SAM development (Phelps-Durr et al. 2005). Microarray hybridization experiments confirmed that the RIK gene was differentially expressed in SAM when compared to whole seedlings. RT-PCR performed on mRNA isolated from thirteen different maize tissues verified that RIK was expressed in SAM-enriched tissue, as well as other developmentally distinct tissues. An approximately 500 bp region of RIK containing both exonic and intronic sequence was amplified and sequenced from seven North American inbreds, five New Mexican open-pollinated landraces, five Mexican open-pollinated landraces and nine accessions of teosinte. When compared to the majority of similarly analyzed maize genes RIK showed low nucleotide diversity, which is consistent with selection at this locus.

P81

Differential Expression of Actin Isovariants During Maize Seed Development

(submitted by Magdalena Segura-Nieto <msegura@ira.cinvestav.mx>)
Full Author List: Godinez-Martinez, Jose-Luis[1]; Segura-Nieto, Magdalena[1]
[1] Departamento de Ingenieria Genetica. Cinvestav Campus Guanajuato. Irapuato, Gto. Mexico. PO Box 629. CP 36500

Most plant and animal Cytoskeleton genes are members of gene families that are differentially expressed and encode diverse protein isovariants. These isovariants can be divided in two classes, depending on their expression pattern: vegetative and reproductive. Maize has eight functional actin genes. Our studies show differential expression of the ZmACT1, ZmACT81 and ZmACT87 genes during kernel development. The ZmACT1 gene is mainly expressed in embryo rather than endosperm. In the embryo, its expression increases slightly while in endosperm it was kept almost constant decreasing up to 35 days after pollination (DAP). This gene is not expressed in the ovary, indicating that it is vegetative. ZmACT81 shows strong expression in the ovary, which decreases after the pollination. A low level of expression remains even at 35 DAP, however no expression of ZmACT81 in endosperm from 15 DAP onwards was observed, supporting their classification as a reproductive gene. The gene ZmACT87 is highly expressed in embryos of 20 DAP and this expression levels remains at 35 DAP. Expression of this gene should be to be studied until seed is observed mature. In endosperm a similar pattern of expression with lower intensity is observed. We do not detect ZmACT87 expression in leaves and anthers, suggesting that this gene is vegetative tissue specific. A polyclonal antibody against gamma-actin from non muscle cells, detected a 43 kDa band of maize actin in a Western blot, from a total protein endosperm extract, but it was barely detectable in the embryo, suggesting differential expression of an actin isovariant between these tissues.

P82

Differential Gene Expression of Sbp-Box Genes in *Zea mays mop1-1* Mutants

(submitted by Judd Hultquist <judd.hultquist@mu.edu>)
Full Author List: Hultquist, Judd F.[1]; Dorweiler, Jane E.[1]
[1] Marquette University, Department of Biological Sciences, Milwaukee, WI 53201-1881

Regulation of the transition from vegetative to reproductive states in *Zea mays* is largely enigmatic. One regulator of this pathway is hypothesized to be *mediator of paramutation 1* due to the varied and abnormal phenotypes displayed by plants homozygous for the recessive, nonfunctional *mop1-1* mutation. The *mop1* mutation was identified based upon its ability to up-regulate the expression of *B'*, resulting in darkly pigmented plants. The *mop1* mutants also tend to display around a week's delay in flowering time, and may be sexually compromised in the tassel becoming either barren or feminized. It has been a focus of our lab to identify genes downstream of *mop1* in the floral transition pathway by searching for differential gene expression among the variant tassel phenotypes of assorted candidate genes.

We are currently studying numerous genes with high sequence similarity to the *SPL* (*SQUAMOSA Binding Protein-Like*) gene family in Arabidopsis. This family is characterized by the presence of the unique DNA-binding motif called an SBP-box (*SQUAMOSA Binding Protein*). Expression analyses were carried out using Real-Time RT-PCR on a number of immature tassel samples dissected from individuals of a family segregating for the *mop1-1* mutation. The three genes *tga1* (*teosinte glume architecture1*), *SBP5*, and *SBP6* were found to be up-regulated specifically in individuals with a feminized tassel, signifying a potentially indirect, downstream effect of the absence of the MOP1 protein. A unique feature shared by these three genes is the presence of a putative miR156 binding site, suggesting a potential role of microRNAs in their regulation. Future work will focus on elucidating the normal regulatory mechanism of these SBP-box genes, and how that may be disrupted by the lack of *mop1* function.

P83

Discolored1 (*DSC1*) Function in Maize Kernel Development

(submitted by Elizabeth Takacs <emt32@cornell.edu>)
Full Author List: Takacs, Elizabeth M[1]; Suzuki, Masaharu[2]; Scanlon, Michael J.[1]
[1] Department of Plant Biology, Cornell University, Ithaca, NY 14850
[2] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611

Defective kernel (dek) mutants disrupt the development of both the endosperm and the embryo. Although they exhibit clonally separate development following double fertilization, it is possible that the endosperm and embryo interact throughout kernel development. Therefore, developmental analyses of dek mutants afford an opportunity to investigate developmental signaling mechanisms during kernel morphogenesis. Several studies of dek mutants have identified hundreds of genes involved in kernel development although relatively few of these genes have been investigated further to determine function of their corresponding proteins. One such gene, discolored1 (dsc1), was identified in a screen of dek mutants from Mutator (Mu) stocks. dsc1 mutants have discolored brownish kernels with a rough texture and a poorly developed embryo that aborts after initiation of one or two leaf primordia. Previously, a fragment of the dsc1 locus was cloned by Mu transposon tagging. The dsc1 full length cDNA was obtained using RACE RT-PCR with primers anchored in an EST encoded within this genomic fragment. This corresponding sequence putatively encodes an ADP-ribosylation factor GTPase activating protein (ARF-GAP). ARF-GAPs are involved in vesicle membrane trafficking and actin remodeling through the hydrolysis of an active GTP bound ARF to an inactive GDP form. Arabidopsis dsc1 homologue SCARFACE/VASCULAR NETWORK DEFECTIVE3 (SFC/VAN3) studies have revealed that SFC encodes an ARF-GAP which is necessary for auxin efflux and vascular patterning. To better characterize the function of DSC1 in kernel development, we will perform detailed investigations of the dsc1 mutant embryo and endosperm phenotypes, generate additional mutant alleles through reverse genetics, examine DSC1 function during vesicle trafficking and auxin efflux, and perform complementation studies of Arabidopsis scf/van3 mutants.

P84

Expression and Functional Characterization of *zmco1*, a Putative Floral Regulator

(submitted by Theresa Miller <theresa.miller@marquette.edu>)
Full Author List: Miller, Theresa A.[1]; Dorweiler, Jane E.[1]
[1] Marquette University; Department of Biological Sciences; Milwaukee, WI, USA 53201

Very little is known about what regulates the transition to reproductive growth in *Zea mays*. We are studying a gene homologous to the *Arabidopsis thaliana CONSTANS*, a floral activator. The maize gene, *zmco1*, also has significant sequence similarity to *Hd1*, a rice floral regulator. Given the similarity *zmco1* shares with *CONSTANS* and *Hd1*, we hypothesize that *zmco1* also plays a role in floral regulation and may provide information about floral induction in maize.

To understand the function of *zmco1*, we are first characterizing its expression. *CONSTANS* and *Hd1* exhibit circadian rhythms. Circadian rhythms are periodic fluctuations of approximately 24 hours, established and regulated by the circadian clock. The clock is "set" in response to environmental cues such as diurnal light cycles, thus fluctuations are maintained even after loss of diurnal input. Here, we report that *zmco1* demonstrates diurnal fluctuations but fails to maintain a free-running rhythm. Given these results, *zmco1* does not appear to be regulated by the circadian clock.

The hypothesized function of *zmco1* is being tested using complementation studies. The maize gene will be transformed into the *constans* and *hd1* mutants of *Arabidopsis* and rice, respectively. This will be done using the *zmco1* cDNA under control of a strong promoter and also by using the *zmco1* native promoter and genomic sequence. The studies involving the *zmco1* cDNA are in progress; others hinge upon complete *zmco1* sequence information. In order to obtain the genomic sequences, we utilized of the synteny between the genomes of maize and rice and the oat-maize addition lines to confirm the location of *zmco1*. Furthermore, we identified a series of maize BAC clones likely to span the *zmco1* region. We have confirmed the presence of *zmco1* in one clone, have sub-cloned a fragment of this BAC containing *zmco1*, and are proceeding with additional complementation studies.

P85

Genetic and Molecular Characterization of Two Pollen-Specific *stk* Paralogs

(submitted by Jun Huang <junhuang@waksman.rutgers.edu>)
Full Author List: Huang, Jun[1]; Park, Wonkeun[1]; Dooner, Hugo K.[1]
[1] Waksman Institute, Rutgers University, Piscataway, NJ 08854
[2] Dept. of Plant Biology, Rutgers University, New Brunswick, NJ 08901

Paternal transmission is an exquisitely sensitive assay of pollen gene function. Mutants in genes that function in the male gametophyte often show reduced male transmission. We report here on two pollen-specific *stk* (serine threonine kinase) paralogs that may perform partially redundant functions in pollen development.

stk1 is the proximalmost gene in the *bz* gene island on chromosome 9. Its predicted protein has high similarity to serine/threonine protein kinases, hence its name. *stk2* is a closely related gene located on long arm chromosome 4 (BAC ZMMBBc0137B12). Both *stk1* and *stk2* are expressed only in pollen and the mature tassel and not at all in other tissues. The *stk* genes likely function in pollen tube growth since mutations of *stk1* show reduced pollen transmission only when competing with wild type pollen. Three *stk1* mutations, generated by *Ac* transposition events, reduce pollen transmission efficiency by as much as 40%. Possibly, *stk2* has a redundant role and complete elimination of both results in pollen lethality. We are trying to isolate mutations in *stk2* to test this possibility.

Maize is considered an allotetraploid that arose < 10 MYA. With 86% nucleotide identity and 68% amino acid identity throughout the entire sequence, one would assume that *stk1* and *stk2* are the orthologous genes of the two progenitors. However, each of these two genes has a remarkably closer counterpart in *Oryza sativa*, which diverged from maize 50 MYA. The respective STK proteins in rice are 83% identical to STK1 and 78% identical to STK2. Hence the gene duplication happened before the maize-rice speciation event and the *stk1* and *stk2* genes are paralogs, not orthologs.

P86

Identification of Genes Involved in Lateral Root Formation in Zea mays

(submitted by Roman Zimmermann <roman.zimmermann@zmbp.uni-tuebingen.de>)
Full Author List: Zimmermann, Roman[1]; Hochholdinger, Frank[1]
[1] University of Tuebingen, ZMBP, Dept. of General Genetics, Auf der Morgenstelle 28, 72076 Tuebingen, Germany

In contrast to other plant model systems, cereals such as Zea mays develop a complex root system architecture. The root system of the young maize seedling is established during embryogenesis and consists of a radicle and a variable number of seminal roots. Early during post-embryonic development, embryonic roots are functionally replaced by a shoot-borne root system which constitutes the major backbone of the adult maize root stock. As a common characteristic, all root types form lateral roots. Lateral roots significantly enhance the overall surface of the root stock and thus ensure sufficient uptake of water and nutrients as well as anchorage of the plant in the soil. Lateral root formation therefore is an essential determinant for plant vigour and crop yield and represents a key process in the genetic control of root development.

The study of the molecular basis of lateral root formation in cereals has only recently been initiated. The phenotypes of two maize mutants, lateral rootless 1 (lrt1; Hochholdinger and Feix, 1998) and rootless with undetectable meristems 1 (rum1; Woll et al., 2005) suggest that lateral root initiation at the primary root and the shoot-borne root system may in part be controlled by different genetic pathways

To gain a deeper understanding of its molecular network, we initiated different strategies to identify novel genes involved in lateral root formation in maize. Sequence and expression data for three genes isolated in a homology-based approach suggest different roles during early stages of lateral root formation in all main root types. Two other projects will focus on uncovering pathways potentially conferring lateral root formation in a root-type specific manner.

P87

Interplay Between Light, High Temperature, and *Hsp101* Activity in the Control of the Emergence of Adventitious Roots at the Coleoptilar Node in Maize Seedlings

(submitted by Jorge Nieto-Sotelo <jorge@ibt.unam.mx>)
Full Author List: Lopez-Frias, Guillermo[1]; Martinez, Luz Maria[1]; Nieto-Sotelo, Jorge[1]
[1] Instituto de Biotecnologia; Universidad Nacional Autonoma de Mexico; Cuernavaca, Morelos, Mexico 62210

Adventitious roots (AR) constitute the prevalent root system of adult maize plants. AR originate from stem nodes located below and above ground and little is known about their triggering factors. We found that a single heat shock induced the formation of AR in young maize seedlings grown in the dark. Since the emergence of the first AR from the coleoptilar node appeared to correlate with the loss of viability of the primary root, we studied the influence of the primary root or the root cap on AR formation. We found that the loss of viability of the primary root or the root cap are not the primary signal for AR formation.

Young (36 and 60 h) seedlings required higher heat-shock temperatures to achieve maximal induction of AR than old seedlings (84 h). This observation paralleled the higher basal heat-shock resistance of young versus old seedlings and correlated positively with the levels of embryo-accumulated heat shock protein Hsp101. Moreover, the higher resistance of the coleoptile, relative to the primary root, also correlated with the levels of Hsp101, which persisted for a longer time in the coleoptile during post-germinative growth.

In a homozygous hsp101-m5::Mu1 mutant, AR emerged in seedlings grown at optimal temperature. At 36 and 60 h, the emergence of AR was maximal in hsp101-m5::Mu1 seedlings after heat shocks between 40 to 45 C, whereas in the wild type maximal response was between 45 to 49 C. At 84 h, AR induction, in both mutant and wild type seedlings, occurred between 28 and 45 C. If exposed to light, wild type seedlings also triggered the emergence of AR at optimal temperature. We suggest that Hsp101 assists a developmentally-regulated switch that represses the appearance of AR at the coleoptilar node in young seedlings and that light can abrogate this negative effect at any moment.

P88

Intraspecific Evolution of Regulatory Factors Controlling Leaf Identity in Maize

(submitted by Stephen Moose <smoose@uiuc.edu>)
Full Author List: Moose, Stephen P.[1]; Zhang, Wei[1]; Lauter, Nick[2]
[1] University of Illinois; Department of Crop Sciences; Urbana, IL, 61801
[2] USDA-ARS; Corn Insects and Crop Genetics Research; Ames, IA, 50011

Shoot development in many higher plant species is characterized by phase change, where meristems and organs transition from one set of identities to another. In maize, the first 5-6 juvenile leaves differ from the upper adult leaves in their expression of epicuticular waxes, specialized cell types such as macrohairs, and cell wall characteristics. These leaf identity traits provide tolerance to environmental stresses and resistance to disease, insect pests, and herbivory. Thus, the regulation of leaf identity may contribute to environmental adaptation in both natural populations and crop plant species.

Molecular genetic analyses in maize and Arabidopsis have identified two classes of microRNA-transcription factor interactions that regulate vegetative phase change and leaf identity. The onset of miR172 expression during early maize shoot development downregulates the expression of the AP2-like gene GLOSSY15 to promote adult leaf identity; conversely, Arabidopsis miR156 reduces the expression of SQUAMOSA BINDING PROTEIN-LIKE (SPL) genes that are required for adult leaf identity. Significant phenotypic diversity in maize exists for the timing of the transition from juvenile-to-adult leaf identity, both among defined mutations and breeding populations, which we hypothesize to be regulated primarily by the relative activities of the miR172-AP2 and miR156-SPL interactions. Support for this model is obtained from analyses of molecular diversity in haplotypes and expression patterns among GLOSSY15 and maize members of the miR172, miR156, and SPL gene classes. Further characterization of this genetic network will elucidate how dosage-dependent interactions among antagonistic regulatory factors can specify phenotypic diversity in a developmental pathway.

P89

Is ZmTCRR-1 a Molecular Messenger Connecting Different Seed Compartments?

(submitted by Gregorio Hueros <gregorio.hueros@uah.es>)
Full Author List: Muniz, Luis M.[1]; Bergareche, Diego[1]; Baudot, Gaelle[2]; Rouster, Jacques[2]; Paul, Wyatt[2]; Gerentes, Denise[2]; Hueros, Gregorio[1]
[1] Dpto. Biologia Celular y Genetica. Universidad de Alcal. Alcal de Henares, 28871 (Madrid). Spain.
[2] Cereal Functional Analysis Group. Biogemma SAS. 8, Rue des Freres Lumiere 63028 Clermont-Ferrand. Cedex 2. France.

Response regulators are signal-transduction molecules present in bacteria, yeast and plants, acting as relays for environmental challenges and hormonal regulation of cell metabolism. We present the characterization of ZmTCRR-1, a Zea mays gene that codes for a member of the type-A response regulator class of proteins. The gene was found to be expressed exclusively in the endosperm transfer-cell layer 8-14 days after pollination, when transfer-cell differentiation is most active. The promoter of ZmTCRR-1 contains motifs similar to the recently described BETL-box, and we show a strong transactivation by the transfer cell-specific transcription factor ZmTCI-1, a SHAQYF-type BETL-specific transcription factor. At developmental stages later than 11 DAP the protein is detected not in the transfer cell layer but in inner areas of the endosperm, where no transcript is detected. This suggests that two-component systems might be involved in intercellular signal transmission in the maize seed, providing a developmentally regulated signalling mechanism between BETL and starchy endosperm development. We have started the characterization of ZmTCRR-1 function using both ubiquitous and seed specific RNAi technology.

P90

Map-based Cloning of *ragged seedling2*: A Gene Required for Lateral Leaf Expansion in Maize

(submitted by Ryan Douglas <rnd4@cornell.edu>)
Full Author List: Douglas, Ryan N[1]; Henderson, David C[2]; Wiley, Dan[2]; Scanlon, Michael J.[1]
[1] Cornell University, Plant Biology Department, Ithaca, New York 14853
[2] University of Georgia, Plant Biology, Athens, Georgia 30602

RAGGED SEEDLING2 (RGD2) plays a role in lateral leaf expansion in maize from the point of leaf initiation and continues throughout development. Plants homozygous for the recessive mutation rgd2-R exhibit mutant phenotypes that can vary from mildly narrow leaves to more severe radial leaves. Unlike other radial leaf mutants, rgd2 radial leaves contain both adaxial and abaxial tissue types, though some swapping of tissue types may occur. rgd2 vasculature also maintains collateral alignment (xylem is adaxial to phloem) in radial leaves, whereas other radial leaf mutants have amphivasal (xylem surrounds the phloem) or amphicribal (phloem surrounds the xylem) vascular alignment in addition to adaxialized or abaxialized leaves. RGD2 may play a role in establishing and/or maintaining transcript accumulation of adaxial/abaxial identity genes. The morphology of plants harboring mutations in rgd2 and leaf bladeless1 (lbl1; which encodes a maize SGS3 ortholog and is required for the production of ta-siRNAs) are similar. Double-mutant analysis for rgd2-R/lbl1-rgd1 was performed and suggests these two genes function in separate, but redundant or overlapping, pathways. A map-based approach is being utilized to clone rgd2. New markers for mapping have been developed from BAC and BAC-end sequences. rgd2 is currently placed within a ~900 kbp region between BAC-end c0276M21 CAPS1 (ctg 19) and BAC-end b0586C02 SNP1 (ctg 20) on the short arm of chromosome 1. We are using the RescueMu lines and Ac/Ds mutagenesis in an attempt to obtain additional alleles of rgd2.

P91

Mapping and Characterization of the *Fascicled ear1* Mutation in Maize

(submitted by China Lunde <lundec@berkeley.edu>)
Full Author List: Lunde, China F[1]; Ma, Lan[1]; Hake, Sarah[1]
[1] UC Berkeley Plant Gene Expression Center, Albany, CA, USA 94710

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. The reference allele was discovered in a Mexican maize population by Dr. Paul Weatherwax and reported in 1947. Traits were evaluated in families carrying *Fas1-R* and a second allele, *Fas1-SH*, in two genetic backgrounds (A188 and B73). Observed tassel traits included: rachis length, length of the branching zone, number of main branches, secondary branches, and spikelet multimers as well as the density of spikelets, number of single spikelets on a defined section of rachis, number of florets per spikelet and number of stamens per floret. Observed ear traits included: length, number of terminal points, percentage of the ear affected, and number of spikelets in a cross-section 1cm from the ear tip(s). Vegetative traits including plant height, leaf number and size were measured but did not differ between mutant and normal plants. Significant differences were observed for rachis spikelet density and number of secondary tassel branches, ear spikelet density and number of terminal points in the ear. Crosses are underway to put each allele into the same genetic background in order to distinguish allele-dependent phenotypic differences from background-dependent differences. Genetic mapping by David Jackson in 1998, placed *Fas1* 27cM proximal of *Rld1* on chromosome arm 9L. Through map-based cloning, the *Fas1* locus has been determined to be flanked by markers designed from ESTs AY109485 and AY109819, placing it at the junction of contigs 389 and 390 in the maize FTP map. This is in bin 9.06 between 492.02 and 526.00 on the IBM2 2005 neighbors genetic map.

P92

Maternal Regulation of Transfer Cell Development and Seed Size in Maize

(submitted by Liliana Costa <liliana.costa@plants.ox.ac.uk>)
Full Author List: Costa, Liliana M.[1]; Perez, Pascual[2]; Dickinson, Hugh G.[1]; Gutierrez-Marcos, Jose F.[3]
[1] Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK.
[2] Biogemma, 24 Avenue des Landais, 63170 Aubiere, France.
[3] Warwick-HRI, University of Warwick, Wellsbourne, Warwick, CV35 9EF, UK.

The triploid endosperm is the product of a double fertilisation event that is unique to the flowering plants. Genetic evidence suggests that endosperm development is under strong maternal control. We therefore screened for genes that are preferentially expressed from the maternal alleles in the maize endosperm. From this screen we identified meg1, which encodes a small cystein-rich protein that is localised to the specialised wall ingrowths of the basal endosperm transfer cells. To gain insight into the function of MEG1, we followed a transgenic approach to alter the expression of MEG1. This resulted in a reduced kernel phenotype, with kernels displaying cellular defects and abnormal differentiation of the endosperm transfer cells. Our data support a signalling role for MEG1 in regulating transfer cell development and, ultimately, seed size.

P93

Microarray Analysis of Juvenile, Adult and Culture-Rejuvenated Leaf Primordia Identifies Candidate Genes Underlying Vegetative Phase Change in Maize

(submitted by Josh Strable <joshua-strable@uiowa.edu>)
Full Author List: Strable, Josh[1]; Borsuk, Lisa A.[2]; Nettleton, Dan[2]; Schnable, Patrick S.[2]; Irish, Erin[1]
[1] University of Iowa, Iowa City, IA, 52242
[2] Iowa State University, Ames, IA, 50011

Vegetative phase change is the developmental transition from the juvenile phase to the adult phase during which a plant becomes competent for sexual reproduction. Coincident with the gain of ability to flower are changes in patterns of differentiation in newly forming vegetative organs. In maize, juvenile leaves differ from adult leaves in anatomy and cell wall composition. The normal sequence of juvenile followed by adult is repeated with every sexual generation. This sequence can be altered by isolation and culture of the shoot apex from an adult phase plant; an "adult" meristem so treated consequentially reverts to forming juvenile vegetative organs. To understand the molecular mechanisms underlying phase change, we compared gene expression in two juvenile samples, leaf 4 and culture-derived leaves 3-4, to an adult sample (leaf 9) using DNA microarrays. All leaf samples were primordia at plastochron 6. We scored a gene as "phase specific" if it was up- (or down-) regulated in both juvenile samples compared to the adult sample with at least a 2 fold-change in gene expression at $P < 0.005$. We identified 221 juvenile-specific and 28 adult-specific genes. RNA blot analysis has confirmed the microarray results for several juvenile- and adult-specific genes. We have now examined the expression of selected phase-specific genes in the mutants d1 and Tp2, which delay phase change, and in gl15, in which phase change is hastened. We are using this combination of gene expression data with phase change mutations to elucidate the genetic networks underlying vegetative phase change.

P94

Molecular Analysis of Embryonic and Postembryonic Root Development in Maize (Zea mays L.)

(submitted by Muhammad Saleem <muhammad.saleem@zmbp.uni-tuebingen.de>)
Full Author List: Saleem, Muhammad[1]; Woll, Katrin[1]; Liu, Yan[1]; Hochholdinger, Frank[1]
[1] Eberhard-Karls-University, Center for Plant Molecular Biology (ZMBP), Department of General Genetics, Auf der Morgenstelle 28, 72076 Tuebingen, Germany

Maize root system formation can be divided in an embryonic and a postembryonic phase. Primary and seminal roots are formed during embryogenesis. Lateral roots are initiated postembryonically from pericycle cells of all root types. The aim of this study is to identify genes involved in the development of embryonic and postembryonic roots in maize.

The rum1 mutant of maize lacks embryonically formed seminal roots and postembryonically formed lateral roots on the primary root. To identify proteins differentially expressed during seminal root development we have performed a proteome analysis of 30 day-old immature embryos of self-pollinated green-house grown rum1 and wild-type plants via 2-DE. 27 proteins were detected to be differentially expressed by more than two fold in either wild type or rum1. 23 proteins were preferentially expressed in wild type, while 4 proteins were predominantly expressed in rum1. Differentially expressed proteins were identified via ESI MS/MS.

Branching and re-branching of roots takes place through lateral root development. Lateral root initiation takes place via re-entry of xylem-pole pericycle cells into the cell cycle. We performed comparative proteome and transcriptome analyses of pericycle and non-pericycle cells of the inbred line B73. Cells were isolated via laser capture microdissection (LCM). Microarray and Northern-blot experiments confirmed 32 genes to be pericycle specific. In addition, pericycle specific proteome analyses have identified the 26 most abundant soluble pericycle proteins.

Based on these proteome and trancriptome analyses of seminal and lateral root development a number of genes have been selected for RT-PCR analyses and further reverse genetic studies.

P95

Molecular Genetics of Mitochondrial Biogenesis in Maize

(submitted by Terry L. Kamps <kampstl@yahoo.com>)
Full Author List: Kamps, Terry L.[1]; Zhao, Liming[1]; Chamusco, Karen[1]; Read, Victoria[1]; Andersen, Ashley[1]; Hannah, L. Curtis[1]; McCarty, Donald R.[1]; Gabay-Laughnan, Susan[2]; Chase, Christine D.[1]
[1] Horticultural Sciences Department, University of Florida, Gainesville, FL USA 32611-0690
[2] Plant Biology, University of Illinois, Urbana, IL USA 61801

The mitochondrial genome encodes proteins essential for mitochondrial respiration and ATP synthesis. Nuclear gene products, however, are required for the expression of mitochondrial genes and the elaboration of functional mitochondrial protein complexes. We are exploiting a unique collection of maize lines developed from plants selected for nuclear mutations disrupting mitochondrial functions to understand how the nuclear genome contributes to mitochondrial biogenesis and function in plants. These mutations gametophytically restore male fertility to plants with the mitochondrial-encoded, cytoplasmic male sterility trait type S (CMS-S). Although restorer mutations rescue CMS-S pollen function, many are homozygous lethal for maize kernel development. The functions that these nuclear restorer gene products normally perform in mitochondrial biogenesis were investigated by assaying mitochondrial RNA and protein accumulation in starch-filling pollen collected from normal (N)-cytoplasm plants and CMS-S plants carrying seven independent restorer mutations. Two mechanisms for fertility restoration are suggested by the results of these assays. Five unlinked restorer mutations conditioned global loss of mitochondrial gene products through post-transcriptional mechanisms. These restorer mutations are hypothesized to effect fertility by also conditioning the loss of the mitochondrial gene product responsible for CMS-S. Restorer mutations having no obvious effects on mitochondrial gene expression are thought to disrupt mitochondrial-signaled cell death events associated with pollen collapse. Assays for both mechanisms of fertility restoration are under development. To further elucidate the nature of fertility restorer genes and their functions, we are also pursuing molecular cloning strategies. Fifty independent, Mutator (Mu) transposon-induced, seed-lethal mutants were tested by seed phenotype for allelism with the above described seven restorer mutants. Twenty-two independent, Mu transposon-induced, seed-lethal mutants were directly tested for fertility restoration in S-cytoplasm. Nine of the 22 mutants restored fertility to CMS-S plants. These nine mutants were intercrossed to test for allelism. The molecular analysis and allelism test results will be presented.

P96

Organ Polarity in Maize is Regulated by Small RNAs

(submitted by Ananda K. Sarkar <sarkara@cshl.edu>)
Full Author List: Sarkar, Ananda K.[1]; Madi, Shahinez[1]; Lu, Pengcheng[2]; Scanlon, Michael J.[3]; Schnable, Patrick S.[2]; Timmermans, Marja CP.[1]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Rd, Cold Spring Harbor, New York, USA, 11724.
[2] Iowa State University, Dept. of Genetics Cells & Cell Biology, 2035B Roy J. Carver Co-Laboratory, Ames, IA 50011-3650.
[3] Cornel University, Dept. of Plant Biology, 140 Emerson Hall, Ithaca, NY 14853.

Higher plants continuously produce organs, such as leaves, from the flank of the shoot apical meristem (SAM). Leaves are dorso-ventrally (adaxial/abaxial) patterned structures composed of distinct cell types within their upper and lower domains. Recent studies indicate that members of the HD-ZIPIII gene family and the opposing activity of two classes of small RNAs are necessary for leaf polarity (adaxial/abaxial identity). microRNA166 (miR166) contributes to abaxial fate by restricting the expression of Rolledleaf1 (Rld1), a HD-ZipIII member, to the adaxial domain. Trans-acting small interfering RNAs (tasiRNAs) are another class of small RNAs shown to be involved in plant organ development and patterning. Our recent work showed that leaf bladeless1 (Lbl1) gene encodes a homolog of Arabidopsis SUPPRESSOR OF GENE SCILENCING (SGS3) and is essential for tasiRNA biogenesis and is required for adaxial cell fate. Severe alleles of lbl1 fail to establish adaxial identity of leaves and are embryolethal (fail to form SAM). Here we present our results on the role of Lbl1 in embryogenesis and identification of the potential downstream elements of Lbl1 using laser capture microdissection (LCM) and microarray analysis.

P97

Polar Auxin Transport in Grasses - PIN1 Localization and NPA Treatments

(submitted by Devin O'Connor <devo@nature.berkeley.edu>)
Full Author List: O'Connor, Devin[1]; Hake, Sarah[1]
[1] University of California; Plant Gene Expression Center; Albany, CA, USA 94710

In the model dicot Arabidopsis, polar localization of the PIN1 auxin transporter creates an auxin stream out of the shoot meristem epidermis that is required for lateral organ initiation. Later during leaf development, the position of the initial auxin stream defines the leaf midvein while subsequent auxin streams out of the developing margin further define the reticulate vascular pattern characteristic of dicot leaves. In monocots, such as grasses, leaf initiation and vascular patterning are drastically different, and the role of PIN proteins in these important plants is not yet known. Grass leaves initiate as a ring around the meristem circumference and have a parallel, not reticulate, leaf venation pattern. These differences between monocot and dicot leaf initiation and vascular patterning could reflect differences in PIN protein-mediated auxin patterning. In this work, PIN1 was localized by fluorescent immuno-labeling maize and Brachypodium shoot apexes. These data show the possible importance of multiple auxin fluxes out of the L1 around the circumference of young leaves. Normal development was also perturbed with an inhibitor of auxin transport, NPA. The resulting leaf initiation and vascular patterning defects further support the importance of auxin flux in establishing several of the major characteristics of grass leaves.

P98

Programmed Cell Death Genes Are Differentially Expressed in Tapetal Cells of cms-T Maize During Fertility Restoration

(submitted by Jun Cao <juncao@iastate.edu>)
Full Author List: Cao, Jun[1]; Emrich, Scott J.[1]; Lu, Pengcheng[1]; Borsuk, Lisa A.[1]; Schnable, Patrick S.[1]
[1] Iowa State University, Ames, IA 50011-3650

Over 150 flowering plant species exhibit cytoplasmic male sterility (cms). In those cms systems that have been characterized, cms is a consequence of the expression of abnormal mitochondrially encoded proteins that interfere with normal pollen development. In many cms systems nuclear genes can rescue (i.e., restore) fertility to cms lines by altering the expression of the abnormal mitochondrial gene or by counteracting its effects *via* yet to identified processes. In several cms systems restoration occurs *via* the action of pentatricopeptide repeat (PPR) proteins that affect transcription of the abnormal mitochondrial genes. Male-sterile T-cytoplasm (cms-T) maize lines accumulate the abnormal mitochondrial encoded protein T-URF13 which blocks pollen development. Fertility of cms-T maize lines can be restored *via* the sporophytic action of dominant alleles of the *rf1* and *rf2a* nuclear genes. The *rf2a* gene does not encode a PPR protein and does not affect the transcription of the *urf13* gene; rather it encodes a mitochondrial aldehyde dehydrogenase (mtALDH; Cui *et al*, 1996, Liu *et al*, 2001, 2002). Hence, *rf2a* restores fertility *via* a novel pathway. As a step towards identifying this pathway global patterns of gene expression in laser-capture microdissected tapetal cells from T-cytoplasm plants that were unrestored (male sterile) or restored (fertile) *via* the action of *Rf2a* were assayed using a custom spotted cDNA microarray and 454 sequencing. These experiments established that genes involved in the programmed cell death (PCD) pathway are differentially expressed during restoration, suggesting a general role of PCD in *rf2a*-mediated fertility restoration.

P99

Progress on Characterization and Positional Cloning of the *gametophyte factor1* (*ga1*) Locus

(submitted by Michael Muszynski <michael.muszynski@syngenta.com>)
Full Author List: Muszynski, Michael G.[1]; Murua, Mercedes[2]; Ritchie, Renee[2]; Dunn, Molly[3]; Yu, Ju-Kyung[2]; Altendorf, Paul[2]; Plunkett, David[2]; Grier, Steve[2]
[1] Syngenta Seeds, 2369 330th St., Slater, IA, USA 50244
[2] Syngenta Seeds, 317 330th St, Stanton, MN, USA 55018
[3] Syngenta Biotechnology Inc., 3054 Cornwallis Rd, Research Triangle Park NC, USA 27709

Interactions between the pollen and pistil leading to compatible or incompatible fertilization have been extensively studied in several solanaceous and cruciferous species. Such studies have illuminated the molecular mechanisms governing self-compatibility and self-incompatibility in species of tobacco, tomato and many brassicas. In general, Zea mays is a self-compatible species; although loci that prevent reciprocal crossing have been identified. One such locus is *gametophyte factor1* (*ga1*), first identified by Demerec in 1929. Genetic studies showed certain popcorn varieties could only be successfully pollinated with their own pollen and would not set seed when crossed with pollen from dent, flint or sweet varieties. In reciprocal crosses, the popcorn pollen could successfully fertilize dent, sweet or flint varieties, indicating the cross incompatibility was unidirectional. The popcorn varieties carry a dominant *Ga1* allele and can only be successfully fertilized by *Ga1* pollen. Pollen carrying *ga1* alleles either do not fertilize or are very inefficient at fertilizing *Ga1* females. Conversely, both *Ga1* and *ga1* pollen fertilize *ga1* females with equal efficiencies. These results indicate that *Ga1* functions gametophytically in the pollen but sporophytically in the silk (pistil). Although first described nearly 80 years ago, very little is known about the mechanisms of *Ga1* unidirectional cross-incompatibility. To gain insight into the molecular mechanisms underlying *Ga1* function, we are characterizing the effects of this mutation on pollen tube growth in compatible and incompatible crosses and positionally cloning the gene. We will present data on our progress with fine mapping the *Ga1* locus on the short arm of chromosome 4 and our method for phenotyping *Ga1*/*ga1* versus *ga1*/*ga1* recombinant progeny. In addition, we will show preliminary results of our pollen tube growth studies from compatible and incompatible crosses and data indicating that silks from *Ga1* plants have an unusual branched morphology.

P100

Role of Alpha Expansin During Morphogenesis of Juvenile Leaf Epidermal Cells

(submitted by Anding Luo <aluo@uwyo.edu>)
Full Author List: Luo, Anding[1]; Sylvester, Anne[1]
[1] Department of Molecular Biology, University of Wyoming, Laramie WY 82071

Maize leaves undergo distinct cellular, morphological and physiological changes associated with the transition from juvenile to adult growth. During the juvenile phase of growth, leaf epidermal cells in particular show a distinct shape characterized by gently incurved crenulations, abrupt end walls and extensive distribution of epicuticular waxes. Adult epidermal cells on the other hand, have deeply inset crenulations, curved end walls and lack architecturally distinct epicuticular waxes. The mechanism of shape change is not well understood, although prior studies show that the microtubule cytoskeleton helps to maintain wall shape during normal cell morphogenesis. We are investigating wall expansion in maize using a YFP-tagged alpha expansin that localizes to both the cortical cytoplasm and the wall space. We are developing an epidermal peel culture system to test for the role of the cytoskeleton and vesicle transport system in localizing and secreting expansin protein. Developmental and cell specific patterns of expansin localization will be presented.

P101

Root Growth and ABA Phenotypic Diversity Available in Maize's Response to Water Deficits

(submitted by Kristen Leach <kalp55@mizzou.edu>)
Full Author List: Leach, Kristen A.[1]; Maltman, Rachel C.[1]; Hejlek, Lindsey G.[1]; Nguyen, Henry T.[1]; Sharp, Robert E.[1]; Davis, Georgia L.[1]
[1] Division of Plant Sciences, University of Missouri-Columbia, Columbia, Missouri 65211

Today's crops are being exposed to and moving into areas of less water availability. Because of this, it is necessary for breeders to develop lines that will be able to survive these conditions. Root growth maintenance is a key part of the plant's ability to survive water deficits. Twelve maize lines were chosen to investigate the amount of phenotypic diversity available in maize to respond to varying water deficits. Inbreds B73, B97, F2, FR697, IABO78, Lo1016, Lo964, Mo17, OS420, P1, P2, and Polj17 are diverse in their location of origin, pedigree, and ability to respond to drought and/or produce abscisic acid (ABA). Primary root growth rates and ABA levels were determined for well-watered (-0.03 MPa), mild stress (-0.3 MPa), and severe stress (-1.6 MPa) conditions. LS means for well-watered and percent well-watered growth rates were used to compare the lines in each treatment. Under water deficit conditions, P2 was unmatched in its ability to maintain root growth, while B97 was the most sensitive. Further comparative analysis of each line's response to each treatment revealed there may be different response mechanisms or amplification of one response mechanism at increasing levels of stress. Correlation analysis between root growth rate and ABA levels revealed there was no correlation in any of the treatments. P-values for the correlations were 0.697, 0.746, and 0.979 for well-watered, mild stress, and severe stress, respectively. The relatively small fold change in Lo1016 from 1.79 to 3.57, when compared to the other lines, may indicate there is a threshold of ABA needed to maintain root growth. Genetic diversity among the lines was also examined. The genetic diversity data along with root growth data suggests that tropical and temperate germplasm may be an ideal source of alleles that contribute to root growth maintenance. This research was funded by NSF-DBI-0211842.

P102

Searching for Genes Responsible for Dosage Effects in the Maize Endosperm

(submitted by Jerome Martin <jerome.martin@mpl.ird.fr>)
Full Author List: Martin, Jerome[1]; Leblanc, Olivier[1]
[1] IRD, Institut de Recherche pour le Developpement; 911 avenue Agropolis, 34394 Montpellier cedex 5, FRANCE

Seeds of flowering plants typically contain a diploid (2x) embryo and a triploid (3x) endosperm in which the maternal (m) to paternal (p) genomic contributions are 1m:1p and 2m:1p, respectively. In most flowering plants, interploidy crosses produce abnormal seeds after endosperm development failure, a phenomenon referred to as "ploidy hybridization barrier". The most accepted explanation for it assumes that endosperm defects result from the alteration of the 2m:1p ratio (4m:1p and 2m:2p after 4x X 2x and 2x X 4x crosses, respectively) that modifies the functional stoechiometry among the products of two sets of genes regulated through differential parental imprinting. We have shown that cell cycle progression during maize endosperm development is modified after interploidy crosses and that dosage effects differ depending on the direction of genomic excess: paternal excess (2x X 4x) prevents endoreduplication whereas maternal excess (4x X 2x) forces proliferating cells into endocycles. Based on this information, we developed a microarray approach to identify the as-yet-unknown genes underlying the response to dosage effects in the maize endosperm.

P103

Sex Determination Gene *tasselseed1* is a Florally Expressed, Proplastid-Localized Lipoxygenase

(submitted by Ivan Acosta <ivan.acosta@yale.edu>)
Full Author List: Acosta, Ivan F[1]; Moreno, Maria A[1]; Mottinger, John P[2]; Dellaporta, Stephen D[1]
[1] Department of Molecular, Cellular and Developmental Biology; Yale University; New Haven, CT, 06520
[2] Department of Cell and Molecular Biology; University of Rhode Island; Kingston, RI, 02881

The sex determination (SD) pathway of maize includes the formation of double staminate florets in the tassel spikelets and solitary pistillate florets in the ear spikelets through the abortion of pistil primordia by a tasselseed-mediated cell death process. Pistil abortion requires a functional tasselseed 1 gene and mutant ts1 pistils fail to abort. We have previously reported the positional cloning of the ts1 gene. The TS1 protein is predicted to belong to the type 2 lipoxygenase (LOX) subfamily, whose members carry a putative chloroplast transit peptide (CTP) and specifically oxygenate the fatty acid at carbon atom 13 (13-LOX) of the hydrocarbon backbone. Analysis of ts1 transcription by real-time RT-PCR has shown an oscillating pattern of expression in the developing tassel. Unexpectedly, the strongest expression was observed at a stage of floral development well past the timing of pistil abortion. A fusion of TS1 and the fluorescent protein mCherry was transiently expressed in onion epidermal cells and shown to be colocalized with a GFP fusion to the small subunit of ribusco (RbcSnt), suggesting that TS1 is localized in proplastids within the developing maize floret. A recombinant full-length LOX_TS1 heterologously expressed in Escherichia coli has not shown lipoxygenase activity. We will discuss a hypothesis where TS1 has two related roles in maize stamen development, one that functions early in floral development for pistil abortion and another later function for male fertility.

P104

The *empty pericarp4* (*emp4*) Gene, Required for Seed and Plant Development, Encodes a Mitochondrion-Targeted Pentatricopeptide Repeat Protein

(submitted by Gabriella Consonni <gabriella.consonni@unimi.it>)
Full Author List: Gutierrez-Marcos, Jose F.[1]; Dal Pra, Mauro[2]; Giulini, Anna[1]; Costa, Liliana M.[1]; Gavazzi, Giuseppe[2]; Cordelier, Sylvain[3]; Sellam, Olivier[3]; Tatout, Christophe[3]; Paul, Wyatt[3]; Perez, Pascual[3]; Dickinson, Hugh G.[1]; Consonni, Gabriella[2]
[1] Department of Plant Sciences, University of Oxford, Oxford OX1 3RB, United Kingdom
[2] Dipartimento di Produzione Vegetale, Universita` degli Studi di Milano, 20133 Milan, Italy
[3] Biogemma, 63 170 Aubiere, France

The phenotype conferred by the empty pericarp (emp) mutants define a subclass of defective kernel mutants characterized by seeds exhibiting a severe reduction in endosperm size, yet possessing a normal pericarp. These mutants are easily recognizable in segregating mature ears because they have a flattened appearance as a result of compression by the surrounding normal seeds. Of the emp genes so far isolated, emp2, which encodes a heat-shock response regulator, is the only one to have been molecularly characterized so far. Here we describe the molecular and phenotypic characterization of emp4-1, a novel lethal empty pericarp mutation that originated from an active Mutator population. Co-segregation analysis revealed that the mutation arose from the insertion of a Mutator3 element in the emp4 locus. The isolation of the emp4 gene was achieved through a gene tagging approach, while proof of its molecular cloning was obtained from the identification of four independent emp4 alleles arising from a reverse genetics based screening. emp4 is a single copy gene which encodes a pentatricopeptide repeat (PPR) protein. The pentatricopeptide repeat (PPR) family represents one of the largest gene families in plants, with more than 440 members annotated in Arabidopsis. PPR proteins are thought to have a major role in the regulation of postranscriptional processes in organelles. emp4 encodes a 614 amino acid protein containing nine 35-aa pentatricopeptide motif repeats, a mitochondria-targeted sequence peptide motif and two additional domains. EMP4 represents a novel type of mitochondrial-targeted PPR protein, which exhibits an essential, non-redundant function during seed development and plant growth. Our data further suggest that EMP4 is necessary for the correct regulation of mitochondrial gene expression in the endosperm.

P105

The *ra2* Pathway as a Tool for Understanding Axillary Branching in Grass Inflorescences

(submitted by Esteban Bortiri <ebortiri@berkeley.edu>)
Full Author List: Bortiri, Esteban[1]; Hake, Sarah[1]
[1] USDA-ARS, Plant Gene Expression Center, 800 Buchanan Avenue, Albany, California, 94710, USA

ramosa2 (ra2) is a LOB domain transcription factor that is expressed in the groups of stem cells that give rise to axillary branches in inflorescences of maize and other grasses. Mutations in ra2 result in inflorescences that produce axillary branches with increased growth. We raised and purified an anti-RA2 antibody and show that RA2 is cell autonomous. Protein localization is identical to mRNA in maize and rice, and it is mainly localized to the nucleus. Because of its early expression in what will become axillary meristems and its mutant phenotype, ra2 plays a key role in axillary meristem activation. Therefore, we are studying the ra2 pathway with the goal of understanding the molecular events that lead to axillary branching in grass inflorescences. We show that normal auxin transport is necessary for proper inflorescence patterning and ra2 expression. Nevertheless, exogenous auxin is not sufficient to increase steady-state levels of ra2 transcript. To identify genes in the ra2 pathway we performed microarray analyses of wild type (B73) versus ra2 tassel primordia and selected a few differentially expressed genes for confirmation using quantitative PCR. To identify downstream targets of RA2 we expressed and purified a full-length recombinant RA2 and will perform SAAB assays. We are also conducting yeast two hybrid analyses to identify biochemical partners of RA2 and characterize the function of different domains of RA2.

P106

The *tassel sheath* Loci of Maize Control *bract* Suppression in the Inflorescence

(submitted by Clinton Whipple <whipple@cshl.edu>)
Full Author List: Whipple, Clinton[1]; Hall, Darren[2]; Schmidt, Robert J.[2]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Rd, Cold Spring Harbor, NY, USA 11724
[2] UC San Diego, 9500 Gilman Drive, La Jolla, CA, USA 92093

Aerial development in plants reiterates a basic unit containing three distinct parts: the leaf, internode, and an axilary meristem. This unit is known as the phytomer, and modification of phytomer development leads to distinct morphologies that characterize both developmental transitions as well as species differences. One common modification of phytomer development is suppression of leaf development after the transition to reproductive development. Suppression of inflorescence leaves, also known as bracts, occurs in nearly all grasses. A class of maize mutants known as tassel sheath (tsh), fail to repress the bract, indicating that a distinct genetic pathway controls bract suppression. We have begun a characterization of the tassel sheath mutants of maize. Complementation and mapping studies suggest that at least three distinct loci give a tsh phenotype: tsh1-tsh3. The tsh1 tsh2 double mutant has a synergistic phenotype suggesting that these genes act in a common pathway. A map-based cloning strategy using synteny with rice has been used to identify the tsh1 gene, which encodes a protein with a putative GATA zinc finger domain. Expression analysis is underway to clarify how TSH1 acts to block bract development. Interestingly, some grass species in the Andropogoneae, as well as outgroups to the grasses fail to suppress bract development. Understanding the mechanism by which tsh loci control bract development may help explain how this trait has evolved in the grasses. This project was supported by USDA CREES grant 2006-35304-17423 and the NSF Plant Genome award "Regulation of Inflorescence Architecture in Maize".

P107

The Expression and Function of *teosinte branched 1* (*tb1*)

(submitted by Anthony Studer <studer@wisc.edu>)
Full Author List: Studer, Anthony J.[1]; Wang, Huai[1]; Doebley, John F.[1]
[1] Department of Genetics, University of Wisconsin-Madison, 425 Henry Mall, Madison, WI 53706 USA

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 the expression and function of tb1. We are addressing three questions. (1) What is the exact nature of the enhancer of tb1 expression that was identified by Clark et al. and is located ~58-69 kb upstream of the transcription start site? We used Southern blot analysis to look at the differences between maize and teosinte in this region. We observed a large insertion in this region that is present in most teosinte but missing in maize. To identify whether this insertion is the cause of the expression differences between maize and teosinte, recombinants between maize and teosinte haplotypes in the region upstream of tb1 are being generated. (2) Are there any genes tightly linked to tb1 which contribute to the phenotypic differences between maize and teosinte? While Clark et al defined a cis enhancer of tb1 expression, their analysis does not exclude the possibility that other tightly linked genes are contributing to the observed phenotypic differences. We compared the phenotypic effects associated with the teosinte haplotype of the minimal control region defined by Clark et al to the phenotypic effects associated with a large teosinte chromosomal segment carrying both the teosinte control region and an extensive flanking teosinte chromosomal region. This comparison suggests that there are additional genes linked to tb1 that contribute to the phenotypic differences between maize and teosinte. More recombinant data are being collected to accurately map these genes. (3) What are the downstream targets of the tb1 protein? Using qPCR and Electrophoretic Mobility Shift Assays (EMSA), we have found that the tb1 protein inhibits the expression of several cell cycle genes.

P108

The Maize *Floury1* Gene Encodes a Novel Zein Protein-Body Membrane Protein

(submitted by David Holding <dholding@ag.arizona.edu>)
Full Author List: Holding, David[1]; Otegui, Marisa[2]; Dam, Thao[3]; Li, Bailin[3]; Meeley, Robert[4]; Hunter, Brenda[1]; Jung, Rudolf[4]; Larkins, Brian[1]
[1] Department of Plant Sciences, University of Arizona, Tucson, Arizona, 85721
[2] Department of Botany, University of Wisconsin, Madison, Wisconsin, 53706
[3] DuPont Crop Genetics Research, Experimental Station, Wilmington, Delaware 19880-0353
[4] Pioneer Hi-Bred International, 7300 NW 62nd Avenue, P.O. Box 1004, Johnston, Iowa, 50131-1004

We report the cloning of the Floury1 (Fl1) gene which encodes a novel protein specifically localized in the protein body ER membrane. The Fl1 gene was isolated using selective amplification of insertion-flanking fragments (SAIFF) from a Mu-tagged opaque kernel mutant, mto222. FL1 is a unique protein that contains a C-terminal, plant specific domain of unknown function (DUF593) and three predicted N-terminal trans-membrane domains. The gene was mapped to BIN 2.04, coincident with the fl1 mutation, and crosses between mto222 and fl1 confirmed their allelism. In mto222, the Mu insertion in Fl1 is immediately upstream of the start codon and results in a very low transcript level and no detectible protein. The fl1 allele contains a point mutation that changes a serine to glycine in the first trans-membrane domain. Although the RNA level is normal in fl1, the protein is barely detectible. In wild type endosperm, FL1 protein increases from a low level at 10 DAP to a peak at 18-24 DAP, before declining to a low level at kernel maturity. Thus, the presence of FL1 corresponds to the period of zein synthesis and protein body development. Immuno-gold labeling showed that FL1 resides at the periphery of the protein body. Both mto222 and fl1 mutants have protein bodies of normal shape and abundance and no specific or general reductions in zein storage proteins. However, both mutants show abnormal localization of the 22-kD and 19-kD alpha-zeins. Normally, the alpha-zeins occupy primarily the protein body core, whereas in mto222 and fl1, the alpha-zeins occur throughout the protein body, including the membrane region. Pair-wise yeast two-hybrid experiments showed that the C-terminal conserved domain of FL1 interacts with the 22-kD and 19-kD alpha-zeins. Taken together, these data suggest that FL1 participates in protein body development by ensuring that the alpha-zeins are targeted correctly.

P109

The Role of *ramosa1* in the Domestication of Maize and the Evolution of Other *Andropogoneae* Grasses

(submitted by Brandi Sigmon <bsigmon@iastate.edu>)
Full Author List: Sigmon, Brandi[1]; Gillispie, Chris[2]; Farrell, Jacqueline[1]; Brown, Patrick J.[3]; Kresovich, Stephen[3]; Kellogg, Elizabeth A.[2]; Vollbrecht, Erik[1]
[1] Department of Genetics, Developmental, and Cellular Biology; Iowa State University; Ames, IA 50011
[2] Department of Biology; University of Missouri; Saint Louis, MO 63121
[3] Institute for Genomic Diversity; Cornell University; Ithaca, NY 14853

The maize ramosa1 (ra1) gene encodes a putative transcription factor involved in the branching architecture of maize inflorescences. ra1 is a hypothesized domestication gene because its nucleotide diversity is reduced in maize inbreds. Statistical tests indicate that selection for specific versions of the ra1 gene occurred at some point since maize diverged from its common ancestor with Tripsacum, ~5 million years ago. To further investigate the question, nucleotide diversity of ra1 was sampled in a panel of teosintes and maize landraces. HKA tests showed significantly reduced nucleotide diversity for the landraces, but not teosinte, supporting the hypothesis that ra1 may be a domestication locus. In the landraces, the noncoding 5' sequence showed a ten-fold greater reduction of diversity than the coding region, suggesting a 5' element may have been the target of selection. Reconstruction of a teosinte-maize haplotype tree for the 5' region and coding region shows all maize haplotypes fall within two clades, a more neutral topology than expected. However, as with the teosinte branched1 gene, if a more distant and unsampled upstream element was the target of selection, then such a neutral topology may be expected. Thus, the inclusion of more 5' sequence may resolve the topology and reconcile the phylogenetic and HKA analyses. In addition to teosinte and maize landraces, ra1 was sequenced and analyzed for a panel of Andropogoneae grasses and the gene tree was reconstructed. Across the tribe, ra1 is conserved in functional domains of the gene and parts of the promoter. Sorghum, an Andropogoneae grass, is unique because ra1 was recently duplicated and a frameshift mutation found in the upstream copy is not present in some wild accessions. Future work will include RNA expression analysis on select Andropogoneae based on morphology, which will shed light on the evolution of ra1 function among the grasses.

P110

Transcriptome Profiling of Shoot-Borne Root Initiation in Maize (Zea mays, L.)

(submitted by Nils Muthreich <nils.muthreich@zmbp.uni-tuebingen.de>)
Full Author List: Muthreich, Nils[1]; Sauer, Michaela[1]; Hochholdinger, Frank[1]
[1] University of Tuebingen, Center for Plant Molecular Biology (ZMBP), Department of General Genetics; Auf der Morgenstelle 28, 72076 Tuebingen, Germany

The shoot-borne root system makes up the major backbone of the adult root system and secures optimal water and nutrient uptake as well as anchorage of the maize plant in the soil. Crown roots are initiated from the innermost cortical cell-layer of the coleoptilar node (first shoot node) about 5 days after germination. The monogenic recessive mutant rtcs (rootless concerning crown and seminal roots) is affected in the initiation of all postembryonically formed shoot-borne roots and the embryonically formed seminal roots. The rtcs gene encodes an auxin inducible LOB domain transcription factor. Comparative transcriptome analyses of whole coleoptilar nodes and laser capture microdissected cortex cells were performed to identify candidate genes involved in crown root initiation downstream of rtcs. In whole coleoptilar nodes 107 genes were preferentially expressed in WT while 67 genes where predominantly expressed in the mutant rtcs. In cortex cells 34 genes were preferentially expressed in WT while 42 genes were predominantly expressed in rtcs. Based on these transcriptomic studies of crown root development a number of genes have been selected for further reverse genetic studies.

P111

Visualization of Polar Auxin Transport during Maize Vegetative and Reproductive Branching

(submitted by Andrea Gallavotti <gallavot@cshl.edu>)
Full Author List: Gallavotti, Andrea[1]; Zanis, Michael J.[2]; Yan, Yang[1]; Schmidt, Robert J.[3]; Jackson, David[1]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724
[2] Purdue University, West Lafayette, IN, 47907-2054
[3] University of California San Diego, La Jolla, CA, 92093-0116

Recent work in Arabidopsis has established a universal model for the positioning and initiation of lateral organs, which involves the action of auxin efflux and influx carriers. Through the subcellular polar localization of these carriers, gradients of the phytohormone auxin are established to promote organ initiation at the flank of the shoot apical meristem throughout the life cycle. Central to this process are the PIN-FORMED (PIN) proteins. We are using a maize PIN1 gene (ZmPIN1a) expressed during vegetative and reproductive development to monitor the dynamics of auxin transport during the formation of branches and lateral organs in maize. The ZmPIN1a gene is able to almost fully complement the Arabidopsis pin1 mutant and therefore likely acts as a functional auxin efflux carrier. We obtained stable maize transgenic lines expressing a fusion of ZmPIN1a with YFP (yellow fluorescent protein). We are characterizing the localization of ZmPIN1a during all branching events in maize, from the formation of vegetative axillary meristems to the formation of floral organs. In order to visualize auxin gradients directly we are also generating maize transgenic lines carrying a synthetic auxin responsive promoter, DR5rev, fused to RFP (red fluorescent protein). Using these lines we are visualizing and describing a basal mechanism of branch and lateral organ formation throughout maize development. The fluorescent protein reporters are also being crossed to several maize mutants affected in branch formation, to understand how they interact with the auxin pathway. Our analysis shows that, contrary to previous reports (Carraro et al., 2006), the auxin-driven formation of branches and lateral organs is conserved between maize and Arabidopsis.

Carraro, N., Forestan, C., Canova, S., Traas, J. and Varotto, S. (2006) ZmPIN1a and ZmPIN1b encode two novel putative candidates for polar auxin transport and plant architecture determination of maize. Plant Physiol. 142: 254-264

P112

Ufo1 Induces Progressive Loss of DNA Methylation from Tandem Repeats of a *pericarp color1* Allele

(submitted by Rajandeep Sekhon <rss222@psu.edu>)
Full Author List: Sekhon, Rajandeep S.[1]; Chopra, Surinder[1]
[1] Department of Crop and Soil Sciences, Pennsylvania State University, University Park, PA 16803

Maintenance of appropriate levels of DNA methylation during development, and inheritance of the methylation patterns over generations is essential for normal development of an organism. In maize, pericarp color1 gene encodes an R2R3 Myb transcription factor and regulates synthesis of red phlobaphene pigments in pericarp and cob glumes. P1-wr, a multi-copy allele of p1, conditions white pericarp and red cob glume phenotype that is stably inherited over generations. Stable P1-wr expression is associated with methylation patterns in regulatory and coding regions of the allele and disruption of these patterns leads to abolishment of tissue-specific expression. We have previously shown that a dominant epigenetic modifier Unstable factor for orange1 (Ufo1) causes hypomethylation of P1-wr resulting in ectopic phlobaphene pigmentation of pericarp and other plant parts. Further, Ufo1 shows incomplete penetrance and only a subset of progeny plants exhibit such gain of pigmentation in the early filial generations. According to our recent observations, selecting for Ufo1-induced pigmentation phenotypes over multiple generations leads to improved penetrance of this modifier. Further, upon such selection, pericarp pigmentation in individual plants also becomes uniform as opposed to a range of pigmentation observed in early generations. To test if improvement in Ufo1 penetrance and expression is due to P1-wr hypomethylation, we measured the methylation levels. Genomic bisulfite data shows that, when Ufo1 is present in the background, P1-wr becomes progressively hypomethylated over generations. We also examined changes in methylation at p1 sequence in P1-wr Ufo1 plants in embryo and during plant development.

P113

An Insect Vectored Bacteria Mediates Silencing of Several Classes of Maize Defense Genes

(submitted by Kelli Barr <klbxr6@mizzou.edu>)
Full Author List: Barr, Kelli L.[1]; Hearne, Leonard B.[2]; Davis, Georgia L.[1]
[1] Division of Plant Sciences, University of Missouri-Columbia, Columbia, Missouri 65211
[2] Department of Statistics, University of Missouri-Columbia, Columbia, Missouri 65211

Wolbachia spp., an obligate intracellular bacterium, infects numerous nematode and arthropod species. Many *Wolbachia* infected hosts induce or vector serious human diseases resulting in the loss of millions of lives per year. *Wolbachia* also infects several insect species that are of economic importance, which cost the U.S. billions of dollars per year in economic losses and management. It is believed that the presence of *Wolbachia* in insects serves as a reproductive barrier by inducing cytoplasmic incompatibility between *Wolbachia* infected and non-infected populations. Evidence suggests that insect mitochondrial mutation rates can be over ten times greater in *Wolbachia* infected populations than non-infected counterparts. The genus *Diabrotica* arose from the neo-tropical regions of Mesoamerica where maize was domesticated. *Wolbachia* has been identified in several species of *Diabrotica* including *Diabrotica virgifera virgifera* (western corn rootworm). Western corn rootworm is the most serious pest to maize in North America and Europe. It has repeatedly surmounted chemical and cultural control measures in far fewer generations than any other crop pest. A microarray experiment conducted to identify defense genes unique to western corn rootworm feeding implicated genes coding for chromatin remodeling, gene silencing, and microbial defense. Since many of the implicated genes are normally associated with microbes, a second microarray was performed in order to determine whether *Wolbachia* or insect elicitors were responsible for the expression pattern. The second microarray indicated that the presence of *Wolbachia* in the insect resulted in a unique maize expression profile. The expression data indicates that insect vectored *Wolbachia* mediates silencing of several classes of plant defense genes. This work was funded by a grant from the Illinois-Missouri Biotechnology Alliance.

P114

Characterization of Tissue-Specific Expression of Multiple Copies of an Allele of *pericarp color1* Gene

(submitted by PoHao Wang <puw116@psu.edu>)
Full Author List: Wang, PoHao[1]; Sekhon, Rajandeep S.[1]; Chopra, Surinder[1]
[1] Department of Crop and Soil Sciences; Intercollege Graduate Degree Program in Plant Biology, Pennsylvania State University, University Park, PA 16802

The maize p1 gene encodes a Myb-homologous transcription activator which regulates accumulation of phlobaphenes or 3-deoxy flavonoid pigments in floral organs. The p1 gene specified phenotypes can be readily observed in the kernel pericarp and cob glumes of mature maize ears. Alleles of p1 gene have been named according to their pericarp and cob glumes pigmentation pattern. For example, the P1-rr shows red pericarp and red cob and P1-wr exhibits white pericarp and red cob. These two alleles show 99.9% sequence similarity in their coding regions. However, P1-wr allele has been reported to have six or more p1 gene copies while P1-rr only has one copy. The objective of this study is to identify distinct copies of P1-wr that may be expressed in a tissue specific fashion. Reverse transcription-PCR was conducted to amplify P1-wr cDNA products from pericarp and cob glumes for sequence comparisons. In order to distinguish one copy from another, genomic sequencing was performed and characterized for sequence polymorphisms among multiple copies. Our preliminary results show that sequence polymorphisms among different copies are revealed at positions within 5'UTR, exon1, exon2 and exon3. Expression profiling shows copy-type pattern in certain tissues. To further study the frequency of expression among multiple copies, a large set of cDNA clones are being isolated and sequenced. Our preliminary results indicate that certain P1-wr copies are expressed more frequently than the others. The results of the sequence polymorphism and cope-type expression pattern will be presented. We are further characterizing the copy-specific expressions in different tissues in the presence of Unstable factor for orange1 (Ufo1). Results will allow us to develop hypothesis on function of Ufo1 and tissue specific expression of P1-wr.

P115

Epigenetic and Genetic Control of *Mez1* Imprinting

(submitted by William Haun <haunx003@umn.edu>)
Full Author List: Haun, William[1]; Springer, Nathan M.[1]
[1] University of Minnesota - Twin Cities; 1445 Gortner Ave; St Paul, MN 55108

Imprinting is a relatively rare form of gene regulation in which a gene's expression level is influenced by its parent-of-origin. In endosperm tissue, the Mez1 gene is expressed solely from the maternal allele. DNA methylation has been shown to play a role in regulating imprinted loci in both plants and mammals. Recent findings have identified a 550 base pair differentially methylated region (DMR) in the 5' proximal sequence of the Mez1 gene. Bisulfite sequencing, confirmed by methylation-sensitive restriction enzymes and PCR assays, has shown the Mez1 paternal allele to contain a substantial increase in both CpG and CpNpG methylation relative to the maternal allele. The role of other epigenetic marks, including histone modifications, are currently under investigation. In addition to studying the epigenetic regulatory mechanisms of Mez1, we have studied the affect of Mutator-transposon insertion alleles in the 5' regulatory region of Mez1. There is some evidence that the insertion of a Mu element 100-200bp 5' to the transcription start site may result in a loss-of-imprinting for Mez1 expression in the endosperm. Preliminary data for this phenomenon will be presented.

P116

Fluorescent Tagging of Maize Chromatin-Associated Proteins

(submitted by Maria Federico <mlfederico@wisc.edu>)
Full Author List: Federico, Maria L.[1]; Earley, Keith[2]; Stephens, Nicholas[1]; Kaeppler, Shawn[1]; Pikaard, Craig[2]; Kaeppler, Heidi[1]
[1] Department of Agronomy, University of Wisconsin; Madison, WI, 53706
[2] Department of Biology, Washington University; Saint Louis, MO, 63130

In vivo protein expression and subcellular localization patterns are key elements to understanding protein function. We developed a systematic approach for investigating the subcellular localization patterns of chromatin-associated proteins as part of the NSF-funded Maize Chromatin Project. To visualize subcellular localization patterns, the 5' ends of 21 full length cDNAs, representing 5 different chromatin-associated gene families, were translationally fused to the Yellow Fluorescent Protein (YFP). Maize immature embryos were biolistically transformed and localization of YFP-fusion proteins was scored after 18 h (transient expression). In each experiment, 4 plates containing 25 immature embryos were biolistically transformed. At least 2 experiments were conducted for each fusion protein. In addition, nucleic acids were stained with DAPI to confirm nuclear and nucleolar localization patterns. Multiple independent transgenic lines have been generated, to date, for 19 of these YFP-chromatin associated protein fusions. Localization analysis is currently underway on transgenic lines stably expressing the *yfp*-chromatin-associated gene fusions. Preliminary results indicate successful visualization and identification of the subcellular localization patterns of chromatin-associated proteins, and high correlation among observations from both transient and stable expression experiments. Several YFP-fusion proteins displayed subcellular localization patterns different to that of the control construct (YFP alone), which localized to the nucleus and cytosol. These included YFP-fusion proteins accumulating solely in the nucleus or cytosol but also others that localized to discrete regions in the nucleus including the nucleolus. Distinct accumulation patterns let us associate these proteins to specific chromatin regions. Knowledge gained from this research will aid the maize research community in elucidating the biological functions of chromatin-associated proteins.

P117

Genetic Control of Chromatin Structure of the Epiallele *Pl1-Blotched*

(submitted by Kyungju Shin <ksgw3@mizzou.edu>)
Full Author List: Shin, Kyungju[1]; Cone, Karen C.[1]
[1] Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211

Pl1-Blotched is a stable epiallele of the anthocyanin regulatory gene purple plant1 (pl1). Pl1-Blotched plants are variegated, but the amount of pigmentation can be increased in the presence of a modifier called Suppressor of plant blotching1 (Spb1). At the molecular level, the phenotypic effect of Spb1 is due to increased Pl1-Blotched mRNA levels and correspondingly higher levels of mRNAs for the structural genes encoding the anthocyanin biosynthetic enzymes. In addition, Spb1 leads to changes in DNA methylation at several methylation-sensitive restriction sites in the Pl1-Blotched sequence. Based on these observations, we hypothesized that Spb1 is an epigenetic modifier, which acts to increase Pl1-Blotched expression by altering Pl1-Blotched chromatin structure. To evaluate this hypothesis, the effect of Spb1 on Pl1-Blotched chromatin structure was investigated through DNaseI sensitivity assays and chromatin immunoprecipitation (ChIP) assays. The results of DNaseI sensitivity assays showed that Pl1-Blotched plants with Spb1 were slightly more sensitive to DNaseI digestion than without Spb1. For ChIP assays, various antibodies against modified histones (e.g., methylation and acetylation of H3 and H4) were used, and the immunoprecipitated DNA was analyzed by quantitative real-time PCR. The results of these assays provide local genic information about the organization of Pl1-Blotched chromatin in plants with and without Spb1 and insights into the mechanism of epigenetic regulation of this gene.

P118

Novel *pericarp color1* Derivatives of a Multicopy Allele Exhibit Altered Tissue Specificity

(submitted by Michael Robbins <mlr263@psu.edu>)
Full Author List: Robbins, Michael L.[1]; Peterson, Thomas[3]; Surinder, Chopra[2]
[1] Department of Plant Biology, Pennsylvania State University; University Park, PA, 16802
[2] Department of Plant Biology and Department of Crop and Soil Science, Pennsylvania State University; University Park, PA, 16802
[3] Department of Genetics, Development and Cell Biology; Iowa State University; Ames, Iowa, 50011-3260

Tissue-specific expression patterns specified by the *pericarp color1* (*p1*) gene depend upon the allelic constitution. For example, the *P1-rr* allele exhibits red pericarps and red cob glumes, whereas the *P1-wr* allele confers white pericarps and red cob glumes. *P1-wr* contains six or more tandemly-repeated copies of a coding sequence similar to that of the single copy of *P1-rr*. However, it is not known how the presence of multiple gene copies may result in the suppression of pericarp pigmentation. To understand the mechanism involved, we characterized three derivative alleles of *P1-wr* that have altered tissue-specific expression patterns. These alleles were identified in a screen that was designed to find functional recombinants between *P1-wr* and a non-functional allele called *p1-ww:10-443* that has a frame shift mutation in exon 3 of *P1-rr*. However, molecular analysis indicated that these three new alleles contained multicopy gene structures similar to *P1-wr*, and that the gene structure characteristic of *p1-ww:10443* was not present. For two of the new alleles, *P1-wr-d1* and *P1-wr-d2*, the gain of pericarp pigmentation was at the silk attachment point and kernel gown. The third allele, *P1-wr-d3*, frequently had a variegated gain of pericarp pigmentation, and also had an extreme reduction in cob pigmentation. *P1-wr-d3* is structurally different from *P1-wr*; however, at least two gene copies remain intact. To elucidate the mechanism for the altered tissue specific expression, the pattern of DNA methylation of *p1* sequences in the derivative alleles was analyzed by DNA gel blot analysis. The gain of pericarp pigmentation for all three derivative alleles was correlated with hypomethylation at *Hpa*II sites of a distal enhancer region located 4.8 kbp 5' of the *p1* transcription start site. Whereas, the *P1-wr-d3* allele with reduced cob pigmentation exhibits hypermethylation of *Hpa*II sites downstream of the transcription start site as well as in intron 2. Interestingly, the pericarp pigmentation of *P1-wr-d3* is highly variable and is sometimes absent. Therefore, hypomethylation of the distal enhancer is not sufficient to determine pericarp pigmentation. Possibly, the same mechanism that suppresses cob pigmentation also functions in pericarp tissue. We propose a model in which pericarp pigmentation in *P1-wr-d3* is subject to enhancing and suppressing competitive epigenetic mechanisms, thereby yielding novel variegated patterns.

P119

Phylogenetic and Mutational Analysis of *RMR1*, A Novel *Snf2* Protein

(submitted by Jennifer Stonaker <jenne@berkeley.edu>)
Full Author List: Stonaker, Jennifer L[1]; Hale, Christopher J[1]; Hollick, Jay B[1]
[1] Department of Plant and Microbial Biology; University of California; Berkeley, CA, 94720-3102

Maintenance of meiotically heritable epigenetic states at the purple plant1 (pl1) locus involves the Snf2-like protein Required to Maintain Repression1 (RMR1). The Snf2 protein family represents a diverse group of ATP driven motors that function in protein complexes to effect chromatin changes. Phylogenetic analyses place Snf2 proteins into several subfamilies based on conserved helicase domain homologies that are highly correlated to distinct functional roles. Accordingly, RMR1 belongs to the DRD1 subfamily, founded by the Arabidopsis protein DEFECTIVE IN RNA-DIRECTED DNA METHYLATION1. DRD1 maintains cytosine methylation patterns of sequences identical to small interfering RNAs. Comparisons of DRD1-like proteins from maize, rice and Arabidopsis show the subfamily is divisible into three distinct clades based on protein structure. RMR1 and DRD1 are members of different clades, but whether this distinction represents functional diversification is unknown. Genetic analysis of our rmr1 allele series revealed unique phenotypes that correlate with disruption of different protein domains rather than predicted allele strengths. These unique characteristics may represent novel functions for RMR1 and help guide further investigation into the molecular mechanism of RMR1 action.

P120

The Effects of Histone Acetylation on the Epi-Allele *Pl1-Blotched*

(submitted by Paul Ladipo <pble07@mizzou.edu>)
Full Author List: Ladipo, Paul B.[1]
[1] University of Missouri-Columbia; Columbia, MO, USA 65201

In maize, numerous genes are thought to function as modifiers of chromatin structure. To better understand what role these genes play in the epigenetic regulation of gene expression, transgenic lines with reduced chromatin gene expression have been produced using RNA interference (RNAi). Among the RNAi lines are a collection that target genes involved in histone acetylation and histone deacetylation. Histone acetylation typically increases gene expression because the addition of acetyl groups to histones lowers the positive charge, thus weakening the association with the negatively charged DNA. Conversely, histone deacetylation typically decreases gene expression because the removal of acetyl groups increases the positive charge of histones, thus tightening the association with chromatin. To study the effect of altered histone acetylation, we backcrossed the knockdown mutants to Pl1-Blotched, an epi-allele of the purple1 gene which encodes a transcription factor that activates anthocyanin synthesis in the maize plant. Pl1-Blotched normally produces a variegated phenotype that is associated at the molecular level with a unique pattern of DNA methylation and a more closed chromatin configuration than in other pl1 alleles. Because the amount of pigment produced reflects the level of Pl1-Blotched gene expression, this allele serves as a convenient reporter for epigenetic regulation. We found that in some lines with reduced histone deacetylase expression, pigmentation is higher than normal, and in some lines with reduced histone acetyltransferase expression, pigmentation is lower than normal. These results indicate that histone acetylation plays a role in regulating the chromatin state of Pl1-Blotched.

P121

Haplotype Variability of the 22-kDa Zein Gene Locus of Maize Inbreds B73 and BSSS53

(submitted by Jian-Hong Xu <jianhong@waksman.rutgers.edu>)
Full Author List: Xu, Jian-Hong[1]; Messing, Joachim[1]
[1] Waksman Institute, Rutgers University, 190 Frelinghuysen Rd, Piscataway, NJ, 08854, USA

Zeins are a group of alcohol-soluble proteins that are encoded by a large number of genes located on several chromosomes. a-Zeins represent the major fraction of storage proteins in maize endosperm and are subdivided into four subfamilies: z1A, z1B, and z1D, each having a Mr of mostly 19-kDa, and z1C, having a Mr of mostly 22-kDa. The genes for the z1C or 22-kDa subfamily are located at two different sites of the short arm of chromosome 4. While one locus contains one functional gene copy, the other locus is highly variable between different inbred lines in gene number and expression. Non-collinear sequence can amount to about 40%, mainly because of insertions of transposable elements. For instance, only three LTR-retrotransposons and one Helitron are shared between B73 and BSSS53, whereas 16 LTR-retrotransposons and nine DNA type transposons (hAT, CACTA and Helitron) are allele-specific. B73 has lost four gene copies of the zein genes that are present in BSSS53 by homologous recombination, while BSSS53 has gained three additional copies compared to B73 through segmental duplication. Besides seven zein genes, there are also some genes or gene fragments which are different between two inbred lines. B73 has one gene fragment, which is captured by a CACTA transposon; BSSS53 has five genes or gene fragments which are captured by Helitrons. What impact might such haplotype variability have for the evolution of the chromosomal architecture of the maize genome have and therefore for phenotype?

P122

Haplotype Variation of 19 kDa Alpha-Zein Gene Loci

(submitted by Mihai Miclaus <mihai@waksman.rutgers.edu>)
Full Author List: Miclaus, Mihai[1]; Messing, Joachim[1]
[1] Waksman Institute of Microbiology; Rutgers University; Piscataway, NJ, 08854

The _-zeins represent the major fraction of storage proteins in the maize endosperm, providing nitrogen for the germinating seedlings and being an important source of essential amino acids for humans and animals. They are divided into four sub-families, out of which three have the same relative molecular mass in SDS-polyacrylamide gels, and are thus called the 19 kDa alpha-zeins, while the fourth one has a relative molecular mass of 22 kDa. The 19 kDa zeins are further divided into three groups based on sequence homology, and are clustered in 4 loci on chromosomes 1, 4 and 7. Because protein analysis of zeins have shown extensive heterogeneity between different inbred lines, we examined the underlying genomic organization of this gene family in two corn belt inbreds: B73 and BSSS53. Data analysis indicates that some of these loci underwent a divergence process characterized by insertions of retroelements, sequence amplification and indels, in addition to the recombination events, while others are nearly completely conserved. Sequence annotation reveals clusters of nested retroelements larger than 45kb, with their captured genes inside, as well as conserved ancestral genes that could serve as anchor points for combinatorial genetics. This comparative analysis of the 19 kDa zein gene family yields new information about the evolution of its members and provides us with key elements for explaining maize haplotype variability in general.

P123

Recombination in a 100-kb Interval Containing *Helitrons* and Retrotransposons

(submitted by Limei He <limei@waksman.rutgers.edu>)
Full Author List: He, Limei[1]; Dooner, Hugo K.[1]
[1] Waksman Institute, Rutgers University, Piscataway, NJ 08854

There is ample evidence that recombination in maize is largely restricted to genes and occurs rarely, if at all, in repetitive DNA. *Helitrons* are recently discovered transposons that have trapped host gene fragments of variable length and mobilized them around the genome. One of the questions that we have addressed in this study is whether recombination occurs within those gene fragments. We have examined recombination in a 100-kb interval of 9S that contains two *Helitrons* and a large retrotransposon cluster, in addition to several genes. The *Helitrons* comprise 10 kb and the retro cluster ~ 50 kb of the total length of the interval. Both insertion types are structurally homozygous, though sufficiently polymorphic, in the heterozygote where recombination is being measured, enabling us to determine precisely if any recombination junctions fall within the insertions. We find that none do. Analysis of the *Helitron* methylation status reveals that, like retrotransposons, *Helitrons* are methylated at CG and CNG residues. All the junctions are circumscribed to the gene space, where they are distributed in a highly nonuniform manner. The *stc1* gene, which comprises just 4% of the interval, contains more than half of the total number of recombinants. Our data establish that recombination does not occur in *Helitrons*, confirm that it does not either in retrotransposons, and provide further support for the notion that recombination in maize is restricted to genes.

P124

Revised Role of an Old Disease Resistance Gene of Maize as a Savior of Grasses

(submitted by Satya Chintamanani <satya@purdue.edu>)
Full Author List: Chintamanani, Satya[1]; Sindhu, Anoop[2]; Brandt, Amanda S.[3]; Zanis, Michael J.[1]; Scofield, Steven R.[3]; Johal, Gurmukh S.[1]
[1] Department of Botany and Plant pathology, Purdue University, West Lafayette, IN 47907
[2] Department of Plant Pathology, Iowa State University; Ames, IA 50011
[3] Department of Agronomy, Purdue University, West Lafayette, IN 47907

The maize HM1 gene provides protection against a lethal leaf blight and ear mold disease caused by Cochliobolus carbonum race 1 (CCR1). Although it was the first disease resistance (R) gene to be cloned in plants (Johal and Briggs, 1992), it remains a novelty because instead of participating in plant's recognition and response system, as most R genes do, HM1 disarms the pathogen directly (Martin et al., 2003). It does so by encoding HCTR, whose function is to inactivate HC-toxin, an epoxide-containing cyclic tetrapeptide (Walton, 2006), which the pathogen produces as a key virulence factor to colonize maize (Johal and Briggs, 1992; Meeley et al., 1992). While CCR1 is strictly a pathogen of maize, orthologs of HM1 as well as the HCTR activity are present widely in the grass family (Han et al., 1997; Multani et al., 1998), suggesting an ancient and evolutionarily conserved role of this disease resistance trait in plants. Here we provide proof for such a role of HM1 since antiquity, and demonstrate its involvement in nonhost resistance of barley to CCR1. Barley leaves in which expression of the HM1 homolog was silenced became susceptible to infection by CCR1, but only if the pathogen was able to produce HC-toxin. HM1 is a disease resistance gene unique to grasses, which appears to have evolved early in this lineage as a result of threat imposed by an HC-toxin-producing pathogen such as CCR1. Given that CCR1 is a devastating pathogen capable of decimating maize, one sobering implication of this revelation is: What would have happened to cereals, the major source of food for humankind, if HM1 did not exist?

P125

Variability of *bz* Haplotypes in Maize and Its Wild Relatives

(submitted by Qinghua Wang <qinghua@waksman.rutgers.edu>)
Full Author List: Wang, Qinghua[1]; Dooner, Hugo K.[1]
[1] Waksman Institute, Rutgers University; 190 Frelinghuysen Road; Piscataway, NJ, 08854

The organization of the maize genome is remarkably polymorphic. The length and make-up of a given intergenic region can vary greatly among lines, mainly because of differences in 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 this polymorphism.

We have recently carried out a vertical comparison of 8 *bz* haplotypes from Corn Belt and tropical inbreds and tropical land races1. The extent of variation is remarkable. 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 the *bz* genomic region in several maize relatives, from teosintes (*Zea mays*, ssp. *mexicana*; *Zea mays*, ssp. *huehuetenangensis*; *Zea luxurians*; *Zea diploperennis*) to Tripsacum and Sorghum. BAC clones containing *bz* haplotypes from several of these relatives have been isolated and sequenced. The current status of this analysis will be presented.

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

P126

Wheat Genome Analysis Based on Random BAC Sequences

(submitted by Antonio Costa de Oliveira <acostol@uga.edu>)
Full Author List: Costa de Oliveira, Antonio[1]; Xu, Xyangyang[2]; Estill, James C.[1]; Estep, Matt C.[1]; Baucom, Regina[1]; DeBarry, Jeremy D.[1]; Smith, Shavanor[1]; Devos, Katrien[2]; SanMiguel, Phillip[3]; Bennetzen, Jeffrey L.[1]
[1] Department of Genetics, The University of Georgia, Athens, GA, 30602
[2] Dept. of Crop and Soil Sciences, The University of Georgia, Athens, GA, 30602
[3] Genomics Core Facility, Purdue University, West Lafayette, In 47907

Large plant genomes tend to have small islands of genes distributed within seas of repetitive DNA, making their sequencing a challenging task. Considering the amount of work and the cost of such a task, sequencing strategies must be well designed to increase the efficiency in obtaining results. Unfortunately, very little is known about the gene arrangement and content of large plant genomes, making it difficult to design such strategies because of a lack of substantial biological information. The sequencing and annotation of a small sample randomly chosen BACs from a large genome can lead to a better understanding of its properties, allowing an informed choice of a whole genome sequencing approach or approaches. Wheat (Triticum aestivum) and its many Triticeae relatives all have large genomes with a basic chromosome number of seven. More than 80% of the nuclear DNA in these species is repetitive, primarily LTR retrotransposons. The majority of cDNA probes detect loci in syntenic positions on all three genomes, such that gene content and order are highly conserved between the A, B and D genomes, although several translocations have been observed. We are currently sequencing 220 randomly selected BACs from hexaploid wheat. The results indicate a relatively gene-rich genome, with less clustering of genes than had previously been reported.

P127

A Novel DNA Structure Model Providing an Explanation for the Abundance, Diversity and Complexity of Living Organisms

(submitted by Hong-Bin Zhang <hbz7049@tamu.edu>)
Full Author List: Zhang, Hong-Bin[1]
[1] Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas 77843, USA

There are probably trillions of living organisms in the world. Although they are all, except for RNA viruses, formatted by DNA, they could be extremely different in phenotype and complexity. Therefore, what underlying mechanisms are undertaken to make the living organisms so abundant and diverged has long been an interesting question. The discovery of the genetic material, DNA, and its double helix model in the last century has shed light to decipher the mystery of the living world; however, it remains mysterious to date how the double helix DNA can account for the abundant and diverged living world. We have investigated the recent DNA or genome research results and findings, especially those of whole genome sequencing of a number of species, including human, Drosophila, chicken, rice, Arabidopsis, chimpanzee, Caenorhabditis elegans, mosquito, maize, etc., with regard to this question. The results were surprising and led us to develop a novel DNA structure model, named the linear "Jigsaw Puzzle" structure. The new DNA structure model, beyond the Watson and Crick's double helix model, provides an explanation of how the double helix DNA makes the abundant and diverged organisms in the world, i.e., how it works from DNA to abundant and diverged organisms. The DNA structure model has been tested using the classic genetics and recent genome research results of several species. It is believed that the new DNA structure model will lay an essential basis for the abundance, diversity and complexity of living organisms in the world, thus providing new insights into and new concepts for genetics, genomics and breeding of all organisms, including maize.

P128

Application of Designed Zinc-Finger Protein Technology in Plants

(submitted by Zhifang Gao <ZGAO@dow.com>)
Full Author List: Shukla, Vipula[1]; Bauer, Teresa[1]; Gao, Zhifang[1]; Arnold, Nicole[1]; McCaskill, Dave[1]; Mitchell, Jon[1]; Simpson, Matt[1]; Skokut, Michiyo[1]; Worden, Sarah[1]; Yau, Kerrm[1]; Urnov, Fyodor[2]; Miller, Jeffrey[2]; Rock, Jeremy[2]; Moehle, Erica[2]; DeKelver, Russell[2]; Doyon, Yannick[2]; Rebar, Ed[2]; Collingwood, Trevor[2]; Zhang, Lei[2]
[1] Dow AgroSciences; Indianapolis, IN 46268
[2] Sangamo Biosciences; Richmond, CA 94804

The primary sequence of the genome at endogenous loci can be altered with high efficiency in mammalian cells using designed zinc finger nucleases (ZFNs; Urnov et al. Nature 435: 646). Ongoing studies indicate that a precisely-placed double-strand break (DSB) induced by engineered ZFNs can stimulate integration of long DNA stretches into a predetermined genomic location in human cells, resulting in site-specific gene addition. Zinc finger protein technology represents a significant breakthrough relative to the ability to edit and engineer genomes in a precise manner.

In this presentation, results from a collaboration between Dow AgroSciences LLC and Sangamo Biosciences that is focused on applications of designed zinc-finger protein technology in plant species will be described. Multiple zinc-finger proteins, including zinc-finger nucleases and zinc-finger transcription factors, have been designed to target specific genes in model and agriculturally important plant species. Validation of this technology and examples of its utility for plant biotechnology will be discussed.

P129

Assembling the Maize Cell Wall Gene Network

(submitted by Bryan Penning <bpenning@purdue.edu>)
Full Author List: Penning, Bryan[2]; Tewari, Jagdish C[2]; Hunter III, Charles T.[6]; Olek, Anna[1]; Vermerris, Wilfred E.[3]; Davis, Mark[4]; Thomas, Steven[5]; Koch, Karen E.[6]; McCarty, Donald R.[6]; McCann, Maureen[2]; Carpita, Nicholas C.[1]
[1] Department of Botany & Plant Pathology, Purdue University, West Lafayette, IN 47907
[2] Department of Biological Sciences, Purdue University, West Lafayette, IN 47907
[3] Department of Agronomy, University of Florida, Gainesville, FL 32611
[4] National Renewable Energy Laboratory, Golden CO
[5] Ceres, Inc., Thousand Oaks, CA
[6] Horticultural Sciences Department, University of Florida, Gainesville, FL 32611

What is the complete set of genes required to make a cell wall? The cell wall is an integrated composite of structurally complex polymers, many of which are secondary gene products, synthesized in several compartments and assembled extracellularly. Maize and other commelinoid monocots possess cell walls that are distinct from all dicots and most other monocots. A systems approach to cell wall biology requires the integration of our existing knowledge base of cell wall molecular machinery with the gene networks that encode it, and use this foundation to predict the missing elements of these networks. We are using a bioinformatics approach to build gene phylogenies of Arabidopsis, rice, and maize into a distinct functionally classified system of cell wall biogenesis (http://cellwall.genomics.purdue.edu). Comparative genomics reveals distinct differences in polysaccharide and glyosyl transferase gene families between Arabidopsis and maize, indicating possible genes responsible for the novel wall construction in grasses. A powerful means to identify cell wall-related genes is to characterize mutants in which wall composition and/or architecture is altered. We have developed artificial neural networks to classify mutant spectrotypes by Fourier transform infrared and near-infrared spectroscopy. From high-throughput screening of leaves of several thousands of segregating lines from the UniformMu maize population, we identified and confirmed several dozen mutants with visual and/or spectral phenotypes, some of which have been shown to have altered carbohydrate-lignin compositions. In parallel, screening reverse-genetics grids has revealed mutants representing several families of cell wall-related genes, including those with visible phenotypes. Augmented by a growing collection of cell-wall mutants, we provide an essential first step towards unraveling the complexities of the maize genetic system now gaining significant agronomic importance in the increase or alteration of C4 grasses as bioenergy crops.

P130

Comparative Study of Genes Expression During Drought Stress in Maize Genotypes from the Republic of Moldova with Different Drought Tolerances

(submitted by Dumitru Badicean <dbadicean@yahoo.com>)
Full Author List: Badicean, Dumitru V.[1]; Barbacaru, Nicolai I.[1]; Jacota, Anatol G.[1]; Gardiner, Jack M.[1]; Chandler, Vicki L.[1]
[1] Institute of Genetics and Plant Physiology of ASM, 20 Padurii str, Chisinau, MD2002, Moldova
[2] University of Arizona, 303 Forbes Building, Department of Plant Sciences, Tucson, AZ 85721 USA
[3] University of Arizona, 303 Forbes Building, BIO5 Institute, Tucson, AZ 85721 USA

Temperature and rainfall variability from year to year, as well as their geographic distribution, have a negative influence on the agriculture of many regions. Analyses have shown that in Moldova drought conditions are present every third year in general and every second year in the southern region in particular. There are distinct ways of managing water resources, with irrigation being the traditional approach for dealing with water shortages. However, increasing scarcity of water resources requires the search for other solutions to dealing with the lack of annual rainfall. One potential solution is creation of drought resistant crops and/or the development of plant varieties with efficient water-use.

The aim of this work is the evaluation of gene expression in maize genotypes with different degrees of drought tolerance (at the first stages of development) using maize 70-mer oligonucleotide microarrays representing more than 50,000 identifiable maize genes. Using different methods (stress modulation by growth in osmotic solutions, evaluation of membrane thermo-stability, evaluation of FS2 activity, etc.) we have classified maize genotypes, in common use within the Republic of Moldova, into four different stress tolerance groups. We have focused our expression analysis on 6 maize genotypes from the maize germplasm collection at the Institute of Genetics and Plant Physiology of ASM with different degrees of drought stress tolerance: 1) MK01, 2) XL12, 3) DH1, 4) XL12xDH1, 5) DH1xMK01, 6)MK01xRF7. Drought treatment was imposed in greenhouse at the three leaf stage. For microarray analysis we will use a reference design targeted to identify genes that are induced or otherwise important during drought stress.

P131

Compound B-A-A Translocations and the Segmental Analysis of the Maize Genome

(submitted by William F. Sheridan <bill.sheridan@und.edu>)
Full Author List: Sheridan, William F.[1]; Auger, Donald L.[2]
[1] University of North Dakota, Grand Forks, ND, USA, 58202-9019
[2] South Dakota State University, Brookings SD, USA 57007-2142

Because of the frequent nondisjunction of the maize B chromosome centromere at the second pollen mitosis, simple B-A translocations have been used to locate genes to chromosome arms and to analyze dosage effects of specific A chromosome segments. Whereas simple B-A translocations contain a single A chromosome segment, compound B-A-A translocations contain segments of two different A chromosomes and can provide aneuploidy for two chromosome segments. The B-A-A translocations can define much smaller chromosome segments than the simple B-A translocations. Compound B-A-A translocations are created by bringing together a simple B-A translocation and an A-A translocation that share a common A chromosome segment, but with the breakpoint of the B-A proximal to the breakpoint in the A-A. Recombination in the region of shared homology of the A chromosome segments creates a new B-A-A translocation. The chromosome arm breakpoints of the approximately 870 different maize A-A translocation stocks have been characterized by earlier investigators. These stocks provide the potential for creating a large number of B-A-A translocations that can be used for fine scale cytological segmental analysis of the maize genome. Previous investigators produced 17 B-A-A translocations. We have enlarged this group by creating 64 new B-A-A translocations. We will present the distribution of the chromosome breakpoints for the 81 B-A-A translocations and the 163 cytologically defined chromosome segments delimited by them. We will show the localization of repetitive DNA marker probes within selected B-A-A defined segments using fluorescent in situ hybridization. We will also report the effects on plant morphological phenotypes associated with extra doses (hyperploidy) of regions of chromosome arms 1L, 5S, 7L, and 10L.

P132

Development of a Sequence-Indexed mPing Transposon Collection for Rice

(submitted by Kazuhiro Kikuchi <kk376@cornell.edu>)
Full Author List: Kikuchi, Kazuhiro[1]; Ling, Xu[1]; Brutnell, Thomas P.[1]
[1] Boyce Thompson Institute, Cornell University, Ithaca, NY, USA 14853

Active minature inverted-repeat transposable elements (MITEs) have recently been discovered in rice. The non-autonomous mPing elements can be mobilized following anther-culture, cell-culture, _-irradiation or following wide crosses between domesticated and wild-rice cultivars. A putative autonomous element, Ping, has been identified in Japonica cultivars and may have mediated the widespread amplification of mPing elements in this cultivar. Ping maps to chromosome 6 in Nipponbare (Japonica), but not in 93-11 (Indica). Using DNA blot analysis we discovered that several US cultivars do not contain the Ping element and have low copy number of mPing insertions. Currently, we are trying to introduce active copies of Ping into the U.S. germplasm by crossing to Nipponbare. We hope to activate mPing transposition in US rice germplasm through these crosses or by chemical or tissue culture treatments. We will mobilize mPing to create a series of near-isogenic insertion lines and are developing methodologies to rapidly amplify, clone and sequence mPing insertion sites using 454 sequence technology. Our goal is to develop a reverse-genetics resource of non-transgenic lines utilizing US germplasm that will be readily accessible to plant scientists and breeders in the US and abroad.

P133

Expression Profiling of Maize and Sorghum miRNAs Using 454 Sequencing

(submitted by Doreen Ware <ware@cshl.edu>)
Full Author List: Maher, Christopher[1]; Zhang, Lifang[2]; Lu, Cheng[3]; Meyers, Blake[4]; Green, Pamela[5]; Stein, Lincoln D.[1]; Ware, Doreen H.[6]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA; and Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY 11794
[2] Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY 11794
[3] Delaware Biotechnology Institute, University of Delaware, Newark, DE 19711
[4] Delaware Biotechnology Institute, University of Delaware, Newark, DE 19711; and Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19711
[5] Delaware Biotechnology Institute, University of Delaware, Newark, DE 19711; Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19711; and College of Marine Studies, University of Delaware, Newark, DE 19711
[6] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA; and USDA ARS North Atlantic Area Plant Soil & Nutrition Laboratory Research Unit, Ithaca, NY

microRNAs are small noncoding RNAs that play an intricate role in regulating gene expression in eukaryotes through mRNA cleavage and translational inhibition. Within plants, miRNA gene families have been demonstrated to target mRNAs from gene families involved in critical processes such as transcription factor activity, stress response, metabolism, and hormone signaling. In addition to targeting protein-coding genes, some miRNAs target noncoding RNA transcripts, known as trans-acting siRNA transcripts (tasiRNAs). Following cleavage, one of the byproducts is further processed into 20 to 22 nucleotide small RNAs, some of which target protein-coding genes. In our analysis we used 454 sequencing of the maize and sorghum small RNA transcriptomes to validate the expression of known miRNAs, novel gene discovery, and differential expression analysis.

High-throughput sequencing of seedling and inflorescence, two key developmental time points, enabled us to validate the expression of two thirds of the known mature miRNA sequences. In addition, we were able to validate the expression of three maize and two sorghum tasiRNAs. A closer analysis of highly conserved small RNAs between maize and sorghum revealed an addition 45 previously undetected paralogs. We also found that highly conserved miRNAs across species targeted similar gene families and shared similar expression profiles suggesting similar regulatory roles.

While a great effort has been placed in studying highly conserved miRNAs, we present a species-independent methodology to predict recently evolved miRNA genes in incomplete genome sequences. In total, we identified thirteen novel candidate miRNA genes from ten different gene families. Not only will this methodology provide key insights into newly evolved functionalities that may define a species, this approach will be useful for identifying lineage-specific miRNA genes in larger unsequenced cereal genomes. This project was made possible by funds from the USDA Agricultural Research Service and the National Science Foundation.

P134

Expression Profiling of Vegetative and Endosperm Tissues in Hybrid Maize: cis-Acting Effects and Transcriptional Imprinting

(submitted by Robert Stupar <stup0004@umn.edu>)
Full Author List: Stupar, Robert M.[1]; Hermanson, Peter J.[1]; Springer, Nathan M.[1]
[1] University of Minnesota; 1445 Gortner Ave.; St. Paul, MN 55108

A combination of Affymetrix microarray profiling and allele-specific expression approaches were applied to RNA samples isolated from vegetative and endosperm tissues from the maize inbred lines B73 and Mo17 and their reciprocal hybrids. These results were used to assess the level of intraspecific variation in these two maize lines and to understand how this variation combines to affect gene expression levels in hybrids across tissues types. A significant fraction of genes displayed additive expression and allelic cis-varitation in the vegetative tissues of hybrids. Most genes also displayed additive expression and alleleic cis-variation in endosperm tissue, however non-dosage-dependent expression levels were observed in the hybrid endosperm tissue more frequently than in vegetative tissues. These non-dosage dependent expression patterns include maternal-like, paternal-like, dominant high-parent, dominant low-parent and expression outside the range of the inbreds in one or both hybrids. Additionally, endosperm alleles frequently (~10% of genes) exhibited parental affects, and several genes with potentially imprinted expression patterns were identified. These results provide a framework for understanding the prevalence of transcriptional dosage-dependence and imprinting in the maize endosperm.

P135

Gene Discovery and Annotation Using LCM-454 Transcriptome Sequencing

(submitted by Li Li <lilsunny@iastate.edu>)
Full Author List: Emrich, Scott J.[1]; Barbazuk, W. Brad[2]; Li, Li[1]; Schnable, Patrick S.[1]
[1] Iowa State University, Ames, Iowa, 50010
[2] Donald Danforth Plant Science Center, St. Louis, Missouri, 63132

454 DNA sequencing technology achieves significant throughput relative to traditional approaches. More than 261,000 ESTs were generated by 454 Life Sciences from cDNA isolated using laser capture microdissection (LCM) from the developmentally important shoot apical meristem (SAM) of maize (Zea mays L.). This single sequencing run annotated >25,000 maize genomic sequences and also captured ~400 expressed transcripts for which homologous sequences have not yet been identified in other species. Approximately 70% of the ESTs generated in this study had not been captured during a previous EST project conducted using a cDNA library constructed from hand-dissected apex tissue that is highly enriched for SAMs. In addition, at least 30% of the 454-ESTs do not align to any of the ~648,000 extant maize ESTs using conservative alignment criteria. These results indicate that the combination of LCM and the deep sequencing possible with 454 technology enriches for SAM transcripts not present in current EST collections. RT-PCR was used to validate the expression of 27 genes whose expression had been detected in the SAM via LCM-454 technology, but that lacked orthologs in GenBank. Significantly, transcripts from ~74% (20/27) of these validated SAM-expressed "orphans" were not detected in meristem-rich immature ears. We conclude that the coupling of LCM and 454 sequencing technologies facilitates the discovery of rare, possibly cell-type-specific transcripts.

P136

Gene Expression Profiles of Corn Developing Kernels of *Tex6* Using Maize Oligo-Microarray

(submitted by Baozhu Guo <bguo@tifton.usda.gov>)
Full Author List: Guo, Baozhu[1]; Luo, Meng[2]; Liu, Jia[3]; Lee, R. Dewey[2]
[1] USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA
[2] University of Georgia, Department of Crop and Soil Sciences, Tifton, GA
[3] The Institute for Genomic Research, Beltsville, MD

Maize oligonuleotide microarray was used to analyze the temporal patterns of gene expression in late developmental maize kernels of Tex6 after 25 days after pollination (DAP). There was a total of 57,452 70-mer oligonucleotides on a set of two array-slides. Because of the resistant traits of Tex6, we reasoned that this unique maize line would be an interesting candidate for microarray study of gene expression in developing kernels during the time favorable for aflatoxin contamination. In this report, transcriptional profiles of Tex6 kernels at 25, 30, 35, 40, and 45 DAP were compared using the 70-mer maize oligonucleotide arrays based on relative expression quantitation, in which glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. Quatitative real-time PCR was also used for validation of selected genes from microarray data. There were total of 8621 positive array spots with unique IDs and 4247 cross-talking genes identified in all sampling stages. These were averaged at 6218 array spots expressed in each sampling stage. Expression patterns of key genes in several metabolic pathways, including starch, lipid and storage proteins, were analyzed. Among the storage proteins, the expression patterns of defense related genes (2676 unique IDs) were identified as up-regulated, down-regulated, or no-change based on hierarchical analysis. Some defense related genes were highly expressed throughout the late kernel development. Twenty genes with different expression trends from microarray were selected for validation using quantitative real-time PCR. The real-time PCR reproduced the expression patterns of up- and down-regulated genes, but did not completely reproduce the genes without changes as in the microarray study. This study was able to investigate the gene categories at various stages of kernel development, therefore providing further insight into gene expression profiles associated with the reduced aflatoxin contamination of Tex6.

P137

Gene and SNP Discovery Using 454 Sequencing of Methylation-Filtered HpaII Libraries

(submitted by Michael Gore <mag87@cornell.edu>)
Full Author List: Gore, Michael A.[1]; Ersoz, Elhan S.[1]; Hurwitz, Bonnie[2]; Narechania, Apurva[2]; Wright, Mark[3]; Grills, George[4]; Ware, Doreen H.[5]; Harkins, Timothy[6]; Taillon, Bruce[7]; Buckler, Edward S.[8]
[1] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[3] Molecular Biology & Genetics, Cornell University, Ithaca, NY 14853
[4] Biotechnology Resource Center, Cornell University, Ithaca, NY 14853
[5] USDA-ARS, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[6] Roche Applied Science, Indianapolis, IN 46250
[7] 454 Life Sciences, Branford, CT 06457
[8] USDA-ARS, Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853

In our ongoing efforts to bridge the gap between plant genome studies and improved breeding strategies, association approaches offer the most direct means of identifying genes and alleles of agronomic importance. With high levels of naturally occurring genetic diversity and low levels of linkage disequilibrium (LD), diverse maize inbred lines are tremendous resources for association mapping of quantitative traits down to the gene level. An estimated 1 million SNP markers, however, are required to capture most of the LD structure in maize. This project (NSF grant DBI0638566) seeks to: (1) identify 1 to 2 million sequence polymorphisms focused in areas of active recombination in the maize genome, and (2) score these polymorphisms against 27 diverse lines that capture 80% of the common variation in maize. To accomplish these goals, we developed a library preparation method built on the differential cytosine methylation patterns of genes and retrotransposons, which are discriminated by the methylation-sensitive restriction enzyme HpaII. This gene-biased HpaII library construction approach, in conjunction with 454 sequencing, is being used for high-throughput SNP discovery via library oversampling. Preliminary sequence data from B73 and Mo17 HpaII libraries have been generated using a 454 GS-FLX instrument.

P138

Generation and Initial Analysis of a Sequence-Indexed Collection of Endogenous Ds Insertion Lines in Maize

(submitted by Erik Vollbrecht <vollbrec@iastate.edu>)
Full Author List: Hall, Bradford D.[1]; Schares, Justin[1]; Ahern, Kevin[2]; Sabharwal, Mukul[1]; Deewatthanawong, Prasit[2]; Ling, Xu[2]; Kikuchi, Kazuhiro[2]; Conrad, Liza J.[3]; Weeks, Becky[1]; Knoot, Cory[1]; Lamar, Kay-Marie[1]; Brutnell, Thomas P.[2]; Brendel, Volker[1]; Vollbrecht, Erik[1]
[1] Iowa State University; Ames, IA, 50011
[2] Boyce Thompson Institute, Cornell University; Ithaca, NY 14853
[3] Cornell University; Ithaca, NY 14853

As sequencing of the maize genome progresses, reverse genetics resources are among the community's paramount needs. Through NSF funding (http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=0501713), we are creating a sequence-indexed collection of transposable element insertions distributed throughout the maize genome. These insertions provide a platform for both reverse and forward genetics. Using endogenous *Activator* (*Ac*) and *Dissociation* (*Ds*) elements converged into the W22 inbred line, we select for *Ds* transpositions from donor anthocyanin genes, and then identify unlinked reinsertions. DNA that flanks the reinsertion is isolated by inverse PCR and sequenced. We describe several components we have developed and implemented, including large-scale field genetics to create and propagate *Ds* excision events, a DNA sequencing and analysis pipeline, and an informatics infrastructure to manage data in-house and prepare it for public release. Initial analysis of flanking *Ds* (fDs) sequences shows that only a small fraction of fDs contain repetitive sequences (less than 5% by repeat masking, average fDs length ~350 bp). We are fully sequencing some long (4-5 kb) fDs, to produce ~200 kb of high quality DNA sequence which, given the low repeat content of the shorter fDs, should be in and near the W22 gene space and be useful for comparative analysis with the B73 genome sequence. To facilitate identification and distribution of gene knockouts, we are placing fDs sequences on both GSS and the emerging genome assemblies. As predicted from the genetics, the unlinked fDs distribute to random chromosomes relative to the donor location. In parallel, we are characterizing over 200 predicted linked reinsertions, to more fully elucidate the biology of *Ds* transposition. The insertion lines generated by this project are available from the Brutnell lab for a small fee (approx. $40/line) that will cover the costs of propagating and shipping maize kernels.

P139

Genetic Control of Gene Expression in Maize

(submitted by Ruth Swanson-Wagner <swansonr@iastate.edu>)
Full Author List: Swanson-Wagner, Ruth A.[1]; DeCook, Rhonda[2]; Hargreaves, Sarah K.[1]; Guo, Ling[1]; Chen, Hsin D.[1]; Lu, Pengcheng[1]; Nettleton, Dan[1]; Schnable, Patrick S.[1]
[1] Iowa State University; Ames, IA 50011-3650
[2] Present Address: University of Iowa; Iowa City, IA 52242

We have previously used microarray technology to examine global patterns of gene expression in a maize hybrid and its inbred parents (B73 and Mo17). Differential expression was detected for over one thousand genes in at least one of the three genotypes examined (Swanson-Wagner et al., 2006). This experiment, however, did not provide insight to the genetic regulation of the differences in gene expression among the genotypes. The regulation of gene expression levels can be studied in eQTL mapping, a combination of traditional QTL mapping and global expression profiling. The maize IBM population of recombinant inbred lines (RILs) was developed from a cross of inbred lines B73 and Mo17. Each RIL is mosaic, and homozygous for either the B73 or the Mo17 allele at each locus. We have previously published a genetic map containing approximately 3,300 markers (Fu et al., 2006). More recently, we have developed an IBM map that contains over 9,000 genetic markers. This map was used in conjunction with the eQTL analyses to gain insight into the regulation and mechanisms underlying heterosis. As a first step, 30 IBM RILs were crossed onto both B73 and Mo17. In combination with the RILs per se, the resulting lines provided a contrast of gene expression for the heterozygous genotype and both homozygous genotypes across all segregating genomic loci. Four replications of each RIL, B73xRIL, and Mo17xRIL genotype were hybridized to a custom cDNA microarray using a loop design that included all pairwise comparisons among genotypes. Significant associations were identified between genetic markers and gene expression levels, with some markers associated with multiple transcripts. Substantial amounts of both cis- and trans-regulation of expression were detected.

P140

Genomic Responses to a Century of Artificial Selection in Maize

(submitted by Han Zhao <zhaohan@uiuc.edu>)
Full Author List: Zhao, Han[1]; Schneerman, Martha C.[1]; Wrage, Elizabeth[1]; Salas, Ayna[1]; Singletary, George[2]; Moose, Stephen P.[1]
[1] Department of Crop Sciences; University of Illinois; Urbana IL, 61801
[2] Pioneer Hi-Bred International, PO Box 1004, Johnston, IA, 50131-1004

Though prior studies have been able to detect evidence for selection among maize genes associated with domestication from teosinte, documenting responses to selection among maize breeding populations has been problematic, particularly for quantitative traits. The Illinois Protein Strains represent four related populations that have been subjected to 105 cycles of divergent recurrent selection for grain protein concentration. This selection program has produced lines that span the known phenotypic extremes in maize for grain composition and a number of correlated traits, suggesting these populations may reveal mechanistic insights into responses of the complex maize genome to artificial selection.

The premise of this study is that genes which show strong differences in RNA expression among the Illinois Protein Strains are also likely to be functional targets of phenotypic selection. Microarray experiments comparing seed RNA expression from the Illinois High Protein (IHP) and Illinois Low Protein (ILP) lines have identified a set of genes whose expression changes greater than 10-fold between these divergently selected genotypes. Many of these genes are predicted to function in pathways associated with the accumulation of nitrogen by developing maize seeds. These candidate genes for targets of selection are being further characterized by DNA sequencing, RT-PCR assays, and in the case of the genes encoding zein seed storage proteins, transgenic promoter-GUS reporter lines. Additional genetic tests are also being developed to distinguish haplotype and expression variation resulting from genetic drift versus selection, by comparing gene frequencies and expression differences among different cycles of the selection experiment and a high-resolution linkage mapping population derived from the cross of IHP x ILP. The results from these studies will illustrate how phenotypic selection has influence the maize genome and its expression.

P141

High-Density Genetic Map of Maize Genes

(submitted by Hsin Chen <debchen@iastate.edu>)
Full Author List: Chen, Hsin D.[1]; Guo, Ling[1]; Fu, Yan[4]; Emrich, Scott J.[1]; Ronin, Yefim I[2]; Viswanathan, Karthik[1]; Mester, David I[2]; Ashlock, Daniel[3]; Korol, Abraham B[2]; Aluru, Srinivas[1]; Schnable, Patrick S.[1]
[1] Iowa State University, Ames, Iowa, USA 50011
[2] University of Haifa, Mount Carmel, Haifa, ISRAEL 31905
[3] University of Guelph, Guelph, Ontario, CANADA N1G 2W1
[4] Donald Danforth Plant Science Center, St. Louis, Missouri, USA 63132

To facilitate scientific advances and crop improvement, the thousands of maize genes being discovered by the maize genome sequencing project must be ordered relative to a genetic map. As part of the NSF-funded ISU Maize Genetic Mapping Project genes are being genetically mapped using a PCR-based strategy. The project's latest high-density transcript map, ISU_IBM Map7, contains 5,829 gene-based ISU markers integrated with 3490 markers generated by other projects. This map was generated using MultiMap software and released on January 9, 2007. This map is available at http://magi.plantgenomics.iastate.edu/cgi-bin/cmap/viewer; where it can be viewed via CMap. Detailed annotation regarding all ISU markers is available on the MAGI website. This map is enhancing our understanding of the organization and evolution of the maize genome. These mapped genes also provide sequence-based cross-links to facilitate the alignment of the maize genetic map with both the rice and maize physical maps, and thereby provide a means to predict the linear order of maize genes on maize chromosomes and to anchor BAC contigs. The resulting resource is facilitating map-based gene cloning experiments and the assignment of functions to genes via QTL analyses.

P142

Identification of Maize Grain Yield QTLs Across Distinct Patterns of Genotype-by-Environment Interaction

(submitted by Travis Coleman <tcoleman@uoguelph.ca>)
Full Author List: Coleman, Travis K[1]; Singh, Asheesh K.[1]; Tollenaar, Matthijs[1]; Lee, Elizabeth A.[1]
[1] University of Guelph; Crop Science; Guelph, Ontario, Canada N1G 2W1

In maize, detection and analysis of quantitative trait loci (QTLs) helps to elucidate complex genetic and physiological processes controlling grain yield. Information gleaned from mapping yield QTLs may be put to a variety of ends such as studying wide-scale functional genomics, or assisting in breeding programs. Because of the complex nature of grain yield, maize QTLs often exhibit extensive genotype by environment (GxE) interaction, thus complicating both genomics and breeding efforts. To examine GxE interaction, we are using an unique set of RILs derived from related lines (CG60 x CG108). Because of their nature, 67% of the genome is fixed and many gross phenological differences are minimized. The 123 RILs (Iodent heterotic pattern) were crossed to CG102 (Stiff Stalk heterotic pattern) to form hybrids. This study relies on location (Alma, Elora, and Orangeville ON), year (2004, 2005, 2006), and plant population density effects to generate GxE interactions. Currently 15 "environments" (combination of location, year, and density) resolve themselves into 6 distinct GxE interaction groups in yield trials using our population of 123 hybrid RILs. Preliminary results suggest that many of the grain yield QTLs are specific to a particular environmental group (i.e. GxE pattern). With the addition of 9 unique environments in 2007, this project aims to expand our understanding of yield QTLs and their GxE interactions.

P143

Identifying Zero Sequence Diversity Genes in Maize Using Temperature Gradient Capillary Electrophoresis (TGCE)

(submitted by Julie Meyer <jmm130@truman.edu>)
Full Author List: Meyer, Julie M[1]; Chen, Hsin D.[2]; Shendelman, Josh[2]; Schnable, Patrick S.[2]
[1] Truman State University, Kirksville, MO 63501
[2] Iowa State University, Ames, IA 50011

Maize is one of the most important crops in the world. Genes that exhibit complete sequence conservation, or zero sequence diversity, may be key genes that are the difference between maize and all other wild relatives like teosinte. Among 24 inbred lines chosen, those displaying zero sequence diversity were retested and analyzed using temperature gradient capillary electrophoresis (TGCE). Of the 81 primer pairs designed from Maize Genomic Assembled Islands (MAGI) sequences, data was obtained for 67. 66 were confirmed and one failed to be confirmed to exhibit zero sequence diversity. This indicates that the 66 sequences are highly conserved and might possibly be the genes that set maize apart from its wild relatives. Further testing of teosinte lines will show which of the zero sequence diversity genes in maize are responsible for the domestic phenotype of modern maize.

P144

Laser Microdissection-Microarray Analyses of *ragged seedling2*, a Gene Required for Maize Leaf Patterning

(submitted by Xiaolan Zhang <xzhang@plantbio.uga.edu>)
Full Author List: Zhang, Xiaolan[1]; Zhou, Ruiling[2]; Jin, Hailing[2]; Borsuk, Lisa A.[2]; Nettleton, Dan[3]; Schnable, Patrick S.[2]; Scanlon, Michael J.[4]
[1] Plant Biology Department, University of Georgia, Athens, GA, 30602, USA
[2] Center for Plant Genomics, Iowa State University, Ames, IA, 50011,
[3] Department of Statistics, Iowa State University, Ames, IA, 50011, USA
[4] Department of Plant Biology, Cornell University Ithaca, NY 14853-5904

The recessive mutation ragged seedling2-R (rgd2-R) results in defective lateral development of maize leaves. Although dorsiventrality is established, some swapping of adaxial/abaxial epidermal identity may occur. Molecular analyses indicate that RGD2 is required for normal transcript levels of multiple leaf developmental markers (including NS1, YABBY14, Zm*KANADI2 and ROLLED1), although the domain-specificity and expression patterns of these transcripts are unaffected. These data suggest that RGD2 is required for lateral expansion and the coordination of developmental patterning in maize leaf development. The RGD2 gene product is unknown, and the mechanism of RGD2 function during maize leaf development is completely unknown. Here we investigate the genetic mechanisms of RGD2 gene function using SAM-specific genomic profiling of 28,671 maize cDNAs via laser microdissection-microarray analyses. These analyses identified 280 differentially expressed genes in rgd2-R mutant meristems, including 29 that are predicted to be involved in either signal transduction or SCF-mediated proteolysis as well as 80 genes of unknown predicted function. In situ hybridization was used to characterize the novel expression patterns of two differentially expressed genes of unknown function. Additional qRT-PCR and in situ hybridization verifications of microarray data are in progress. These experiments provide a database of genes and potential genetic pathways implicated during RGD2 function, which may provide novel insight into mechanisms of leaf patterning and lateral expansion.

P145

Maize Full-length cDNA Project

(submitted by Yeisoo Yu <yeisooyu@ag.arizona.edu>)
Full Author List: Yu, Yeisoo[1]; Haller, Karl[2]; Kudrna, Dave[1]; Collura, Kristi[1]; Campos, David[1]; Wissotski, Maria[1]; Lopez, Georgina[1]; Currie, Jennifer[1]; Golser, Wolfgang[1]; Ashley, Elizabeth[1]; Descour, Anne[2]; Morrow, Darren[3]; Fernandes, John[3]; Soderlund, Cari[2]; Walbot, Virginia[3]
[1] Arizona Genomics Institute, University of Arizona, Tucson, AZ 85721
[2] Arizona Genomics Computational Laboratory, University of Arizona, Tucson, AZ 85721
[3] Department of Biological Sciences, Stanford University, 855 California Ave., Palo Alto, CA 94304

Maize is the most important US crop and the complete genome sequencing is in progress. Therefore, the availability of full-length (FL) cDNA sequences becomes crucial for accurate annotation and future functional characterization of the genes. NSF funded maize full-length cDNA project will finish approximately 30,000 FLcDNAs, focusing on reproductive development and stress-induced transcripts. A full-length cDNA library in a Gateway-compatible backbone was built using a pooled RNA from 13 B-73 reproductive tissues, highly enriched for full-length transcripts by cap and tail selection, and then normalized to increase representation of rare transcripts. The second cDNA library from embryo, scutellum and 7-day seedlings with stress inducer treatments was also made following the same methods. Currently, about 23,000 candidate FLcDNA clones for full-length sequencing has been selected based on 360,000 5' and 3' EST assemblies generated in this project by aligning to publicly available ESTs, FLcDNAs and genomic sequences of rice and maize. Iterative primer walking has been employed on each candidate FLcDNA, and a transposon method will also be utilized to complete finish larger insert and difficult clones with phred 40 quality as a finishing standard. In addition, we are collecting expression profile by hybridization of RNA from stress induced seedlings and dissected reproductive tissues on two oligo-microarray platforms. The information derived from this project will be used to better understand gene regulatory networks involved in plant reproduction and environmental stresses leading to higher yielding and broader adaptive crop plants to feed future generations. Maize full-length cDNA project will also help to train teachers and young scientists from underrepresented groups in the art of scientific investigation and discovery through outreach efforts.

P146

Maize Functional Genomics - North

(submitted by Elizabeth Lee <lizlee@uoguelph.ca>)
Full Author List: Lee, Elizabeth A.[1]; Colasanti, Joe[2]; Raizada, Manish[1]; Tetlow, Ian[2]; Tollenaar, Matthijs[1]; Emes, Mike[2]; Rothstein, Steven[2]
[1] University of Guelph, Department of Plant Agriculture, Guelph, Ontario N1G 2W1 CANADA
[2] University of Guelph, Department of Molecular and Cellular Biology, Guelph, Ontario N1G 2W1 CANADA

Modern maize is the result of thousands of years of selection by ancient farmers who produced significant changes in its architecture and growth patterns, and by 20th century breeders who selected for incremental increases in tolerance to environmental stresses. As the next level in corn improvement, the maize group at the University of Guelph is embarking on an inter-disciplinary genomics research project focused on improving grain yield and kernel quality through misexpression of selected candidate genes to further adapt corn for Northern climates. To achieve this we are systematically testing key genes that may increase yield and environmental stress tolerance based on published information about gene function. As in traditional corn domestication we will initially focus on altering architecture and growth rate by misexpressing genes from several categories that may augment plant structure for yield support and extend the grain fill period. Additional gene discovery will be made through phenomic and metabolomic efforts. QTL studies and selection bias studies will identify key genomic regions for subsequent diversity profiling and candidate gene searches for processes influencing grain yield and quality. We invite the maize community to collaborate on this project by submitting suggestions for transgene misexpression.

P147

Maize Genome Sequencing Pipeline

(submitted by Laura Courtney <lcourtne@watson.wustl.edu>)
Full Author List: Courtney, Laura P.[1]; Kruchowski, Scott S.[1]; Minx, Patrick[1]; Tomlinson, Chad M.[1]; Maize Genome Sequencing Consortium, The[2]
[1] Genome Sequencing Center; Washington University School of Medicine; St. Louis, MO 63108
[2] Maize Sequencing Genome Consortium; genome.wustl.edu

The strategy chosen to sequence the maize genome uses a tilepath of mapped BACs. This method of isolating the highly repetitive elements of the maize genome into smaller regions has been successful in assembling maize sequence. Sequence data from the BACs are generated with low coverage, approximately 4-6X, and BACs are then confirmed by end sequence. To improve the fragmented draft assembly, software is used to incorporate fosmid paired end sequence to provide order and orientation. An analysis of retro-transposon scaffolding is also used to improve order and orientation. Since the focus of the maize project is gene space within the genome, the unique, non-repetitive regions are targeted by K-mer analysis software (Ware, D., Cold Spring Harbor Laboratories) for improvement. Automated sequence improvement is performed using Autofinish (Gordon, D., University of Washington) on all gaps and low quality regions within unique sequence data. Additional data in the form of methyl-filtered reads, high-Cot fraction data, EST, and cDNA data is incorporated into the assembly following the automated prefinishing. Manual improvement of miss-assemblies, gaps, and low quality data within unique sequence is then performed using specialty directed sequence reactions. Once the unique sequence has met standards set by the Maize Consortium, the sequence data is submitted to Genbank as Phase-I improved (HTGS_IMPROVED). Over the next 2 years our goal is to develop quicker and more cost effective means of improving the maize sequence data.

P148

Maize Leaf Initiation: A Genomic Analysis

(submitted by Mike Scanlon <mjs298@cornell.edu>)
Full Author List: Brooks III, Lionel[1]; Elshire, Robert J.[1]; Ohtsu, Kazuhiro[2]; Schnable, Patrick S.[2]; Scanlon, Michael J.[1]
[1] Department of Plant Biology, Cornell University, Ithaca, NY, USA 14853
[2] Center for Plant Genomics, Iowa State University, Ames, IA, USA 50011

The maize vegetative shoot apical meristem (SAM) maintains a pool of indeterminate stem cells within the central zone (CZ) and crown, and initiates lateral organs from the peripheral zone (PZ). Deciphering the genetic mechanisms that maintain this crucial balance between stem cell maintenance and organogenesis is a focus of plant developmental analyses. Genetic studies of model organisms have identified a number of genes required for leaf initiation, although embryo/seedling lethal phenotypes present obstacles to analyses of early events in leaf development. Here we describe the use of laser microdissection microarray (LMM) technology in genomic analyses of leaf initiation in maize. Samples encompassing both the initiating maize leaf (P0/P1) and the CZ and crown (SAM proper) were laser microdissected; RNA was extracted from the microdissected tissues and used for microarray analyses. In six biological replicates comprised of samples derived from 10 apices each, over 153 differentially expressed genes (out of > 29,000) were identified at p values < 0.01. Differential expression of control marker genes in the P0/P1 (i.e. maize yabby paralogues; rough sheath2) and in the SAM proper (knotted1) attests to the precision of the LM protocols. The identification of 71 maize genes with no similarity to previously described genes reveals the power of LMM technology for gene discovery. In situ hybridization analyses of selected differentially expressed genes identified novel expression patterns. qRT-PCR analyses are used to identify implicated genes that are also differentially expressed in NPA-treated maize SAMs, which are defective in polar auxin transport and fail to initiate leaf primordia. Genes identified in both these experiments represent strong candidates that are likely to function during early events in leaf initiation. These LMM genomic analyses generated a unique maize database and provide a platform for subsequent reverse genetic and molecular analyses of leaf development in maize and Arabidopsis.

P149

Many Maize Genes are Expressed in an Oat Background Carrying a Specific Maize Chromosome

(submitted by Candida Cabral <cabr0024@umn.edu>)
Full Author List: Cabral, Candida B[1]; Springer, Nathan M.[1]; Rines, Howard W[2]; Phillips, Ronald L[1]
[1] University of Minnesota, Saint Paul, MN 55108, USA
[2] USDA-ARS, Saint Paul, MN 55108, USA

Oat-maize addition (OMA) lines are derived from oat x maize sexual hybrids in which individual maize chromosomes have been retained in plants containing a full complement of oat chromosomes. Many of the OMA lines display specific phenotypes, which indicate that maize genes are likely expressed and capable of altering the phenotype of oat plants. We employed Affymetrix microarrays to determine the frequency of expression for maize genes in an alien background and whether independently derived OMA lines for the same chromosome expressed the same maize genes. We utilized seedling tissue of three OMA lines containing chromosome 5 from the B73 maize inbred line in two oat backgrounds. We identified a set of 438 non-redundant maize genes that were expressed in at least one of the three OMA lines. We found that 96% (420/438) of the genes displayed expression in all three lines. Comparison of expression levels for maize genes in an OMA background with expression levels in B73 maize seedlings revealed that ~24% of the maize chromosome 5 genes that were expressed in maize seedlings were also expressed in the OMA seedlings. Among the genes with no detectable expression in OMA lines, many were highly expressed in maize seedlings. We annotated the non-redundant gene set with regards to map position and putative function. In total, 222 genes had positional information available, and the majority mapped either to chromosome 5 (62%) or chromosomes that share homology with it (17%). We did not find evidence for active or silenced chromosomal domains; neither did we detect a significant over-representation of any specific GO annotation in the non-redundant gene set. So far, we have gathered evidence that a consistent set of maize genes was expressed in seedling tissue across independently derived chromosome 5 OMA lines, regardless of background, chromosome location, or putative function.

P150

Microarray Resources for Maize

(submitted by Jack Gardiner <gardiner@ag.arizona.edu>)
Full Author List: Gardiner, Jack M.[1]; Kaeppler, Shawn[2]; Galbraith, David[3]; Buell, Robin[4]; Iniguez, A. Leonardo[2]; Liu, Jia[4]; Ly, Eugene[4]; Chandler, Vicki L.[3]
[1] Department of Plant Sciences, University of Arizona, Tucson, AZ 85721
[2] Department of Agonomy, University of Wisconsin, Madison, WI 53706
[3] BIO5 Institute, University of Arizona, Tucson, AZ 85721
[4] The Institute for Genomic Research, Rockville, MD 20850

The Maize Microarray Project (http://www.maizearray.org) was initiated to provide low-cost, comprehensive, public sector long-oligonucleotide microarrays for gene expression analysis in Zea mays L. The overall objectives are: 1) Produce an array with 70-mer oligonucleotides for the >30,000 identifiable unique maize genes which should allow better discrimination among the gene duplications common in maize relative to cDNA arrays, and provide a hybridization service, both based on a cost recovery model. 2) Provide a website to distribute microarray information, and archive expression data generated by the project and array users with links to rice and maize genome annotation. 3) Perform expression profiling with a subset of maize tissues to provide a baseline of data and detailed protocols for the community. 4) Utilize the flexible NimbleGen system to experimentally refine oligonucleotide design for the next generation of 70-mer arrays which will achieve better discrimination among gene family members. 5) Develop web based experimental design tutorials and provide experimental design assistance to users as needed. An array with 57,452 70-mer oligonucleotides is available as a slide pair for $125. To date, 3500 slide sets have been ordered and distributed to 65 laboratories in the U.S., England, Italy, China, Canada, Ireland, Venezuela, Australia, Mexico, Poland, France, Belgium, Brazil, and Switzerland. A MIAME compliant relational database, Zeamage, has been constructed to store expression data generated by this project. Replicated baseline expression profiles for 18 tissues have been deposited in Zeamage as have data from 12 additional expression profiling studies representing a total of 468 hybridizations. In April 2007, an additional 7 studies representing 134 additional hybridizations will be deposited into Zeamage. NimbleGen arrays have been used to orient unoriented ESTs, validate AZM expression, and assess antisense transcription. A set of standardized, cross-platform protocols has been developed to allow performance of oligos on NinbleGen arrays to be predictive of performance on spotted arrays. Experimental protocols for the array have been optimized and are available on the website. Five workshops have been held at the University of Arizona and a total of 75 researchers have participated.

P151

Molecular Characterization of the CETS Gene Family in Maize

(submitted by Olga Danilevskaya <olga.danilevskaya@pioneer.com>)
Full Author List: Danilevskaya, Olga N.[1]; Meng, Xin[1]; Simmons, Carl[1]; Ananiev, Evgueni V.[1]
[1] Pioneer Hi-Bred Int., 7250 NW 62nd Ave, PO BOX 552, Johnston, IA50131-552

The CETS gene family (Pnueli et al., 2001) was named after the three plant genes: Antirrhinum CENTRORADIALIS (CEN) (Bradley et al., 1996), Arabidopsis TERMINAL FLOWER 1 (TFL1) (Bradley et al, 1997), and tomato SELF-PRUNING (SP) (Pnueli et al., 1998). The CETS genes encode closely related proteins with similarity to mammalian phosphatidylethanolamine-binding proteins (PEBPs) that act as kinase inhibitors. In plants the most extensively studied CETS proteins function as promoters and repressors of the floral transition. Using TBLASTN search of EST and Genome Survey Sequences (NCBI, GenBank) we identified 24 maize genes that encoded CETS-like proteins. In the rice genome 19 CETS genes were previously found (Chardon and Damerval, 2005). Monocots appeared to have an expanded CETS gene family compared to the 5-7 gene family in dicots. Genomic structure and map location were determined for all maize CETS genes. Phylogenetic analysis grouped maize CETS proteins in 5 clades. The maize genes with predicted proteins forming clades with Arabidopsis TFL and FT have expression patterns consistent with their role in controlling flowering time. However, maize CETS genes from the other clades are expressed in roots, kernels and mature leaves, suggesting they possess novel functions.

P152

Molecular and Functional Diversity of the Maize Genome

(submitted by Jeff Glaubitz <glaubitz@wisc.edu>)
Full Author List: Glaubitz, Jeff[1]; Buckler, Edward S.[2]; Doebley, John F.[1]; Gaut, Brandon[3]; Goodman, Major[4]; Holland, Jim[4]; Kresovich, Stephen[2]; McMullen, Michael[5]; Stein, Lincoln D.[6]; Ware, Doreen H.[6]
[1] Genetics Department, University of Wisconsin, Madison, WI, USA 53706
[2] Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA 14853
[3] Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA 92697
[4] Crop Science Department, North Carolina State University, Raleigh, NC, USA 27695
[5] Department of Agronomy, University of Missouri-Columbia, Columbia, MO, USA 65211
[6] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA 11724

Maize is the number one production crop in the world and is the most diverse crop species. We are examining two questions in this model species: "How has selection shaped molecular diversity?" and "How does this molecular diversity relate to functional trait variation?" To answer these we have performed SNP discovery in 4000 genes. The sequence data from our SNP discovery panel have been used to determine the proportion of genes influenced by artificial selection during the domestication and crop improvement phases of maize evolution. The SNPs are being used to genetically characterize the comprehensive germplasm base of maize, and to perform QTL and association mapping both in maize (Zea mays ssp. mays) and in its wild progenitor (teosinte, Zea mays ssp. parviglumis) for traits of evolutionary, developmental and agronomic importance. Herein we emphasize the key resources for the maize research community that our project is generating: validated SNPs from 4000 genes, fully genotyped QTL and association mapping populations in maize and teosinte, novel statistical methods for association mapping and joint QTL/association mapping, and our project web site - www.panzea.org - where information on all of these key resources can be obtained and where all of our project data is being released.

P153

Natural Antisense Transcripts (NATs) Accumulate for Over 70% of Maize Genes

(submitted by Yi Jia <jiayi@iastate.edu>)
Full Author List: Jia, Yi[1]; Swanson-Wagner, Ruth A.[1]; Emrich, Scott J.[1]; Fu, Yan[2]; Guo, Ling[1]; DeCook, Rhonda[3]; Nettleton, Dan[1]; Schnable, Patrick S.[1]
[1] Iowa State University, Ames, Iowa
[2] Current address: Donald Danforth Plant Science Center, St. Louis, Missouri
[3] Current address: University of Iowa, Iowa City, Iowa

Natural Antisense Transcripts (NATs) can regulate gene expression by virtue of their ability to form double-stranded RNA duplexes. To investigate NATs in the maize transcriptome, seedling cDNAs from two inbred lines (B73 and Mo17) were hybridized to an oligonucleotide microarray designed to validate in silico detected NATs and to screen for NATs that can anneal to a random set of 3' UTRs and selected UTR repeat regions. Quantitative Real-Time PCR experiments were conducted to identify the minimum detection threshold and to thereby identify genes for which both sense and antisense transcripts accumulate to detectable levels. Two independent approaches, strand-specific real-time PCR and S1 nuclease assays, were conducted to validate the microarray results. Based on these conservative assays, NATs accumulate in seedlings that can anneal to over 70% of a random set of maize genes. In addition, more than 80% of a set of maize repeat sequences also detect both sense and antisense transcripts. Significantly, sense and antisense transcripts exhibit significant differential expression patterns among genotypes and relative to each other. Based on these findings we hypothesize that interactions between sense and antisense transcripts may contribute to the differential patterns of gene expression in maize hybrids (Swanson-Wagner et. al., 2006) and may therefore contribute to heterosis.

P154

Nutrient Acquisition in the Ustilago/Maize Pathosystem

(submitted by Ramon Wahl <wahlra@mpi-marburg.mpg.de>)
Full Author List: Wahl, Ramon[1]; Dohlemann, Gunther[1]; Kahmann, Regine[1]; Kaemper, Joerg[1]
[1] Max-Planck-Institute for Terrestrial Microbiology; Karl-von-Frisch-Str.; Marburg, Hessen, Germany 35043

Maize is one of the most important crop plants worldwide. The agricultural importance gives reason to investigate the molecular interplay between the plant and fungal pathogens that lead to large economical losses every year. *Ustilago maydis* is the causative agent of the smut disease causing the formation of large plant galls. Currently, there is only marginal information how compatibility is established in this pathosystem. To investigate nutrient acquisition by *U. maydis* during the infection process, we are embarking two independent strategies, considering both reprogramming of the host metabolism and nutrient uptake.

Firstly, we are performing DNA array expression analysis for both corn and *U. maydis* during a time course of infection. Initial experiments of infected plant leafs 5 and 9 days after inoculation exhibit around 3000 differentially expressed genes. Among these, metabolic key enzymes are found, indicating a change from a photosynthetically active source to carbon consuming sink tissue. A more detailed analysis comparing infected leafs with non-infected leafs or non-infected leafs showing a systemic response on both transcriptome and metabolome level will enable us to depict the rearrangement of the maize metabolism in detail.

Secondly, we have chosen a candidate gene approach based on in silico identification of 20 *U. maydis* hexose transporters to investigate carbon uptake. Deletion analysis revealed that at least 3 of them have an impact on pathogenicity. A yeast complementation assay and expression analysis identified glucose as a major cargo for two of them. One of the transporters, termed Hxt1, is similar to high affinity glucose transporters from *A. muscari* and *U. viciae-fabae*; the other one, termed Sog1, may function as a sensor, probably triggering a signal transduction cascade leading to a glucose dependent self activation. The third transporter, similar to Itr1 from yeast, most likely functions as inositol facilitator. Apparently, both glucose and inositol play important roles during biotrophic development of *U. maydis*.

P155

Pericycle Specific Gene Expression in Primary Roots of Maize Prior to Lateral Root Initiation

(submitted by Diana Dembinsky <diana.dembinsky@zmbp.uni-tuebingen.de>)
Full Author List: Dembinsky, Diana[1]; Fu, Yan[2]; Hochholdinger, Frank[1]
[1] Eberhard-Karls-University, Center for Plant Molecular Biology (ZMBP), Department of General Genetics, Auf der Morgenstelle 28, 72076 Tuebingen, Germany
[2] Donald Danforth Plant Science Center, St. Louis, MO, USA

Lateral roots have a strong influence on root architecture and are responsible for the major part of water and nutrient uptake of the maize plant due to their branching capacity which significantly increases the root surface. Lateral roots of maize are initiated in the differentiation zone of pre-formed roots after dedifferentiation of already differentiated pericycle cells. So far only little is known about genes that are involved in lateral root initiation of maize. The goal of this study was to identify genes that are preferentially expressed in primary root pericycle cells and might thus been related to lateral root initiation.

Pericycle-specific gene expression was studied by comparing transcriptional profiles of pericycle cells versus non-pericycle (central cylinder) cells of 2.5 day-old primary roots of the maize inbred line B73. Cells have been isolated via laser capture microdissection. The differential expression of the transcriptomes of these cells was analyzed by suppression subtractive hybridization (SSH), microarray experiments and real time PCR.

Moreover, a pericycle specific library of the inbred line B73 was generated and 377 cDNA clones (ESTs) were sequenced and the sequences were compared to 324 ESTs from a non-pericycle library. After clustering and assembling the ESTs into contigs they were functionally annotated.

Last but not least, a comparison between the transcriptome of the pericycle cells of wild type and pericycle cells of the mutant lrt1 that does not initiate lateral roots on the primary root, has been performed via microarray and real time PCR experiments.

P156

PlantGDB and ZmGDB - Resources for Maize Genome Annotation

(submitted by Jon Duvick <jduvick@iastate.edu>)
Full Author List: Duvick, Jon[1]; Dong, Qunfeng[2]; Wilkerson, Matt[1]; Schlueter, Shannon[3]; Brendel, Volker[4]
[1] Department of Genetics, Development and Cell Biology, Iowa State University, Ames IA 50011
[2] Center for Genomics and Bioinformatics, Indiana University, Bloomington, IN 47405
[3] Department of Agronomy, Purdue University, West Lafayette, IN 47907
[4] Department of Genetics, Development and Cell Biology and Department of Statistics, Iowa State University, Ames IA 50011

PlantGDB (www.PlantGDB.org) is a sequence database for plant comparative genomics. Key features include: species-specific sequence repositories and BLAST capabilities for about 70,000 plant species, up-to-date putative unique transcript (PUT) assemblies for approximately 100 plant species, and GSS assemblies for maize and sorghum. Gene models that integrate cDNA and EST evidence are available for 12 crop and model system plant genomes (including maize and sorghum). These can be visualized via PlantGDB through its ancillary "xGDB" browser modules. For the maize community, close links are maintained with MaizeGDB (www.MaizeGDB.org) to enable access to related maize biological information and with PLEXdb (www.PlexDB.org) to facilitate browsing through microarray probe data. At PlantGDB, maize researchers can view transcript assemblies, evaluate alternative splicing using the GeneSeqer spliced alignment tool and query other genome-related features including transposon insertion sites, repetitive sequences, and microarray probes.

ZmGDB is PlantGDB's "xGDB" genome browser for maize. ZmGDB provides unique resources for maize sequence-related information. For example, researchers can browse the most current GenBank-deposited BACs that have been aligned with maize PUTs, ESTs, GSS sequence, Oryza sativa proteins, and probes. Cognate and non-cognate alignments and alternative splicing evidence can be explored using transcript viewing tools. Researchers are then able to contribute novel gene annotations to ZmGDB's community annotation repository through the easy-to-use yrGATE Annotation Tool.

Future plans for PlantGDB and ZmGDB include deploying additional tools for exploring gene space in maize and other plant species, and we welcome suggestions from maize researchers concerning what new features would be most useful for their research. PlantGDB and its ancillary projects and databases are funded by the National Science Foundation (DBI- 0606909).

P157

Proteomic Analysis of Hexaploid Maize Derived from Inbred Oh43

(submitted by Hong Yao <yaoho@missouri.edu>)
Full Author List: Yao, Hong[1]; Kato, Akio[2]; Birchler, James A.[1]
[1] Division of Biological Sciences, University of Missouri, Columbia, MO 65211
[2] Faculty of Agriculture, Kyoto Prefectural University, Kyoto-shi, Sakyo-ku, Shimogamo Hangi-cho 1-5, Kyoto 606-0823, Japan

Hexaploid (6N) plants derived from maize inbred Oh43 are morphologically distinct from the diploid (2N) and tetraploid (4N) Oh43 plants. The hexaploid plants exhibit decreased stature and growth rate, late flowering and reduced fertility, increased size of stomata and pollen. To begin to understand the molecular basis of the morphological changes associated with the increased ploidy level, we compared the profiles of protein expression in adult leaf among the 2N, 4N and 6N plants. Specifically, total proteins were extracted from pooled leaf tissues collected from four individual plants at the same ploidy level. In each comparison (e.g. 2N vs. 4N), equal amount of two different protein samples were labeled with Cy3 and Cy5, respectively and subjected to the 2-D DIGE (Difference Gel Electrophoresis) analysis. No large differences of protein expression were found between the 2N and 4N samples. In contrast, large differences of protein expression were detected in the 4N vs. 6N and the 2N vs. 6N comparisons. The detected differences between 6N and the other ploidies in these two comparisons are similar.

P158

RNA Interference as a Functional Genomics Tool in Maize

(submitted by Karen McGinnis <mcginnis@ag.arizona.edu>)
Full Author List: McGinnis, Karen M.[1]; Murphy, Nicholas J.[1]; Chandler, Vicki L.[1]; Carlson, Alvar[2]; Hermanson, Peter J.[3]; Kovecevic, Nives[2]; McGill, Annie[3]; Cone, Karen C.[4]; Kaeppler, Shawn[2]; Kaeppler, Heidi[2]; Springer, Nathan M.[3]
[1] Department of Plant Sciences, University of Arizona, Tucson, Arizona 85721
[2] Department of Agronomy, University of Wisconsin, Madison, Wisconsin 53706
[3] Department of Plant Biology, University of Minnesota, Saint Paul, Minnesota 55108
[4] Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211

A large-scale functional genomics project was initiated to study the function of chromatin-related genes in Zea mays. Transgenic lines containing short gene segments in inverted repeat orientation designed to reduce expression of target genes by RNA interference (RNAi) were isolated, propagated, and analyzed in a variety of assays. Analysis of the selectable marker expression over multiple generations revealed that most transgenes were transmitted faithfully, whereas some displayed reduced transmission or transgene silencing. A range of target-gene silencing efficiencies, from non-detectable silencing to nearly complete silencing, were revealed by semi-quantitative RT-PCR analysis of transcript abundance for the target gene. In some cases, the RNAi construct was able to cause a reduction in the steady state RNA levels of not only the target gene, but also another closely related gene. Correlation of silencing efficiency with expression level of the target gene and sequence features of the inverted repeat did not reveal any factors capable of predicting the silencing success of a particular RNAi-inducing construct. The frequencies of success of this large-scale project in maize, together with parameters for optimization at various steps, should serve as a useful framework for designing future RNAi-based functional genomics projects in crop plants.

P159

SNP Discovery Via 454 Transcriptome Sequencing

(submitted by Scott Emrich <semrich@iastate.edu>)
Full Author List: Emrich, Scott J.[1]; Barbazuk, W. Brad[2]; Chen, Hsin D.[1]; Schnable, Patrick S.[1]
[1] Iowa State University; Ames, IA, 50010
[2] Danforth Plant Science Center; St Louis, Mo, 63132

A massively parallel pyrosequencing technology commercialized by 454 Life Sciences Corporation was used to sequence the transcriptomes of shoot apical meristems isolated from two inbred lines of maize using laser capture microdissection (LCM). A computational pipeline that uses the POLYBAYES polymorphism detection system was adapted for 454 ESTs and used to detect SNPs (Single Nucleotide Polymorphisms) between the two inbreds. Putative SNPs were computationally identified using 260,000 and 280,000 454 ESTs from the B73 and Mo17 inbreds, respectively. Over 36,000 putative SNPs were detected within 9,980 unique B73 genomic anchor sequences (MAGIs). Stringent post processing reduced this number to >7,000 putative SNPs. Over 85% (28/33) of a sample of these putative SNPs were successfully validated via Sanger sequencing. Considering only B73/Mo17 EST alignments that contained at least 3X coverage for both inbreds, the validation rate was >90% (21/23). Based on these validation rates, this pilot experiment conservatively identified >6,000 valid SNPs within >2,700 maize genes. An additional set of 19 validated SNPs were converted to genetic markers and used in combination with a mass spectrometry technology commercialized by Sequenom to SNPtype 91 B73 X Mo17 RILs. These RILs were independently genotyped at the SNP-containing loci via temperature gradient capillary electrophoresis. The call rate with Sequenom technology was 99%. The agreement rate between the Sequenom- and TGCE-based genotypes was also ~99%. These results demonstrate that 454-based transcriptome sequencing is an excellent method for the high-throughput acquisition of gene-associated SNPs that can be efficiently converted into accurate, high-throughput genetic markers.

P160

Shedding New Light on the Secrets of the *U. maydis* / Maize Interaction by Confocal Microscopy and Global Expression Profiling

(submitted by Gunther Doehlemann <doehlemann@mpi-marburg.mpg.de>)
Full Author List: Doehlemann, Gunther[1]; Wahl, Ramon[1]; Kamper, Jorg[1]; Kahmann, Regine[1]
[1] Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., D-35043 Marburg, Germany

The basidiomycete Ustilago maydis causes smut disease in maize. After penetration of the maize cuticle, the biotrophic phase is established in which the plant plasma membrane invaginates and surrounds the intracellularly growing hyphae. U. maydis does not form feeding structures but establishes an extensive mycelial network within the plant tissue. This coincides with tumor induction and during this stage the fungal hyphae proliferate mostly between host cells.

A variety of factors which control different stages of fungal development have been described in detail, but the determinants for compatibility, i. e. gene products which redirect host metabolism to the site of fungal proliferation, suppress plant defenses and induce tumours, have remained mysterious. Although the biology of Ustilago has been studied extensively there are still contradictory reports on the proliferating stage within the plant tissue.

To address this issue, intracellular and apoplastic development as well as the early phases of tumor induction have been analysed in detail by confocal microscopy using GFP tagged strains and fungal specific fluorescence dyes. To link this with gene expression, whole genome expression profiling has been performed by microarray analysis of both U. maydis and maize during all specific steps of biotrophic development. From these data we expect to obtain insights into maize expression programms that are altered by the pathogen, in particular with respect to defense reactions and infection related changes of metabolic pathways. Combining these complementary approaches will shed light on how U. maydis reprograms its host during the biotrophic phase.

P161

The 5' Stem-loop and Its Role in mRNA Stability in Maize S Cytoplasmic Male-sterility

(submitted by Yonglian Zheng <yonglianzheng@gmail.com>)
Full Author List: Xiao, Hailin[1]; Zhang, Fangdong[1]; Zheng, Yonglian[1]
[1] State Key Laboratory of Crop Genetic Improvement and National Center of Crop Molecular Breeding, Huazhong Agricultural University, Wuhan, Hubei, China, 430070

In flowering plants, cytoplasmic male-sterility (CMS) is a maternally-transmitted defect in pollen production. CMS is prevalent in higher plants, and was shown to be associated with chimeric orfs in the mitochondrial genome. These orfs usually contain novel nucleotide sequences adjacent to partial or entire mitochondrial genes. The suppression of CMS by nuclear Rf genes provides a model to investigate communication between the nucleus and mitochondria.

Post-transcriptional control plays an important role in the regulation of plant mitochondrial gene expression. In studies of CMS, several reports indicated that the steady-state levels of CMS-associated orfs were related to their 5_ or 3_ processing, which is modulated by certain nuclear genes. Improper processing of precursor mRNAs in organelles will lead to certain defects in translation or mRNA stability. The 5_ and 3_ untranslated regions (UTRs) had been identified as the key determinants of organellar mRNA stability.

The co-transcribed orf355-orf77 region of the mitochondrial genome is associated with S cytoplasmic male-sterility (CMS-S) in maize; the amounts of its 1.6 kb and 2.8 kb transcripts were previously shown to be greatly reduced in fertility-restored microspores relative to the amounts in sterile plants. To investigate the mechanism underlying this reduction, detailed analysis of the 5_ and 3_ termini of these transcripts was conducted. Using 3_ RACE analysis, the polyadenylation sites of the 1.6kb and 2.8kb transcripts were mapped adjacent to a 3_ stem-loop, which may play an important role in stabilizing their 3_ ends. No difference between the polyadenylation sites in sterile and fertility-restored microspores was found that could account for the differences in orf355-orf77 transcript levels. The 5_ terminus of the 1.6kb transcript was further studied by primer extension; the result revealed that there was a deletion of 9 nucleotides only in fertility-restored microspores, and that this deletion eliminated a 5_ stem-loop sequence. We propose that the elimination of the 5_ stem-loop in the fertility-restored microspores could be the cause of the degradation of the 1.6kb transcript. Because the 2.8kb transcript can be cleaved to generate the 1.6kb transcript, the amount of the 2.8kb transcript is also reduced in fertility-restored microspores.

P162

The Development of a Set of Maize SNP Markers for the Illumina Platform

(submitted by Elizabeth Jones <liz.jones@pioneer.com>)
Full Author List: Jones, Elizabeth[1]; Ayele, Mulu[1]; Ho, Julie[1]; Yourstone, Ken[1]; Chu, Wen-Chy[1]; Smith, Stephen[1]
[1] Pioneer Hi-Bred, Maize Product Development, 7300 NW 62nd Ave, Johnston, IA 50131

We have identified SNP loci that are highly effective and polymorphic across a broad range of public maize germplasm. Gene loci were selected for sequencing from the public unigene set. Consensus sequences were assembled from sequence data for 61 publicly bred maize genotypes representing a broad range of germplasm. SNP loci were further selected using broad marker design criteria to enable design with a number of SNP chemistries: 100 flanking bases upstream and downstream from the SNP site, 25 bp around the target SNP with no polymorphism and a GC content of 40-60% using consensus sequence. This selection resulted in 640 SNPs being targeted in 540 sequences, with an average minor allele frequency of 0.35 and expected heterozgosity of 0.26 across the inbred genotypes. SNPs and sequences have been deposited at http://www.panzea.org with the prefix PHM to denote their origin from Pioneer Hi-Bred. These 640 PHM SNPs and 895 PZA SNPs (selected largely from the "unbiased" set at http://www.panzea.org) were submitted to Illumina for marker design. 768 (484 PHM and 292 PZA) loci amenable to design were further selected and validated using the GoldenGate(tm) technology with 480 public and Pioneer-proprietary maize inbreds. 88% of loci had less than 20% missing data and were considered high quality. A list of useful loci will be available.

P163

The Grass Transcription Factor ORFeome Project

(submitted by John Gray <jgray5@uoft02.utoledo.edu>)
Full Author List: Anderson, Richard[1]; Palaniswamy, Saranyan K.[2]; Davaluri, Ramana V[3]; Grotewold, Erich[2]; Gray, John[1]
[1] Dept. of Biological Sciences, Univ. of Toledo, Toledo, OH 43606
[2] Dept. of Plant Cellular and Molecular Biology, Plant Biotechnology Center, The Ohio State Univ., Columbus, OH 43210
[3] Dept. of Mol. Virology, Immunology and Medical Genetics, The Ohio State Univ., Columbus, OH 43210

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 in the regulation of gene expression is the identification of these regulatory networks in which TFs participate. It is estimated that the number of TFs in maize could be up to 10% 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. The initial goal of this project is to generate an ORFeome collection for approximately 400 rice, maize, 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 many of these clones will be recombined into destination (pDest) vectors suitable for overexpression and protein production. Clones for these TFs are being annotated and are being made publicly available to researchers. Information on available clones is being posted at the GRASSIUS (www.grassius.org) web resource (see accompanying poster). GRASSIUS will serve as a central database for the comparative regulomics across grass species. The project has a strong educational component that aims to Foster the Integration of Research with Education (FIRE). In Fall 2006, all 56 of the BIOL 3020 (Molecular Genetics Laboratory) students at the University of Toledo participated in this project and learned database mining, gene annotation, and Gateway cloning skills. In future years it is anticipated that this ORFeome project will be serve as a core to the teaching laboratory curriculum.

P164

The Maize TILLING Project: 2006-2007 Update

(submitted by Rita-Ann Monde <rmonde@purdue.edu>)
Full Author List: Monde, Rita-Ann[1]; Till, Bradley J[2]; Chambers, Courtney[1]; Daniel, Dacia[1]; Sahm, Heather[1]; Conners, Lisa[1]; Eichstedt, Michael[1]; Weisser, Sara[1]; Xavier, Theresa[1]; Greene, Elizabeth[2]; Henikoff, Jorja[2]; Weil, Clifford F.[1]
[1] Purdue University, West Lafayette, IN 47907
[2] Fred Hutchinson Cancer Research Center, Seattle, Washington, 98109-1024

The Maize TILLING Project (MTP, http://genome.purdue.edu/maizetilling/) has a collection of ~11,000 EMS-mutagenized lines generated in the B73 and W22 inbreds that can be used as both a reverse and a forward genetics resource. Data from MTP, including TILLed mutations and photographs of mutant plant and ear phenotypes are integrated into MaizeGDB.

In the past year MTP added 1152 B73 and 1152 W22 (3522 and 2304 total lines respectively) lines to our screening population. As of January 2007, we have delivered 441 mutations (223 of them non-silent) for 73 gene targets. An additional 52 requests are in the TILLING or primer pre-screen stage. Most of the screening population contain an average of 1.0 mutations/kb/1000 families screened and one of the W22 populations has ~ 2.0 mutations/kb/1000 families. We estimate our lines contain an average of 163,300 mutations within protein coding exons. This summer MTP will generate a B73 population with a higher mutation density, aiming for ~ 7-10 mutations/kb/1000 families.

MTP has made two recent improvements. First, we have added Eco-TILLING of 48 Maize Diversity Lines (representing ~ 70% of the diversity in maize germplasm) to our service. Eco-TILLING uses TILLING methodology to survey natural variation (SNPs, small indels) and provides additional information about allelic diversity available for functional genomics, as well as data on selection and domestication processes. Second, by adding low molecular weight amines to the PCR we can now TILL targets up to 72% GC without compromising TILLING gel quality.

As sequencing throughput becomes faster, read lengths improve and costs decrease, we are exploring ways to better query our mutant collection. High-throughput, massively parallel sequencing technologies now make targeted re-sequencing of any given gene across thousands of mutant lines feasible. We are determining how well massively parallel, sequence-by-synthesis methods can be used for cost-efficient discovery of point mutations.

P165

The TIGR Rice Genome Annotation Database

(submitted by Matthew Campbell <campbell@tigr.org>)
Full Author List: Campbell, Matthew[1]; Ouyang, Shu[1]; Zhu, Wei[1]; Lin, Haining[1]; Hamilton, John[1]; Childs, Kevin[1]; Thibaud-Nissen, Francoise[1]; Orvis, Joshua[1]; Haas, Brian[1]; Wortman, Jennifer[1]; Buell, C. Robin[1]
[1] 9712 Medical Center Drive, Rockville, MD 20850

We have been funded by the National Science Foundation to annotate the rice genome and to provide the community with access to the annotated genome. We have created an annotation database for rice called Osa1 that houses sequence and annotation information. We released version 5 of our genome annotation in January. Using 12 pseudomolecules that represent ~95% of the total genome, we have created gene models using a combination of ab initio gene finders and experimental evidence. The Program to Assemble Spliced Alignments (PASA) was used to improved the structural annotation of these models with ESTs and FL-cDNAs. Recent upgrades in PASA have allowed for a comprehensive analysis of alternative splicing in rice and comparison to Arabidopsis. We have generated a series of other annotations for the rice genome that include gene ontologies, paralogous families, alignment with flanking sequence tags, synteny with other Poaceae species, and a variety of expression data. In order to complement our annotation effort, we have implemented a Community Annotation tool that allows community experts to refine our structural and functional annotation. The annotation is publicly available at the TIGR Rice Genome Annotation website (http://rice.tigr.org) where the annotation can be viewed in the Rice Genome Browser or downloaded through the Data Extractor Tool.

P166

A Gaspe Flint B73 Introgression Library for the Genetic Dissection of Flowering Time in Maize

(submitted by Nicola Carraro <nicola@sidoine.net>)
Full Author List: Salvi, Silvio[1]; Corneti, Simona[1]; Bellotti, Massimo[1]; Carraro, Nicola[1]; Tuberosa, Roberto[1]
[1] DiSTA - University of Bologna, Viale Fanin, 44, Bologna, 40127, Italy

The genetic control of flowering time and other morphological traits in maize was dissected using an introgressione library (IL) developed from the cross B73 x Gaspe Flint. B73 is an elite dent line of medium-late flowering time while Gaspe Flint is a flint, extremely early variety of current no agronomic value. The IL is based on 70 lines developed by five cycles of marker-assisted backcross using B73 and Gaspe Flint as recurrent and donor parent, respectively. Each IL line retains 30-40 cM of Gaspe Flint genome and an estimated 70% of Gaspe Flint genome appears represented within the IL. Based on trait differences between the donor and the recipient genotypes, the IL collection will serve as a permanent source of nearly isogenic material for QTL analysis and cloning for many morpho-physiological traits such as flowering time, plant and root architectures, yield and yield components, etc.

A replicated field trial was carried out during summer 2006 for evaluating flowering time, plant height, number of leaves and other agronomic traits. Major differences on root architecture between the parental lines were also observed at the seminal level. Such root traits are being analysed using a simple hydroponic system based on paper-roll. Results of QTL analysis based on IL lines evaluation in the field and in hydroponics will be reported.

P167

Amylose Content in Amylomaize VII Varied in Different Generations and at Different Locations

(submitted by Yusheng Wu <yshmh2@yahoo.com>)
Full Author List: Wu, Yusheng[1]; Campbell, Mark[3]; Yen, Yang[2]; Gustafson, Dawn[1]; Wicks III, Zeno[1]
[1] South Dakota State University, Plant Science Department, Brookings, SD, 57007
[2] South Dakota State University, Biology and Microbiology Department, Brookings, SD, 57007
[3] Truman State University, Division of Science, Kirksville, MO, 63501

Amylose has a number of specialty uses in the production of such things as biodegradable plastics, adhesives and more recently in the production of resistant starches. Specifically, Amylomaize VII corn contains starch with at least 70% amylose as the result of being homozygous for the ae1 (amylose extender1) allele and possessing modifier genes. In this study the inheritance and gene action of these modifier genes was elucidated with a generation mean analysis specific for triploid endosperm tissue. Seeds of individual ears of P1 (H99ae, 55% of amylose), P2 (GUAT209ae, 70% of amylose), F1 (P1 x P2), F1r (P2 x P1), F2, F2:3 (from the selfed F2), BC1P1 (F1 x P1) and BC1P2 (F1 x P2) were sampled at two locations (Brookings, SD and Kirksville, MO) in 2005. Kernel samples of each generation were scanned with NITS (near infrared transmittance spectroscopy) to establish the models for predicting amylose content of unknown samples. Purified starch from the seed samples was analyzed using a colorimetric amylose-iodine method following wet-milling of the corn. Amylose content in both parents was constant across locations but significantly different from each other. The amylose content of the F2:3 at the two locations (n=315 in SD and n=283 in MO) both showed normal distributions between about 55% of P1 and about 70% of P2. Sixteen transgressive individuals in SD and seven in MO were found. The maternal effects on amylose content and interactions between genotypes and environments were observed at the two locations in terms of the mean differences of those generations. There was no correlation between amylose content and plant height, ear height or 100-kernel weight. Broad sense heritability of amylose content was high in SD and low in MO. Regression models established in this study can be used to predict amylose content of unknown samples.

P168

An Investigation of Non-destructive Methods for Determining the Presence of Low Phytate Maize Genotypes

(submitted by Courtney Bonney <ceb005@truman.edu>)
Full Author List: Bonney, Courtney E.[1]; Ponder, Jessica N.[1]; Raboy, Victor[3]; Duvick, Susan A.[2]; Campbell, Mark[1]
[1] Truman State University, Division of Science, 100 E Normal, Kirksville, MO, 63501
[2] Iowa State University, USDA-ARS, G212 Agron, Ames, IA, 50011-1010
[3] USDA-ARS, 1691 S 2700 W, Aberdeen, ID, 83210

Environmentalists now recognize the effect of phosphorus pollution on watersheds and are making the connection between agriculture management practices and phosphorus pollution. The recessive alleles lpa-1 and lpa-2 have been found to increase inorganic phosphorus, improving bioavailability of phosphorus from corn for non-ruminants which, therefore, can reduce the need for supplemental dietary phosphorus and reduce the amount of phosphorus released into the environment. Our long term goal is to introduce these low phytate alleles in the tropical germplasm from the USDA-GEM program in order to identify favorable genetic backgrounds to overcome yield drag associated with low phytic acid genes. To facilitate this goal we are searching for a non-destructive method for identifying low phytic acid genotypes. Our first approach was to construct a near-infrared calibration using a set of materials segregating either lpa-1 or lpa-2. Near-infrared Transmittance spectroscopy (NITS) and reflectance spectroscopy (NIRS) studies on ground or whole bulk samples and single maize kernels were accomplished, but all studies produced correlations below values necessary required for effective germplasm screening. A correlation (r) of 0.41 for ground reflectance of bulk samples was the highest obtained. Our present studies focus on other non-destructive methods based on the amount of phosphorus leached from whole single maize kernels. In one study we found that reaction of leachate with Chen's reagent could be used to correctly predict the presence of low phytate kernels seventy percent of the time.

P169

An Update on Gramene QTL Data Module

(submitted by Junjian Ni <jn66@cornell.edu>)
Full Author List: Ni, Junjian[1]; Yap, Immanuel[1]; Jaiswal, Pankaj[1]; Buckler, Edward S.[3]; Youens-Clark, Ken[2]; Hebbard, Claire[1]; Pujar, Anuradha[1]; Casstevens, Terry M.[3]; Bradbury, Peter J.[3]; Ren, Liya[2]; Zhao, Wei[2]; Tecle, Isaak Y.[1]; Ravenscroft, Dean[1]; Tung, Chih-Wei[1]; Avraham, Shulamit[2]; Spooner, William[2]; Wei, Sharon[2]; Canaran, Payan[2]; Liang, Chengzhi[2]; Ware, Doreen H.[3]; Stein, Lincoln D.[2]; McCouch, Susan R.[1]
[1] Department of Plant Breeding, Cornell University, Ithaca, New York, USA 14853
[2] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY USA 11724
[3] USDA-ARS, Institute for Genomic Diversity, Cornell University, Ithaca, New York, USA 14853

We continue to curate and develop the QTL data module within Gramene. The current release of the QTL database (V23) houses trait and map position information for more than 11,000 QTL. Out of them, around 1,700 are maize QTL, including both those integrated from MaizeGDB and maize drought resistance QTL curated internally by Gramene. Gramene uses traits, Trait Ontology (TO), and Plant Ontology (PO) (both Plant Structure and Plant Growth and Development Stage) to define, organize, cluster, and search the QTL. Each QTL's detail includes ontology terms, map position, and links to Gramene's Markers and Maps modules. This assists researchers to retrieve associated feature information for a QTL and perform multiple map or species comparisons. Together, with other modules within Gramene, QTL can be utilized as a starting point for maize researchers developing scientific hypotheses and discovering candidate genes. Users are encouraged to participate in the improvement of the QTL search through suggestions for search utilities and visual displays, as well as direct data submission and curation.

P170

Analysis of Significant Polymorphisms Within Candidate Aluminum Tolerance Genes

(submitted by Allison Krill <amk72@cornell.edu>)
Full Author List: Krill, Allison[1]; Hoekenga, Owen A.[2]; Kochian, Leon[2]; Buckler, Edward S.[2]
[1] Institute for Genomic Diversity, Cornell University Ithaca NY
[2] US Plant, Soil and Nutrition Laboratory, USDA-ARS, Ithaca NY

Aluminum (Al) toxicity is a major constraint to maize productivity on acidic soils throughout the world. Phytotoxic Al becomes soluble at pH < 5.5, inhibiting root growth and function, thus severely reducing yields. Al exclusion and intracellular tolerance are important mechanisms involved in achieving Al tolerance in maize; however, little is known about the underlying genetics. Diverse maize inbred lines, with varying tolerance to Al, provide a useful resource for studying the genetics behind this complex trait. An association approach was used to evaluate candidate genes involved in Al tolerance, which were selected using comparative and physiological genomics-based approaches. We scored polymorphisms from 22 candidate genes across ~300 diverse inbred lines. Six of these candidate genes produced significant associations with net seminal root growth under toxic Al stress conditions: malic enzyme (ME), isocitrate lyase (ICL), S-Adenosylhomocysteinase hydrolase (SAHH), aluminum activated malate transporter (ZmALMT), multidrug and toxin exclusion transporter (ZmALS),and pectin methylesterase (PME). We will discuss the significant polymorphisms identified and the haplotype diversity contributing to Al tolerance in maize

P171

Association Mapping in Teosinte: How Natural Allelic Variation Controls Phenotypic Variation

(submitted by Allison Weber <allisonweber@wisc.edu>)
Full Author List: Weber, Allison[1]; Clark, Richard[1]; Vaughn, Laura[1]; Sanchez-Gonzalez, Jose de Jesus[2]; Briggs, William[1]; Yu, Jianming[3]; Yandell, Brian S.[4]; Bradbury, Peter J.[5]; Liu, Kejun[6]; McMullen, Michael[7]; Buckler, Edward S.[5]; Doebley, John F.[1]
[1] Department of Genetics, University of Wisconsin-Madison, 425 Henry Mall, Madison, WI 53706
[2] Centro Universitario de Ciencias Biologicas y Agropecuarias, Universidad de Guadalajara, C.P. 45110, Carretera Guadalajara- Nogales, Km. 15.5, Las Agujas, Mpio. de Zapopan, Jalisco, Mexico
[3] Department of Agronomy, Kansas State University, 3004 Throckmorton Plant Science Center, Manhattan, KS 66506
[4] Department of Statistics, University of Wisconsin-Madison, 1300 University Avenue, Madison, WI
[5] USDA-ARS, Department of Plant Breeding and Genetics, Cornell University, 741 Rhodes Hall, Ithaca, Ithaca, NY 14853
[6] GlaxoSmithKline, 5 Moore Drive, P.O. Box 13398, Research Triangle Park, NC 27709
[7] USDA-ARS, Plant Genetics Research Unit and Plant Sciences Unit, University of Missouri, Columbia, MO 65211

Teosinte, the wild ancestor of maize, contains almost 50% more nucleotide diversity than found in maize. We are currently conducting association mapping in two teosinte population samples to assess how this natural genetic variation contributes to phenotypic variation. Our goal is to identify associations between candidate genes and a variety of traits for plant and inflorescence architecture as well as kernel composition. Our first teosinte association mapping population consists of 600 plants representing 75 local populations that were scored for 18 phenotypes and genotyped for SNPs in 12 candidate genes and 495 randomly chosen ESTs, the latter was used to estimate degrees of relatedness among the plants. In this population, we have identified significant associations using a mixed model that controls for multiple levels of relatedness. This model consisted of principal component vectors found to be significant using the Tracy-Widom statistic and a kinship matrix based on the proportion of shared alleles. Among the associations observed was one between plant height and a SNP in zfl2 that is strong enough to survive the conservative Bonferroni correction. Analysis of our second population, which includes 820 plants representing 34 local populations, 28 phenotypes, 157 candidate genes and 434 ESTs, is underway and has greater statistical power to detect associations.

P172

Association Mapping of Central-Metabolism Enzyme Activities in Maize

(submitted by Amit Gur <ag336@cornell.edu>)
Full Author List: Gur, Amit[1]; Gibon, Yves[2]; Sulpice, Ronan[2]; Stitt, Mark[2]; Buckler, Edward S.[3]
[1] Institute of Genomic Diversity, Cornell University, Ithaca, New-York 14853
[2] Max Planck Institute of Molecular Plant Physiology, Science Park Golm, 14476 Golm-Potsdam, Germany
[3] Institute of Genomic Diversity, USDA-ARS and Department of plant-breeding and genetics, Cornell University, Ithaca, New-York 14853

The use of information from intermediates between genes and phenotypes to explain phenotypic variation is becoming common approach. Gene expression and metabolites are being measured using high-throughput methods but it is not clear how informative are these measurements for explaining phenotypes that integrate over time. Protein levels and enzymes activity are more heritable parameters which integrate over time. On this project we use enzyme activity to study the variation in Glycolysis and TCA pathways. The central role of these pathways in energy balance and metabolites distribution makes the genes in them attractive candidates for crop improvement, assuming enough natural functional diversity exists. We took advantage of the extensive diversity in maize to study the natural genetic and phenotypic variations in central metabolism genes and enzyme activities. We initially tested the activity of 10 enzymes across 8 inbred lines and found significant genetic effect for 9 of them. The existence of considerable variation allowed the performance of association mapping experiments for 4 of the enzymes using a panel of 100 diverse inbred lines, in which we have already identified significant associations for 2 of the enzymes. Here we present the results for the NAD-dependent Isocitrate dehydrogenase (NAD-IDH) gene. We were able to identify a single nucleotide polymorphism (SNP) within this gene that encode for amino-acid substitution. This SNP was strongly associated with variation in NAD-IDH activity and 30% difference in activity was found between the two alleles across the 100 lines. The Phenylalanine to Tyrosine substitution is predicted to be located at the protein surface in a conserved site across plants and altogether this site makes a good functional candidate. Associations were also found between SNPs in several central-metabolism genes and yield- and quality-related traits collected in multiple field experiments. The approach of using the diverse germplasm to genetically dissect the variation in target enzyme activities and agronomic traits could be powerful way to directly identify enzymes and their genetic controllers that are relevant for crop improvement.

P173

Automated Image-Based High Throughput Phenotyping of Rowing Corn Plants

(submitted by Joerg Vandenhirtz <joerg.vandenhirtz@lemnatec.com>)
Full Author List: Vandenhirtz, Joerg[1]; Vandenhirtz, Dirk[1]; Eberius, Matthias[1]
[1] LemnaTec GmbH, 18 Schumanstr, Wuerselen, Germany, 52146

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

For this purpose LemnaTec provides the fully integrated high-throughput technology platform Scanalyzer 3D Conveyor. This HTS platform includes conveyor belts for plant supply to the 3D imaging station or for full greenhouse management, imaging stations for visible images, chlorophyll and GFP fluorescence imaging of the full plants, thermal imaging. As integrated modules automatic programmed watering and weighting stations for water use efficiency or drought stress experiments are available.

Quantitative non-destructive assessment of plant phenotype parameters (e. g. leaf area, leaf length, colour, internode length) are extracted at each point of measurement. Additionally phenotypic developments in time like growth rates, stay green and dynamic reversible reaction on drought (e. g. leaf orientation and rolling) or other stressors are automatically quantified. High frequency imaging e. g. for visible performance and chlorophyll measurements in combination with quantitative watering and evaporation measurement allow new insights in dynamic reaction on daily stressors and long term stress.

After harvest imaging under field conditions can provide additional quantitative information on plant performance. Detail imaging on corn ears allow fast quantitative assessment of kernel number, size and e. g. density.

Easy to use databases, fast analysis and automated data transfer provide a new quality in long term experiments and statistically very significant high-content measurement of plant development. Imaging units designed for corn may be used additionally for a quantitative assessment of a wide range of other plants including wheat, barley, rice and e. g. Arabidopsis.

P174

Detection of Pleiotropic QTL Involved in the Integrated Development of Maize Inflorescence Architecture

(submitted by Narasimham Upadyayula <upadyayu@uiuc.edu>)
Full Author List: Upadyayula, Narasimham[1]; Johnson, Richard[1]; Bohn, Martin O[1]; Rocheford, Torbert R.[1]
[1] Department of Crop Sciences, University of Illinois; Urbana, IL 61801

The intermated B73/Mo17 recombinant inbred lines (IBMRILs) were used to investigate the genetic relationship between tassel and ear inflorescence architecture. A comprehensive set of eleven tassel and three ear traits were measured on 292 IBMRILs over five environments. Tassel architecture showed strong morphological integration, with traits falling into two multi-trait groups: tassel size and spikelet number. Through a canonical transformation of data, pleiotropic QTL affecting the two tassel multi-trait groups and the ear traits were detected. Central spike spikelet density and central spike length, among the tassel traits, and kernel row number, among the ear traits, accounted for most of the common variation between tassel and ear architecture. Using canonical correlation analysis, seven QTL having pleiotropic effects on the three traits were detected. The common genetic control between the tassel and ear could potentially be exploited in selection for higher grain yield and increased pollen production on central spike, with the pleiotropic QTL of possible value in marker assisted selection.

P175

Developing Markers for Association Mapping in Biofuel Grasses

(submitted by Denise Costich <dc58@cornell.edu>)
Full Author List: Costich, Denise E.[1]; Gore, Michael A.[1]; Denton, Michelle E.[1]; Ersoz, Elhan S.[1]; Buckler, Edward S.[1]
[1] Cornell University, 175 Biotechnology Building, Ithaca, NY, USA 14853

We are initiating association mapping studies of potential biofuel grasses, including switchgrass (Panicum virgatum L.), reed canarygrass (Phalaris arundinacea L.) and gamagrass (Tripsacum dactyloides L.). A critical first step in this research is the identification of molecular markers that are tightly linked to biomass-related trait loci, thus enabling marker assisted selection and greatly accelerating the breeding programs for enhanced biomass production. In perennial polyploid species such as these, effective marker assisted selection will be key to rapid selection for improved varieties and adaptation to environments across the US. Here we present the first set of results from our marker development study, in which we used complete digestion by HpaII, an enzyme that preferentially cuts unmethylated low-copy gene regions, followed by DNA fragment size selection (100-600bp) to eliminate the large fragments containing the highly methylated retrotransposons, as the first steps to enrich for the genic fraction. These fragments were ligated together and non-selectively amplified using a GenomiPhi(tm) kit. The sequence libraries that were generated were then analyzed by BLAST searches. For switchgrass and reed canary grass, 87% of the cloned DNA fragments were genic or unknown sequence, 5% retrotransposon, 8% chloroplast. These preliminary data show that this HpaII library preparation approach works for polyploid grasses. Using next generation sequencing technologies and methyl-filtration of the genomes, we plan to identify thousands of SNPs for these polyploid grasses, for which there are currently limited genetic resources.

P176

Development of Informative Markers through Association Mapping in Maize to Improve Drought Tolerance in Cereals

(submitted by Jianbing Yan <j.yan@cgiar.org>)
Full Author List: Yan, Jianbing[1]; Sawkins, Mark[1]; Setter, Tim[2]; Buckler, Edward S.[3]; Xu, Yunbi[1]; Gore, Michael A.[4]; Heather, Yates[4]; Grudloyma, Pichet[5]; Gethi, James[6]; Khosa, Ester[7]; Li, Wanchen[8]; Magorokosho, Cosmos[1]; Huerta, Eva[1]; Martinez, Carlos[1]; Pastrana, Jose Simon[1]; Warburton, Marilyn[1]
[1] International Maize Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico
[2] Department of Crop and Soil Sciences, Cornell University, Ithaca, New York 14853, USA
[3] Institute for Genomic Diversity, Cornell University, Ithaca, New York 14853, USA/United States Department of Agriculture-Agricultural Research Service (USDA-ARS)
[4] Institute for Genomic Diversity, Cornell University, Ithaca, New York 14853, USA
[5] Nakhon Sawan Field Crops Research Center (NSFCRC), Takfa, Nakhon Sawan, Thailand 60190
[6] Kenya Agricultural Research Institute (KARI), RRC Mtwapa, P.O. Box 16, Mtwapa
[7] Sustainable Infrastructure Research and Development Center (SIRDC), P.O. Box 6640, Harare Zimbabwe
[8] Maize Research Institute, Sichuan Agricultural University, Yaan 625014, Sichuan

Drought and low soil fertility are the major limiting factors for cereal-crop production in developing countries. Association analysis through linkage disequilibrium may be a useful new tool for dissection of drought tolerance and identification of superior alleles. A total of 600 diverse maize lines were selected for evaluation and grown in Mexico in 2004 and 2005 under drought stress conditions. Five thousand samples were harvested each year for three tissues (ear tips, silks & leaves) at two timepoints (0 & 7 days after anthesis) and levels of sucrose, glucose, starch, abscisic acid and ABA-glucose ester were measured. Hybrids were produced by crossing 349 lines selected from the 600 with the tester line CML312.The hybrids were divided into three maturity groups based on flowering time and are now being evaluated in five locations in China, Kenya, Mexico, Thailand, and Zimbabwe. Yield components and secondary traits of interest will be measured for the hybrids under water stress and well-watered conditions across the five locations for two planting seasons (2006 and 2007). Forty six SSR markers distributed randomly across the gnome have been used to evaluate population structure of the lines. More than 300 candidate genes related to biotic or abiotic stress resistance or with the carbohydrate and ABA pathways that might be associated with drought tolerance were selected for association tests. To date, 60 genes have been sequenced in a panel of 48 highly diverse inbred maize and teosinte lines to develop SNP markers. These and other neutral SNPs will be used to scan the drought association mapping panel using the Illumina?genotyping system. Any positive associations found in this project will provide useful information for drought tolerance breeding in maize and potentially other cereal crops as well.

P177

Development of a Real-Time Destructive Sugar Biosensor and Its Application to Corn Stover Hydrolysis

(submitted by Lisa Haney <lhaney@iastate.edu>)
Full Author List: Haney, Lisa J.[1]; Scott, M. Paul[2]; Lamkey, Kendall R.[1]; Kirkpatrick, Krystal[1]; Coors, James G.[3]; Lorenz, Aaron J.[3]
[1] Department of Agronomy, Iowa State University, Ames, IA 50011
[2] Corn Insects and Crop Genetics Research Unit, ARS, USDA
[3] Department of Agronomy, University of Wisconsin-Madison, Madison, WI 57306

Lignocellulose is said to be the most abundant compound on earth and is an excellent renewable feedstock for the production of ethanol as an alternative fuel source. Corn stover is made of lignocellulose and may prove to be an economical renewable energy resource. Current methods to evaluate the conversion potential of corn stover to ethanol involve lengthy fermentations and require special equipment to create anaerobic conditions. A high throughput screening method for predicting conversion potential of corn stover to ethanol has been developed using a real-time destructive sugar biosensor. The biosensor is based on E. coli strain CA8404. This strain produces green fluorescent protein (GFP) constitutively and is capable of catabolism of both five- and six-carbon sugars. The application of this biosensor to corn stover hydrolysis involves dilute sulfuric acid pretreatment and a process we call simultaneous saccharification and catabolism (SSC). In this process, enzymes are used to break down the hemicellulose and cellulose fractions of the stover to sugars which are metabolized by our biosensor. Growth of this strain is limited by carbon produced in the hydrolysis reaction, so fluorescence is proportional to the level of sugars released by hydrolysis. We reason that the level of sugars produced in this hydrolysis should be a good predictor of suitability of a sample as a fermentation feedstock. The germplasm selected for evaluation by the assay represents a broad spectrum of biomass yield based on yield trials and composition based on in vitro digestibility tests. This high throughput method may enable breeders to select varieties with improved suitability for lignocellulose conversion and could lead to higher ethanol yields.

P178

Dual Testcross QTL Analysis: A Solution to the Current Rate-Limiting Steps of Positionally Cloning QTL in Maize

(submitted by Nick Lauter <nickl@iastate.edu>)
Full Author List: Hessel, David[1]; Lauter, Nick[1]
[1] USDA-ARS, Corn Insects and Crop Genetics Research and Iowa State University, Ames, IA, 50011

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

P179

Gene Expression Profile Analysis on Developing Maize Kernels with Different Levels of Starch

(submitted by Sofia Silva <ssilva@uiuc.edu>)
Full Author List: Silva, Sofia[1]; Johnson, Richard[1]; Moose, Stephen P.[1]; Schneeberger, Richard[2]; Rocheford, Torbert R.[1]
[1] Department of Crop Sciences, University of Illinois, Urbana, IL 61801
[2] Formerly, Ceres, Inc., Thousand Oaks, CA 91320

Maize starch is a macronutrient of great importance not only for human and animal nutrition but also for its numerous industrial uses. Starch is a very complex trait having a well characterized pathway, although regulatory loci controlling fluxes through the pathway still need to be identified. In an attempt to better understand the genetic control of starch in maize, we produced a mapping population for starch concentration. Cycle 90 of Illinois Low Protein (ILP) strain, which exhibits very high levels of starch, was crossed to B73. The F1 was backcrossed to B73 and then selfed to produce a mapping population. The BC1S1 lines were selfed two more generations producing BC1S3 lines which were then used in testcross evaluation. QTL mapping for starch, protein, oil, and kernel weight/yield in the testcross was performed. The higher and lower lines for starch on both the BC1S1 and testcross populations were selected and planted in 2004 and 2005. Ears and leaf tissue were collected 10, 15 and 20 days after pollination and microarray analysis was performed on RNA from the materials. Preliminary microarray data analysis revealed over 100 ESTs that were differentially expressed in developing kernels of the high vs. low starch lines in both the per se and testcross materials. Further analysis will involve gene validation through qPCR as well as mapping these genes in the per se and IBM population. In this experiment we expect to identify new genes that could assist in a better manipulation of the corn plant to produce desired amounts of starch. Furthermore, these results could possibly be helpful in explaining the genetic changes that occurred in the ILP strain during 90 cycles of selection.

P180

Genetic Analysis of Popping Expansion Volume in a Popcorn X Dent Maize Population

(submitted by Thanda Dhliwayo <tdhliwa@iastate.edu>)
Full Author List: Dhliwayo, Thanda[1]; Lee, Michael[1]; Erazo-Barradas, Mauricio[1]; Ziegler, Kenneth E[1]; Woodman-Clikeman, Wendy[1]
[1] Department of Agronomy; Iowa State University, Ames, IA, 50011

Popcorn (Zea mays L) is inferior to dent corn in agronomic performance and disease resistance. Dent corn may be used to improve the agronomic performance of popcorn, but its utility is limited by the potential for losing popping expansion volume (PEV), an important quality trait of popcorn. The risk of losing PEV may be minimized by using genomics-aided breeding approaches if genetic information is available a priori. To investigate the genetic basis of popping ability, QTL for PEV and kernel weight (KNWT) were mapped using SSR loci in a population of 189 F3 lines derived from B104 (dent) and 95:2 (popcorn). Phenotypic (rp) and genotypic (rg) linear correlation coefficients between the two traits were statistically significant and negative (rp = 0.55+/-0.04; rg = 0.68+/-0.05). Seven QTLs were detected for PEV on chromosomes 1S, 1L, 2S, 3S, 5L, 8L, and 9L. For all but one QTL (8L), the allele from the 95:2 increased PEV. Five QTLs were detected for KNWT on 1S, 6L, 7L, 8L and 9L, and for all loci, the allele from B104 increased KNWT. KNWT QTLs on 1S and 9L were detected at the same genetic map positions as those for PEV. The two QTLs jointly accounted for 40% of the phenotypic variance of each trait, indicating that the observed linear correlation may be due to pleiotropy. The results reveal genomic regions that may be important for KNWT and PEV in popcorn x dent maize crosses. Coupled with QTL information for important agronomic traits, these loci may be used to design genomics-aided breeding strategies for improving agronomic performance of popcorn using dent maize germplasm while maintaining or possibly increasing PEV.

P181

Genetic Diversity of Regionally Adapted Maize Germplasm

(submitted by Lewis Lukens <llukens@uoguelph.ca>)
Full Author List: Lukens, Lewis[1]; Guerra Peraza, Orlene[1]; Liu, Kang[1]; Lee, Elizabeth A.[1]
[1] University of Guelph, Department of Plant Agriculture, Guelph, Ontario, Canada N1G2W1

Maize is a productive crop across diverse environments, and a selection program for yield in single geographic area is expected to influence genetic diversity. We characterized maize germplasm consisting of inbred lines developed for a short season growing area in southeastern Ontario, Canada. The population of 95 inbred lines was surveyed at 122 loci with SSR primer pairs. The number of alleles within this regional germplasm was low relative to American and tropical lines. However, the average allelic diversity within this regional germplasm was similar to American and tropical germplasm. Genomic DNA from three representative inbred lines hybridized onto an oligonucleotide array with over 27,000 probes revealed that probe signal intensities were highly similar across inbreds. Nonetheless, the inbred lines that had the highest similarity based on marker data also had the smallest number of probe signal differences. Our results show that this regional germplasm has likely undergone a bottleneck that has reduced allele number. Different alleles have subsequently been maintained at high frequencies within the germplasm. The association between comparative hybridization differences and genetic distance suggests that changes in gene copy number occur gradually and at low frequency among isolated inbreds.

P182

Genetic and Biochemical Analysis of Iron Bioavailability in Maize

(submitted by Owen Hoekenga <owen.hoekenga@ars.usda.gov>)
Full Author List: Hoekenga, Owen A.[1]; Mwaniki, Angela[2]; Buckler, Edward S.[1]; Glahn, Ray[1]; Kochian, Leon[1]
[1] US Plant, Soil and Nutrition Lab, USDA-ARS, Cornell University, Ithaca NY USA 14853
[2] Dept. of Food Science, Cornell University, Ithaca NY USA 14853

Maize is a major cereal crop widely consumed in developing countries, which have a high prevalence of iron (Fe) deficiency including anemia. The major cause of Fe deficiency in these countries is inadequate intake of bioavailable Fe, of which poverty is a major contributing factor. Therefore, biofortification of maize has great potential to alleviate this deficiency. Maize is also a model system for genetic and genomic research and thus allows the opportunity for gene discovery. Here we describe an integrated genetic and physiological analysis of Fe nutrition in maize kernels, to determine the genes and molecular processes that influence seed Fe content and bioavailability. Quantitative trait locus (QTL) analysis was used to dissect seed Fe concentration (FeSC) and Fe bioavailability (FeSB) from the Intermated B73 x Mo17 (IBM) recombinant inbred (RI) set of maize. FeSB was determined by an in vitro digestion/Caco-2 cell line bioassay. Loci associated with increased Fe bioavailability were identified on chromosomes 3, 6 and 9 while those associated with increased seed Fe content were identified on chromosomes 1, 2 and 5. Models obtained explained ~25% of the variance in Fe bioavailability and ~20% of the variance in seed Fe content. Seed Fe concentration was not correlated with Fe bioavailability. Iron bioavailability was also not correlated with the levels of seed phytate, as estimated by testing RI at the extremes of the observed Fe bioavailability. Comparative genomic analysis identified several candidate genes for each of the observed QTL, which will be briefly discussed.

P183

Genetic and QTL Analysis of Pericarp Thickness and Ear Inflorescence Architecture in Fresh Market Waxy Corn Germplasm

(submitted by Eunsoo Choe <echoe1@uiuc.edu>)
Full Author List: Choe, Eunsoo[1]; Rocheford, Torbert R.[1]
[1] Department of Crop Sciences; University of Illinois at Urbana Champaign; Turner Hall 1102 S. Goodwin Ave. Urbana, IL, 61801

Waxy corn is widely consumed in Asia for its unique properties in tenderness, sweetness, and stickiness. Due to increasing Asian-American population in the U.S. and lack of information of waxy corn for fresh consumption, breeding and genetic research on waxy corn for U.S. fresh market is needed. Marker Assisted Selection (MAS) of Quantitative Trait Loci (QTL) for introducing taste quality characteristics from exotic Korean varieties to more adapted U.S. backgrounds would enhance breeding success. This research was conducted to understand associations among pericarp thickness traits, which are relevant to tenderness, as well as for ear inflorescence architecture traits, which are associated with consumer preference. We wish to detect QTL for both traits and examine relationships. Pericarp thickness on five different regions of kernels, and nine ear inflorescence architecture traits were measured on 264(BH20XBH30)F3 families. Univariate and multivariate (Principal Component Analysis) approaches were used to detect QTL from linkage map based on 100 SSR markers. All five pericarp thickness traits were highly correlated and one PC was explaining most of the total phenotypic variation was detected. Number of QTL were clustered closely on bins 1.07, 1.10, 2.06, 3.00, 4.01, 4.08, 6.05, and 9.03. The additive effect of QTL for thin pericarp thickness came from both BH20 and BH30. We plan to reduce pericarp thickness by pyramiding favorable alleles from both parents. Nine ear inflorescence architecture traits were reduced into four independent PCs. Number of QTL were clustered closely and some PC-QTL were detected closely to the QTL. Some PC-QTL were found on additional regions that were not detected in univariate QTL would suggest the increase of power of detecting QTL. Based on the QTL information, four lines were selected and crossed with each other so that we could pyramid favorable alleles through marker assisted selection.

P184

Germplasm Survey of Stomata Density

(submitted by Tyler Huffman <thg7f@mzzou.edu>)
Full Author List: Huffman, Tyler[1]; Bush, Dana[1]; Grzekowiak, Nicole[1]; Musket, Theresa[1]; Davis, Georgia L.[1]
[1] Division of Plant Sciences, University of Missouri, Columbia, MO 65211

Millions of bushels of maize are lost annually to drought. Under drought stress maize will respond in many ways, including stomata closure. Stomata facilitate gas exchange in plants. During stomate opening transpiration causes water loss. Thus increased stomata density may increased loss of water occurs during stomata opening. In addition, previous work has demonstrated that stomata density can be altered by a variety of environmental changes such as altered CO2 content and light cues, as well as, by hormone levels, particularily, absiscic acid and ethylene. Previous research identified a number of QTL for stomata density using a subset of 94 IBM mapping lines over two years. In this experiment; eighty-nine lines of the maize diversity set were grown to the seventh leaf stage at the Genetics Research Farm in Columbia, Missouri over two years. Epidermal casts were made from the abaxial epidermis of the fifth leaf of the plant. Stomate density was then taken by counting the number of stomates in a square millimeter on four plants from each line in each of two replications per year. Five replicates of the one millimeter square area were counted per plant. Statistical analysis was performed with the GLM procedure in SAS. Model variables included year, replicate, line, sub-sample, and associated interaction terms. Significant variability was identified among lines in this material indicating the potential to select for high or low stomata density lines for both genetic studies and in breeding. In agreement with work of other laboratories indicating that environmental cues can significantly alter stomata density, significant variation was observed between years in our study pointing towards the need to measure this trait in multiple years or highly controlled environments to achieve better estimates of the genotypic contribution to stomata density.

P185

Identification of QTL for Nitrogen Use Efficiency in the IBMRI x IHP1 Population

(submitted by Devin Nichols <dmnichol@uiuc.edu>)
Full Author List: Nichols, Devin[1]; Below, Fredrick E.[1]; Moose, Stephen P.[1]
[1] Department of Crop Sciences, University of Illinois; 1201 West Gregory Drive; Urbana, IL, USA 61801

Nitrogen (N) is often a yield-limiting nutrient in the production of maize. The identification of genes involved in nitrogen use efficiency (NUE) and the use of such genes to develop hybrids with greater NUE could have both economic and environmental benefits. The objective of this study is to identify QTL controlling NUE and its component traits. The mapping population used is a set of hybrids developed by crossing lines from the IBMRI population to the Illinois High Protein (IHP1) inbred. This population takes advantage of the high mapping resolution of the IBMRIs and the high N uptake phenotype of IHP1. A set of 243 hybrids was grown with and without supplemental N in an N-responsive environment. Measurements were made for a number of phenotypic traits that are components of NUE, including grain yield, plant biomass, and kernel composition. Results from the QTL analysis of these traits will be presented here.

P186

Identification of Traits Correlated with Lower Aflatoxin Accumulation using a Diverse Maize Germplasm Survey

(submitted by Dana Bush <dlw3f9@mizzou.edu>)
Full Author List: Bush, Dana[1]; Musket, Theresa[1]; Davis, Doug[1]; Brooks, Thomas[2]; Williams, Paul[2]; Windham, Gary[2]; Krakowsky, Matthew[3]; Davis, Georgia L.[1]
[1] Division of Plant Sciences, University of Missouri-Columbia, Columbia, Missouri 65211
[2] USDA-ARS-CHPRRU, Mississippi State University, Mississippi 39762
[3] USDA-ARS-CGBR, USDA Coastal Plain Experiment Station, Tifton, Georgia 31794

Aspergillus flavus has low host specificity and can infect a wide range of plants, insects, and animals. Mycotoxin-related crop losses are estimated to be almost a billion dollars each year. The FDA mandates the allowable toxin level to be < 20 parts per billion (ppb) in grain for human consumption and < 0.5 ppb in milk products. The objective of this experiment was to conduct a survey of aflatoxin levels in maize germplasm lines. A subset of 86 lines, from the Maize Diversity Project, was examined for aflatoxin accumulation. Two replications of each line were grown over two years in one location. To further examine this material, Mississippi and Georgia locations were added to the experiment in year two. Line accounted for the largest proportion of variation at all locations. Line, rep, year, year by rep, line by rep, year by line, year by line by rep were all highly significant variables in the analysis of variance for the combined years at the Missouri location. In the Missouri location resistant lines came from mixed, non-stiff stalk, and tropical groups. Correlations were conducted between other agronomic trait data and aflatoxin levels. Ear husk coverage had a -27.8% (P

P187

Joint Linkage and Association Mapping of Genome-wide Variation in Total DNA 5mC Methylation in Maize

(submitted by Elhan Ersoz <ee57@cornell.edu>)
Full Author List: Ersoz, Elhan S.[1]; Holmberg, Karin J.[1]; Thannhauser, Theodore W.[2]; Buckler, Edward S.[2]
[1] Institute for Genomic Diversity,Cornell University, Ithaca, NY, 14853
[2] USDA-ARS, Ithaca, NY, 14853

In plants, 5-methylcytosine (5mC) residues are found predominantly at symmetric CpG and CpNpG sequences. DNA methylation patterns attributable to 5mC are established and maintained by DNA methyltransferases, which catalyze the transfer of a methyl group from S-adenosylmethionine (SAM) to the 5th position in the pyrimidine ring of cytosine. Methylation patterns of DNA is not only correlated with gene expression but is also associated with inactivation of transposable elements, instances of imprinting and paramutation as well as transgene silencing in plants. Furthermore, several developmentally regulated quantitative traits such as flowering time are regulated through the methylation status of transcriptional regulators. In order to dissect the genetic basis of variation in total levels of 5mC-DNA methylation in the maize genome, we have adopted a joint linkage and association mapping approach where total 5mC nucleotide concentration of genomic DNA will be quantified with high-pressure liquid chromatography (HPLC). To achieve this goal, we will first phenotype the Intermated-B73-Mo17 (IBM) population for the 5mC levels and map large effect QTLs that influence genome-wide methylation levels. Concurrently, we will take a candidate gene approach to detect associations between single nucleotide polymorphisms (SNPs) from the most likely candidate genes that influence this trait and variation in total 5mC levels from 302 diverse inbred lines. Various DNA methyltransferases, chromomethylases, and enzymes involved in SAM metabolism have been designated as candidate genes and are being sequenced to identify candidate SNPs for genotyping. Progress to date will be presented.

P188

Linkage Disequilibrium and Associations with Forage Quality at Loci Involved in Monolignol Biosynthesis in Breeding Lines of European Silage Maize (*Zea mays L.*)

(submitted by Jeppe Reitan Andersen <jepper.andersen@agrsci.dk>)
Full Author List: Andersen, Jeppe R.[1]; Zein, Imad[2]; Wenzel, Gerhard[2]; Kruetzfeldt, Birte[3]; Eder, Joachim[3]; Ouzunova, Milena[4]; Luebberstedt, Thomas[1]
[1] Department of Genetics and Biotechnology, University of Aarhus, Research Center Flakkebjerg, Slagelse, Denmark
[2] Department of Agronomy and Plant Breeding, Technical University of Munich, Freising-Weihenstephan, Germany
[3] Bavarian State Research Center for Agriculture, Freising-Weihenstephan, Germany
[4] KWS Saat AG, Einbeck, Germany

During recent decades, breeding efforts have lead to a substantial increase in whole plant yield of silage maize. However, during the same period of time there has been a steady decrease in cell wall digestibility, and, consequently, in feeding value of elite hybrids. Cell wall digestibility is influenced by both lignin content and lignin structure. Thus, genes involved in the lignin biosynthetic pathway are considered promising candidate genes for improving digestibility of silage maize.

Partial genomic sequences of 10 genes involved in biosynthesis of monolignols have been obtained in a number of inbred lines currently employed in European silage maize breeding. Different levels of nucleotide diversity and linkage disequilibrium (LD) were found, indicating different levels of selection pressure on individual genes of the monolignol pathway. Individual polymorphisms were tested for association with four quality-related traits to identify candidate functional markers for forage quality. Significant associations were identified, both when including and excluding population structure in the analysis. However, discrimination of effects of individual polymorphism was in some cases not possible due to extended LD. Studies in larger and/or broader sets of maize germlasm could decrease LD and validate candidate functional markers for forage quality identified in the present study.

P189

MetaQTL: New Computational Methods for QTL Meta-Analysis

(submitted by Alain Charcosset <charcos@moulon.inra.fr>)
Full Author List: Veyrieras, Jean-Baptiste[1]; Goffinet, Bruno[2]; Charcosset, Alain[1]
[1] INRA, UMR de Genetique Vegetale, Ferme du Moulon, 91190 Gif sur Yvette, France
[2] INRA, BIA, Chemin de Borde Rouge BP27 31326 Castanet Tolosan Cedex, France

Integration of multiple results from Quantitative Trait Loci (QTL) studies is a key point to understand the genetic determinism of complex traits. Up to now many efforts have been made by public database developers to facilitate the storage, compilation and visualization of multiple QTL mapping experiment results. However, studying the congruency between these results still remains a complex task. Presently, the few computational and statistical frameworks to do so are mainly based on empirical methods (e.g. consensus genetic maps are generally built by projection methods).

We present a new computational and statistical package, called MetaQTL, for carrying out whole-genome meta-analysis of QTL mapping experiments. Contrary to existing methods, MetaQTL offers a complete statistical process to establish a consensus model for both the marker and the QTL positions on the whole genome. First, MetaQTL implements a new statistical approach to merge multiple distinct genetic maps into a single consensus map which is optimal in terms of weighted least squares and can be used to investigate recombination rate heterogeneity between studies. Secondly, assuming that QTL can be projected on the consensus map, MetaQTL offers a new clustering approach based on a Gaussian mixture model to decide how many QTL underly the distribution of the observed QTL.

We demonstrate using simulations that the usual model choice criteria from mixture model literature perform relatively well in this context. As expected, simulations also show that this new clustering algorithm leads to a reduction in the length of the confidence interval of QTL location provided that across studies there are enough observed QTL for each underlying true QTL location. The usefulness of our approach is illustrated on published QTL detection results of flowering time in maize. Finally, MetaQTL is freely available at http://bioinformatics.org/mqtl.

P190

Natural Genetic Variation for Water Stress Responses in *Zea mays* Roots

(submitted by Tina Wambach <twambach@uoguelph.ca>)
Full Author List: Wambach, Tina[1]; Lukens, Lewis[1]
[1] University of Guelph, Guelph, ONT, N1G 2W1, Canada

Elite maize germplasm has variable responses to abiotic stress. Some genotypes yield well in a wide range of environments while other genotypes yield well in a small number of environments. As a model system to investigate the basis for variability among genotypes across environments, we are studying seedling root growth rates in response to water stress among a population of maize inbred lines. Root growth rates across different growth conditions vary in a genotype specific manner ($P<0.001$). Increased concentration of the amino acid proline in the root tip enhances a plant's osmotic adjustment, and there is a significant correlation between proline and root growth under osmotic stress. Analyses of root tips from twelve inbred lines indicate that proline concentrations can differ over 5-fold among lines under control growth conditions with a range from 8.13 (inbred line SD80) to 43.85 (CG73) micromoles per gram dry weight tissue ($P<0.001$). Although the amount of proline per dry weight increases in all genotypes under water stress conditions as compared with control conditions (ranging from 25% higher (CG108) to close to 300% higher (SD80)), some lines have lower proline concentrations under water stress conditions than others do under control conditions. Using 122 microsatellite loci distributed across chromosomes 1 through 10, we have identified linkage disequilibrium between root growth traits and loci within the maize genome. We are examining expression profiles across lines and conditions to determine if genetic variation for expression profiles is as great as the genetic variation observed for physiological traits.

P191

Near-Isogenic Lines for the Genetic Dissection of Quantitative Resistance to Southern Leaf Blight and Grey Leaf Spot in Maize

(submitted by Peter Balint-Kurti <peter_balintkurti@ncsu.edu>)
Full Author List: Balint-Kurti, Peter J.[1]; Zwonitzer, John C.[1]
[1] USDA-ARS, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616, USA

Quantitative disease resistance is a poorly understood trait in plants, from both a molecular and a physiological perspective. We have developed several near-isogenic line pairs (NIPs) differing in resistance for one of two foliar maize diseases; southern leaf blight and gray leaf spot. In specific cases, the lines within a NIP appear to differ for both diseases. Within each NIP, the genetic background is fixed, allowing the effects of specific quantitative trait loci for disease resistance to be directly evaluated. In addition, the analysis of families derived from these pairs allows fine-mapping of the genes conferring quantitative resistance. We will present our current progress in these areas.

P192

P-glycoprotein-Mediated Auxin Efflux and the Control of Plant Architecture in Sorghum

(submitted by Patrick Brown <pjb34@cornell.edu>)
Full Author List: Brown, Patrick J.[1]; Franks, Cleve[2]; Rooney, William L.[3]; Kresovich, Stephen[1]
[1] Institute for Genomic Diversity, 157 Biotechnology Building, Cornell University, Ithaca NY 14850
[2] USDA-ARS Cropping Systems Research Laboratory, 3810 4th St, Lubbock TX 79415
[3] Dept. of Crop and Soil Sciences, Foundation Seed Building, 2474 TAMU, College Station TX 77843

Sorghum Dw3 encodes a P-glycoprotein (PGP)-class auxin efflux carrier. Most commercial US grain sorghum carries a recessive dw3 allele with an 882 bp tandem duplication in the last exon. We used association mapping to investigate the effects of the recessive dw3 allele on plant and inflorescence architecture in a panel of 378 sorghum lines. The tandem duplication in dw3 shortens vegetative internodes but lengthens inflorescence structures including both the rachis and primary branches. The basis for inflorescence elongation in recessive dw3 plants is unknown, but expression data is consistent with a rescue of dw3 function in the inflorescence. Plant height variance in recessive dw3 lines is drastically reduced, suggesting that dw3 is epistatic to the other major plant height loci in sorghum. We present association results for several gibberellin metabolism genes in sorghum and demonstrate that their phenotypic effects are contingent upon a wild-type dw3 background.

P193

Phenotypic Analysis of Intermated B73xMo17 (IBM) Populations.

(submitted by Victor Abertondo <vja@iastate.edu>)
Full Author List: Abertondo, Victor J[1]; Lee, Michael[1]
[1] Iowa State University, Agronomy Hall, 100 Osborn Drive, Ames, Iowa 50011-1010

Random mating within a mapping population creates more opportunities for recombination. Therefore, the probability of observing recombination events between linked loci is increased and more reliable genetic maps may be produced. The intermated B73xMo17 (IBM) population is a widely used resource for maize mapping. It was developed by intermating the F2 for four generations before recombinant inbred lines were derived. In order to increase the number of recombinants to reach a higher map resolution, a second population of double haploid lines was created after six additional cycles of intermating, and it was termed IBM-10. It is known that intermating was effective in creating a higher frequency of recombinants in IBM-10 than IBM (Jaqueth, 2003). However, there is no information about how intermating affects the phenotypic variation of these populations. In this study, IBM and IMB-10 populations are compared in terms of phenotypic variance, population means and correlation coefficients for a set of traits. The populations were grown in adjacent experiments at the Agronomy Agricultural Engineering Research Center (AAERC), Ames, Iowa in 2006. The results indicate a trend for increased phenotypic variance for IBM-10 for most of the traits. Moreover, the variance confidence intervals for % of oil and number of tillers per plant of both populations did not overlap. Changes in population means and phenotypic correlation between some pairs of traits were also detected.

P194

Physiological Mechanisms Underlying Grain Yield QTLs

(submitted by Andrea Chambers <aarmst03@uoguelph.ca>)
Full Author List: Chambers, Andrea J.[1]; Singh, Asheesh K.[1]; Tollenaar, Matthijs[1]; Lee, Elizabeth A.[1]
[1] University of Guelph, Guelph, Ontario, Canada, N1G 2W1

We are currently utilizing a novel QTL mapping population structure (IBD-limited RILs) to identify grain yield QTLs and subsequently elucidate their underlying physiology. The key features to this approach are that gross phenotypic differences (e.g., plant height and flowering date) are minimized and population size is substantially reduced. Currently we have identified grain yield QTLs and are beginning our efforts to dissect the underlying physiology of how these regions influence grain yield. A subset of 80 identical-by-descent recombinant-inbred-lines (IBD-RILs) from the CG60 x CG108 mapping population crossed to CG102 were grown in a 3 rep, 2 year (2005-06) physiology trial at Elora, Ontario. Given that grain yield is essentially the ability of the corn plant to accumulate dry matter and then partition it to the ear (i.e., harvest index), we are beginning by examining dry matter accumulation during development and harvest index in this mapping population. All of the genetic variation for dry matter accumulation in this population is due to physiological differences occurring after silking. Genetic differences in grain yield are due both to differences in dry matter accumulation during the grain filling period and to differences in harvest index. Preliminary analyses suggest that some "physiological QTLs" cancel out one another, resulting in those regions not influencing grain yield. Once associations between "grain yield QTLs" and the underlying physiological mechanism (i.e., "physiological QTLs") have been made we can further examine how the expression of these "physiological QTLs" change under different environmental conditions and begin to investigate candidate genes.

P195

QTL Analysis of the Shade Avoidance Response in Maize

(submitted by Patrice G. Dubois <pgd7@cornell.edu>)
Full Author List: Dubois, Patrice G.[1]; Olsefski, Greg O.[1]; Hoekenga, Owen A.[2]; Brutnell, Thomas P.[3]
[1] Cornell University, Ithaca, NY 14853.
[2] US Plant, Soil and Nutrition Laboratory, USDA-ARS, Ithaca, NY 14853.
[3] Boyce Thompson Institute for Plant Research, Ithaca, NY 14853.

In a typical maize field, high density planting affects the intensity and spectral quality of the incident light. Shade created by the canopy is characterized by a reduced ratio of red light (R) to far-red light (FR) and by a lower PAR (photosynthetically active radiation). The low R:FR ratio can be explained by the selective absorption of R by chlorophyll. Reductions in R:FR lead to a series of morphological adaptations known as shade avoidance responses. Typical shade avoidance responses include increased apical dominance, upward leaf orientation, elongation of internodes, reduced lateral branching, and the acceleration of flowering. This repartitioning of resources is made at the expense of reproductive development, ultimately lowering yield. The phytochrome photoreceptors are responsible for the perception of the altered R:FR ratio. Under a low R:FR ratio phytochromes will be maintained in an inactive (Pr) conformation. R induces an isomerization of Pr into an active (Pfr) form, allowing its import into the nucleus where it acts as a protein kinase repressing photomorphogenesis. A better understanding of the mechanisms controlling shade avoidance responses could benefit breeding efforts that strive to increase planting density. To examine the genetic variation that contributes to shade avoidance responses, we initiated a QTL analysis using the well-characterized intermated B73 x Mo17 (IBM) population. In growth chambers, canopy shade was mimicked using end-of-day-FR treatments. Highly responsive traits such as 1st leaf sheath length and mesocotyl length were measured. The results of QTL mapping, together with comparative mapping and first pass candidate gene identification, will be presented.

P196

QTL Mapping of Prolificacy and Related Traits in a Dent x Popcorn Population of Maize

(submitted by Maria Laura Mayor <mlmayor@iastate.edu>)
Full Author List: Mayor, Maria Laura[1]; Erazo-Barradas, Mauricio[1]; Woodman-Clikeman, Wendy[1]; Lee, Michael[1]
[1] Department of Agronomy; Iowa State University; Ames; IA; 50011

Popcorn is used for human consumption, thus, selection and breeding are focused in popping expansion and quality of the kernel and flakes (see poster from Dhliwayo et al.). Also, popcorn plants differ from dent corn in several aspects. Popcorn tends to be prolific (increased number of ears per plant) and produces tillers. The objectives of this experiment are to find genomic regions associated with prolificacy, plant stature, maturity and number of tillers per plant in a population derived from the cross between B104 (dent inbred) and 95:2 (a prolific popcorn inbred). B104 is shorter and late at flowering than 95:2. 189 F2:3 families were planted in a row-column alpha-lattice design with two replications in two environments (years). The traits assessed were: plant height (HT), Growing degree days to anthesis (GDA), number of ears per plant (EP) and number of tillers per plant (NTP). The genetic map was created from 134 SSR loci evenly distributed throughout the maize genome. The population HT averaged 197cm across both environments, while GDA, EP and NTP were higher in the first year compared to the second year (727, 2.04 and 0.67 vs. 678, 1.67 and 0.19 respectively).Several genomic regions were found to be associated with these traits. Linkage of maturity and plant stature DNA sequences with those related to ear morphology and development was observed when C103AP, a prolific inbred, was developed. Further studies are being conducted in this and in the (C103xC103AP) mapping populations for a better understanding of the genetic basis underlying prolificacy and ear development.

P197

Quantitative Trait Loci Associated with Northern Corn Leaf Blight Resistance in Waxy Corn (*Zea mays var. ceratina*)

(submitted by Juthaporn Khampila <kjuthapo@uiuc.edu>)
Full Author List: Khampila, Juthaporn[1]; Theerakulpisut, Piyada[1]; Rocheford, Torbert R.[2]; Pataky, Jerald[2]; Lertrat, Kamol[1]; Sanitchon, Jirawat[1]
[1] Khon Kaen University, Khon Kaen 40002 Thailand
[2] University of Illinois at Urbana-Champaign, Urbana 61801 USA

Exserohilum turcicum (Pass.) K.J. Leonard & E.G. Suggs causes northern corn leaf blight (NCLB), an important disease occurring on maize (Zea mays L.) throughout the world. The development of E. turcicum resistant cultivars has become a high priority for maize breeding programs. Marker-assisted selection (MAS) enables breeders to improve selection efficiency. The objective of this study was to identify quantitative trait loci (QTL) associated with NCLB resistance in waxy corn using simple sequence repeats (SSRs) markers. A population of 187 F2:3 lines derived from a cross between a highly susceptible inbred line (209W) and a resistant inbred line (241W), were used for linkage map construction. Phenotypic data was collected for 2 replications planted at different times in greenhouse for NCLB resistance. In the combined analysis of both replications, five regions distributed in the maize genome were significantly associated with NCLB resistance (chromosome 1.01, 5.05, 6.05, 7.02 and 8.05) describing 24.6% of the total phenotypic variability. Individual QTL explained between 8.3 and 13.7% of the phenotypic variability. Some of the QTL associated with genes controlling resistance to NCLB have been reported previously, and some are described here for the first time. The resistant allele at these QTL originated from 241W resistant line. On chromosome 8.05 a QTL mapped to the same region as the major race-specific gene Ht2.

P198

Roles of Stolbur phytoplasma and *Reptalus panzeri* (Cixiinae, Auchenorrhyncha) in the epidemiology of Maize redness in Serbia

(submitted by Margaret Redinbaugh <redinbaugh.2@osu.edu>)
Full Author List: Jovic, J.[1]; Cvrkovic, T.[1]; Mitrovic, M.[1]; Krnjajic, S.[1]; Redinbaugh, M.G.[2]; Pratt, R.C.[3]; Gingery, R.E.[2]; Hogenhout, S.A.[3]; Tosevski, I.[4]
[1] Institute for Plant Protection and Environment, Department of Plant Pests, Banatska 33, 11080 Zemun, Serbia
[2] USDA, ARS Corn and Soybean Research, Ohio Agriculture Research and Development Center, Wooster, OH 44691
[3] The Ohio State University, Ohio Agriculture Research and Development Center, Wooster, OH 44691
[4] CABI Bioscience, Centre Switzerland, 1 Rue des Grillons, 2800 Delmont, Switzerland

Maize redness (MR), a disease causing midrib, leaf and stalk reddening and abnormal ear development in maize, has been reported from Serbia, Romania and Bulgaria for 50 years. Recent epiphytotics reduced yields by 40-90% in southern Banat, Serbia. MR was recently associated with the presence of the stolbur phytoplasma (Duduk and Bertaccini Plant Dis. 90: 1313), although the epidemiology of the disease remained unknown. Diseased fields in southern Banat were surveyed for potential vectors of the phytoplasma during 2005 and 2006, and high populations of *Reptalus panzeri* were found. In affected fields, 20% of the *R. panzeri* individuals and 85% of symptomatic maize plants carried the stolbur phytoplasma. When stolbur phytoplasma-infected *R. panzeri* were introduced into insect-free mesh cages containing healthy maize plants, midrib and leaf reddening developed on 48% of plants and stolbur phytoplasma was detected in 90% of the symptomatic plants. No symptoms or phytoplasma-positive plants were found in cages without insects. These data indicate that MR symptoms are associated with the stolbur phytoplasma. To identify potential reservoirs of pathogen, *Convolvus arvense* and several other perennial weeds collected from the test plots in southern Banat were tested for the presence of stolbur phytoplasma, but none were infected. However, *R. panzeri* larvae collected from the roots of infected maize plants in late October, 2006 were positive for the phytoplasma. These results indicate that *R. panzeri* is likely to be a major vector of MR, as it is abundant in affected fields, can transmit the stolbur phytoplasma, and the overwintering form of the insect is infected with the pathogen.

P199

Strategies for Reaching Targets of Maize Kernel Carotenoid Content Using Visual Selection and Molecular Markers

(submitted by Robyn Stevens <allschei@uiuc.edu>)
Full Author List: Stevens, Robyn[1]; Dias de Carvalho, Ines[2]; Bermudez Kandianis, Catherine[1]; Wurtzel, Eleanore[3]; Buckler, Edward S.[4]; Rocheford, Torbert R.[1]
[1] Dept. of Crop Sciences; University of Illinois; Urbana, IL 61801
[2] Seccao Autonoma Eng Ciencias Agrarias; Faculdade de Ciencias da Universidade do Porto; Porto, Portugal
[3] Dept. of Biological Sciences; Lehman College; The City University of New York; New York City, NY 10468
[4] Institute for Genomic Diversity; Cornell University; Ithaca, NY 14853

Micronutrient deficiencies affect millions of people worldwide. Our goal is to perform genetic research to facilitate breeding to increase provitamin A and carotenoid concentrations in maize grain. Maize contains carotenoids, a group of lipid-soluble antioxidant compounds, including vitamin A precursors (alpha-carotene, beta-carotene, and beta-cryptoxanthin), and xanthophylls (lutein and zeaxanthin). Visual selection for dark orange color in synthetic populations has resulted in increased levels of total and individual carotenoids, in the 45-65ug/g range. We present data on the correlations observed between visual color score and carotenoid concentrations obtained by HPLC for an A619xSC55 F2:3 population. SC55 is pale yellow and A619 is dark yellow. We performed composite interval mapping (CIM) on visual score and ranking in the A619xSC55 population which identified QTL in bins 6.01, 9.02, and 9.07. The largest QTL was for rank and color was located in bin 9.07, explaining 35% and 36% of variation, respectively. Notably, the white cap1 locus maps to bin 9.07. This gene encodes a cleavage enzyme responsible for the degradation of carotenoids and is homologous to carotenoid cleavage dioxygenase1 (CCD1) in Arabidopsis. The QTL in bin 9.07 may represent different alleles at the white cap1 locus. Although SC55 does not carry the wc1 mutant allele, discrete color classes can be observed in F2 and F3 materials. Further CIM to identify QTL affecting total and individual carotenoid concentrations in this population will be presented. Assessment of candidate genes possibly related to QTL identified in the A619xSC55 and W64a x A632 F2:F3 (Wong et al, 2004) mapping populations was performed using structural carotenoid biosynthesis genes as probes. Results of phytoene synthase (PSY1) and lycopene epsilson cyclase (LYCe) will be presented. Strategies to use allelic variation at candidate genes in the selection program will be discussed.

P200

Targeted Discovery and Characterization of QTL for Resistance to Northern Leaf Blight and Other Foliar Fungal Diseases in Maize

(submitted by Chia-Lin Chung <cc435@cornell.edu>)
Full Author List: Chung, Chia-Lin[1]; Nelson, Rebecca J.[1]
[1] 303G Plant Science Bldg., Dept. of Plant Pathology, Cornell University, Ithaca, NY, USA 14853

With the aim of analyzing and dissecting quantitative resistance for northern leaf blight (NLB), the heterogeneous inbred family (HIF) strategy (Tuinstra et al. 1997) was used for targeted QTL mapping and NIL development. The tropical maize line CML52 was chosen for analysis based on its superior resistance to NLB, gray leaf spot (GLS), southern leaf blight (SLB), and ear rot. Chromosomal regions associated with multiple disease resistance (MDR) were selected based on a consensus map of disease QTL in maize (Wisser et al. 2006). For 94 individuals in 19 F5 families derived from the cross of B73 x CML52, 66 markers covering 38 bins associated with MDR were analyzed and heterozygous lines were identified for each. From 2005 to 2006, 15 F6, 7 F7, and 2 F8 families (a total of 24 HIFs) were evaluated for NLB resistance parameters, including incubation period, lesion number, diseased leaf area, and area under disease progress curve (AUDPC). Significant phenotypic contrasts in near isogenic line (NIL) pairs were detected and verified in bins 1.06, 1.07-1.08, 5.03, 6.05, and 8.02-8.03. Except for QTL in bin 5.03, CML52 alleles were associated with NLB resistance. To more precisely localize the QTL, a total of 44 flanking SSR markers were used to estimate the start and end points of heterozygous regions in F5 families. The QTL are co-localized with NLB-QTL previously detected in the other 3 maize populations: B52 x Mo17 (Freymark et al. 1993), D32 x D145 (Welz et al. 1999), and IL731a x W6786 (Brown et al. 2001). In the future, NIL pairs contrasting for each identified NLB-QTL will be chosen from corresponding HIFs and evaluated for resistance to other important fungal diseases. The practical considerations of using HIFs for QTL analysis will also be discussed.

P201

The Analysis of Quantitative Resistance to Foliar Diseases of Maize and Evidence for Multiple Disease Resistance Loci

(submitted by Peter Balint-Kurti <peter_balintkurti@ncsu.edu>)
Full Author List: Balint-Kurti, Peter J.[1]; Johal, Gurmukh S.[2]; Kolkman, Judith[3]; Nelson, Rebecca J.[3]; Wisser, Randall[1]; Zwonitzer, John C.[1]
[1] USDA-ARS, Dept of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616
[2] Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN.
[3] Dept. of Plant Pathology, Cornell University, Ithaca, NY.

Quantitative resistance is the dominant form of resistance utilized in cultivated maize and QTLs for resistance to many diseases have been identified. However, very little is known about the molecular genetic or physiological basis of quantitative resistance in maize or any other crop. Working with the foliar diseases gray leaf spot (GLS), southern leaf blight (SLB) and northern leaf blight (NLB), we are identifying and mapping new sources of resistance from diverse germplasm. From a synthesis of the previously published literature on the locations of disease QTL, we have identified several chromsomal segments associated with QTL for multiple diseases. We have also shown a significant genetic correlation between resistance to SLB, GLS and NLB in a diverse set of 300 inbred lines. Using the maize IBM population we have precisely delimited GLS and SLB QTL which in some instances co-localized with each other, allowing the identification of candidate genes for multiple disease resistance. We have also identified several novel QTL for GLS and SLB at both the seedling and mature plant stages. Finally, we have shown that the disease lesion mimic phenotype conferred by the recombinant resistance gene Rp1-D21, is profoundly affected by its genetic background. We have mapped several loci which modify the phenotype conferred by Rp1-D21. These loci likely represent important genes in the defense response pathway

P202

The Genetics of Complex Traits

(submitted by Gael Pressoir <ghp5@cornell.edu>)
Full Author List: Pressoir, Gael[1]; Yu, Jianming[3]; Zhang, Zhiwu[1]; Buckler, Edward S.[2]; Kresovich, Stephen[1]
[1] Cornell University, Ithaca, NY 14853-2703
[2] USDA-ARS, Cornell University, Ithaca, NY 14853-2703
[3] Dept of Agronomy, KSU, Manhattan, KS 66506-5501

Identity by descent between pairs of individuals or _ij can be inferred from known pedigree relationships. Cattle geneticists have long used mixed models to estimate genetic variance components (Henderson mixed model); assuming that all individuals belong to a same population, a coancestry matrix can then be used in order to estimate correctly the additive genetic variance component. Unfortunately, in many species or population, accurate estimates are often hard if not impossible to obtain. Furthermore, founding effects (founding of a population, establishment of a heterotic group, a pool) using a limited number of individual are generally unaccounted for when estimating _ij. In crops there is an additional problem arising when dealing with inbreds, selection favoring one of the parental genomes will not be accounted for. In addition to these concerns regarding the estimation of _ij from pedigrees, in populations, common designs for estimating variance components generally assume that all individuals or families are equally (un)related; however, in many cases this assumption is incorrect. Recent progress in population genetics now allows estimating relatedness and population structure using molecular markers. We show that current estimates of IBD and population structure already allow for an accurate partitioning of the genetic variation. In this paper we pull together an (1) animal genetics inspired mixed model approach along with (2) recent developments in population genetics; used jointly, these methods allow to predict Key genetic parameters such as narrow sense heritability, genetic correlations and breeding values (it will prove very useful for managing a breeding program or evaluating a germplasm collection).

P203

The Other NPGS Maize Collection - A Rich Source of Maize Genetic Diversity

(submitted by Candice Gardner <gardnerc@iastate.edu>)
Full Author List: Millard, Mark J.[1]; Gardner, Candice A.C.[1]
[1] USDA-ARS Plant Introduction Research Unit, NCRPIS, ames, IA 50011

The maize collection at the North Central Regional Plant Introduction Station, Ames, IA is comprised of over 18,300 accessions from all over the world. Of these, 16,000 are maize accessions with population level genetic diversity and over 2,000 are inbred lines with little segregation. The collection includes teosintes, an ancestor of maize, Tripsacum and Coix, and expired plant variety protected inbreds. The collection is available for distribution to researchers worldwide. Accessions vary in size from the 2-3 foot tall Gaspe Flint to the 15-20 foot tall tropical collections. They are differentiated from the maize genetic stocks collection, which is maintained at Urbana, IL.

Maize curation project objectives include acquisition of landraces, inbreds and populations which represent the breadth of maize genetic diversity, conservation of these resources and their genetic integrity, and providing diverse genetic resources and associated passport, provenance, characterization and evaluation data for research and educational purposes which support agricultural research applications.

Approximately 25% of the collection is distributed annually to support a wide array of research and educational objectives, including evolutionary biology and genetics, breeding for increased yield and agronomic traits, silage quality, ethanol use, sweet corn flavor, popcorn expansion, physiological traits, disease and insect resistance, archaeology and ethnobotany, and organic crop production.

Collection information can be accessed via the GRIN Database, http://www.ars.usda.gov/main/site_main.htm?modecode=36-25-12-00.

P204

Trait Evaluation of Candidate Genes Underlying Maize Brace Root QTL

(submitted by Michael Gerau <mjgf36@mizzou.edu>)
Full Author List: Gerau, Michael J.[1]; Davis, Georgia L.[1]
[1] Division of Plant Sciences, University of Missouri-Columbia, Columbia, Missouri 65211

Brace roots play many roles in the mature maize plant, they facilitate water and nutrient uptake and provide mechanical anchorage to the terrain. Previous work has identified various QTL segregating in the intermated B73 x Mo17 (IBM) mapping population for brace root traits. The goal of this experiment was to test for candidate genes underlying brace root variation by evaluating root traits on mutants which map near QTL or that where implicated in the same pathway as a candidate gene. Candidate genes were selected using the IBM2 2005 Neighbors map (http://www.maizegdb.org/). Families segregating for a mutation in a candidate gene were grown in the field and evaluated for number of nodes with brace roots and number of brace roots at each node. Means comparison tests were then performed to identify statistically significant differences between wild type and mutants using SAS. The three gibberellic acid insensitive dominant dwarf mutations, *D8*, *D9*, and *D10* were significantly different than wild-type for all traits measured. *ramosa1* had fewer nodes with brace roots and fewer brace roots at node one than wild type. *teopod1* and *teopod2*, two heterochronic mutants of maize, had significantly more brace root nodes and a larger number of brace roots at nodes two and three than the wild-type. Each of these mutants are either, involved in gibberellin response, or are restored to wild-type phenotypes by application of gibberellic acids, implicating this hormone in brace root formation. Association mapping will allow us to validate these candidate genes in the future.

P205

UV Modulation of Nitrate Reductase Activity is Controlled by Multiple Interacting Genes

(submitted by Lee Richbourg <hlr7003@uncw.edu>)
Full Author List: Richbourg, Lee[1]; Morrison, Kristin[3]; Simmons, Susan[2]; Stapleton, Ann[1]
[1] 601 S. College Rd. Wilmington, NC 28401 Department of Biology & Marine Biology UNCW
[2] 601 S. College Rd. Wilmington, NC 28401 Department of Mathematics & Statistics UNCW
[3] 10900 Euclid Ave. Cleveland, OH 44106 Genetics Department School of Medicine, Case Western Reserve University

Nitrogen is an important constituent of many biomolecules in plants & is therefore often a limiting factor affecting yield in a variety of agricultural systems. Nitrate reductase is a critically important and well characterized enzyme in plants, which is responsible for converting soil nitrate assimilated by the roots into nitrite which is further reduced to ammonium, the primary source of nitrogen for building the amino acids necessary for the plant's metabolism. UV radiation has been shown to have a negative impact on the activity of nitrate reductase in leaf tissue as well as root tissue.

What are the factors signaling this environmental responsiveness of nitrate reductase? To address this question, we performed nitrate reductase activity assays on crude extracts of leaf and root tissue from the IBM94 recombinant inbred mapping population. The randomized block experimental design incorporated UV-B stress and control treatments and measurements on multiple individuals per line. Linear-model-based analysis and recursive partitioning allowed sensitive and high-confidence detection of multiple genomic loci controlling nitrate reductase response to ultraviolet radiation stress.

P206

TED, A New Autonomous *MULE* with a High Postmeiotic Excision Frequency

(submitted by Yubin Li <yubin@waksman.rutgers.edu>)
Full Author List: Li, Yubin[1]; Harris, Linda[2]; Dooner, Hugo K.[1]
[1] Waksman Institute, Rutgers University, Piscataway, NJ 08855, USA
[2] Agriculture and Agri-Food Canada, Ottawa, ON, Canada

The new *bz* mutable allele *bz-m175* was isolated in stocks derived from Rhoades and Dempsey's High-Loss x High-Knob stocks, known to carry active transposon systems from different superfamilies. Genetic and molecular characterization of the transposon in *bz-m175* show it to be an autonomous member of the *Mutator* superfamily, which we have named *TED* (for *T*ransposon *E*llen *D*empsey). *TED* is 3959 bp long, ends in 191-bp terminal inverted repeats (TIRs) and causes a 9-bp duplication of the target site. *TED* is predicted to encode a 700-amino-acid protein, TEDA, that is highly homologous to MURA, the putative *MuDR* transposase. However, unlike *MuDR*, *TED* does not encode a B function, which has been postulated to play a role in *MuDR* reinsertion after excision. To assess the effect of the absence of a B function on the reinsertion of *TED*, we have set up a large experiment to isolate and characterize *Bz'* germinal revertants from *bz-m175*. Putative germinal revertants were identified as fully purple kernels in *bz-m175* ears. We find that most of these are nonconcordant kernels with a Bz endosperm and a bz-m embryo and, therefore, represent postmeiotic reversions in the embryo sac which are not recovered in the egg nucleus. The frequency of such events is high: ~ 1 per 1500 kernels. If *TED* reinserts after excision and the reversion event occurred in the megagametophytic division that gives rise to the egg and its sister polar nucleus, it may be possible to recover a *tr TED* element in the bz-m embryo of nonconcordant kernels. We are currently analyzing these exceptions for *tr TED* element.

P207

mPing Transposition Requires Two Proteins

(submitted by C Nathan Hancock <cnhancock@plantbio.uga.edu>)
Full Author List: Hancock, C Nathan[1]; Yang, Guojun[1]; Wessler, Susan R.[1]
[1] University of Georgia, Athens; Athens, GA, USA 30605

Miniature Inverted Repeat Transposable Elements (MITEs) are the most abundant nonautonomous DNA transposons in the *Oryza sativa* genome with ~90,000 copies. Despite their abundance in rice and other plant and animal genomes, it is not currently known how they are able to attain such high copy numbers (up to several thousand/family). *mPing*, the only active MITE isolated to date, has recently been shown to be mobilized by the autonomous elements *Ping* and *Pong*. These elements belong to a novel superfamily of DNA transposons that encode a second open reading frame (ORF 1) in addition to the proposed catalytic protein (ORF 2). Our aim is to determine the role of these two ORFs in the transposition of *mPing*. We expressed Ping ORF 1 and ORF 2 (using the 35S promoter) in Arabidopsis containing an *mPing*:GFP reporter construct. Transposition was only observed when ORF 1 and ORF 2 were expressed together, indicating a requirement for both proteins. The use of non-native promoters further suggests that ORF 1 is not necessary for expression of ORF 2 and is likely to have a functional role in mobilization. Experiments are underway to determine whether the ORF 1 and ORF 2 proteins interact *in vivo*. In addition, we are using mutagenesis to identify the functional domains of the ORF 1 and ORF 2 proteins.

P208

Accessing the Abundance and Diversity of Helitrons in the Maize Genome

(submitted by Natalie Jameson <nataliejameson@gmail.com>)
Full Author List: Jameson, Natalie M[1]; Chaudhry, Sadia[1]; Krisko, Ashlee[1]; Hannah, L. Curtis[2]; Lal, Shailesh K.[1]
[1] Department of Biological Sciences, Oakland University, Rochester, MI 48309-4401
[2] Plant Molecular & Cellular Biology, University of Florida, Gainesville, FL 32611

The propensity of Helitrons to capture and mobilize gene sequences likely played a major role in creating the unprecedented lack of gene collinearity in maize lines. To further determine the extent of abundance and diversity of these elements, we searched the maize genome database for other Helitron members and annotated the structure and origin of genes captured by these elements. We also designed Helitron specific primers and used them to detect the presence/absence of thirteen candidate Helitrons in nine different land inbred lines and teosinte. The presence of most of the analyzed Helitron at conserved positions in all inbred lines and in teosinte points to their ancient insertion. In several cases we amplified other closely related members of a particular Helitron family. Family members contained different permutations of internal, captured gene sequences. The data generated from these studies will be presented.

P209

Activator/Dissociation (Ac/Ds) Derivative Formation and Epigenetic Regulation in Maize

(submitted by Liza Conrad <ljc28@cornell.edu>)
Full Author List: Conrad, Liza J.[1]; Bai, Ling[1]; Ahern, Kevin[2]; Olsen, Marika[2]; Brutnell, Thomas P.[2]
[1] Cornell University, Ithaca, NY, USA 14853
[2] Boyce Thompson Institute, Ithaca, NY, USA 14853

Barbara McClintock first reported on the phenomenon of active Ac elements undergoing mutations to become non-autonomous Ds elements over 50 years ago. To capture these rare events we have developed high-throughput genetic and molecular methods that can be utilized to select newly formed Ds elements from any Ac insertion in the maize genome. Using these methods we have identified 15 new Ds elements derived from Ac insertions at 8 different loci. Approximately half of the Ds elements contain filler DNA inserted at the deletion junction, however, this filler DNA does not always originate from within the Ac. In contrast to previous reports, several of these Ds elements lack direct repeats flanking the deletion junctions and filler DNA. We will present a model for the formation of these Ds elements that illustrates how mispairing during DNA synthesis can account for the deletion and insertion of filler DNA in the absence of direct repeats at the deletion junction. Interestingly, one of these deletion derivatives, bti00252::Ac(D1), is predicted to encode a functional transposase protein due to an in-frame deletion of Ac sequence. Genetic and molecular assays indicate that this element encodes a transposase protein with altered activities. Although several Ac derivatives have been reported, this is the first derivative containing a deletion of coding sequence that retains its ability to make a functional transposase protein. Two of the five Ac derivatives previously described, Ac-stabilized1 and Ac-stabilized2 display altered expression patterns due to methylation within the element. We have discovered five additional derivatives of Ac that have altered expression patterns. Genetic and molecular evidence suggests the altered expression levels may also be due to methylation within the element. These studies provide insight into the genetic and epigenetic regulation of transposable elements in the maize genome.

P210

Approaches and Progress Toward Annotating Repetitive Regions of the Maize Genome

(submitted by Joshua Stein <steinj@cshl.edu>)
Full Author List: Stein, Joshua[1]; Narechania, Apurva[1]; SanMiguel, Phillip[2]; Pasternak, Shiran[1]; Ware, Doreen H.[3]; Maize Genome Sequencing Consortium, The[4]
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
[2] Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Indiana 47907
[3] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; and USDA-ARS NAA Plant, Soil & Nutrition Laboratory Research Unit, Ithaca, NY 14853
[4] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; Arizona Genomics Institute, University of Arizona, Tucson, AZ 85721; Iowa State University, Ames, IA 50011; and Genome Sequencing Center, Washington University, St. Louis, MO 63108

The goal of the Maize Genome Sequencing Project is to provide high quality sequence, annotation, and positional information for all of maize's ~50,000 genes. In the process a vast quantity of repetitive sequences will also be acquired, assembled, and displayed within the maize genome browser (http://www.maizesequence.org). Approximately two-thirds of the genome is comprised of repetitive DNA, mostly in the form of retrotransposons that proliferated over the last several million years. Along with other classes of mobile elements, this fraction represents a highly dynamic portion of the genome and a potential source of novel functional diversity. Thus, aside from the practical necessities of identifying such sequences, there is considerable interest in characterizing their structure and prevalence within the genome. Anticipating these needs, we describe several approaches to annotating repetitive elements that accomplish the following: 1) Discrimination of transposon-encoded gene models based on protein-homology; 2) Quantification and graphical display of copy number by using k-mers queried against the DOE JGI whole shotgun sequence; 3) Classification of transposons using publicly available databases; and 4) Visualization of transposable elements in a fashion that reveals nested structures and transposition history. The combination of these annotations, to be incorporated in future releases of the maize browser, would allow users to see relationships between copy number and other features, such as transposon class, coding regions, and long terminal repeats, from which hypotheses could be drawn and tested. As an example application of these methods, we identified transposon coding sequences that are not highly prevalent in the genome and have not been characterized extensively in maize. This work was funded by the NSF/DOE/USDA "Sequencing The Maize Genome" project (NSF #0527192).

P211

Capture of an Active Maize Cytochrome P450 Monooxygenase Gene by a Helitron

(submitted by Sara Martens <chops10blop@yahoo.com>)
Full Author List: Martens, Sara, G[1]; Fouladbash, Eric, M[1]; Hannah, L. Curtis[1]; Lal, Shailesh K.[1]
[1] Department of Biological Sciences, Oakland University, Rochester, MI 48309-4401
[2] Plant Molecular & Cellular Biology, University of Florida, Gainesville, FL 32611

The mass movement of Helitron-mediated gene sequences may have significantly contributed to the molecular basis of heterosis or hybrid vigor in maize. However, with one exception, all reported captured genes are truncated versions of their progenitor and lack coding capacity for biologically-active proteins. Mobilization of a cytidine deaminase gene by a Helitron was recently reported (Xu and Messing, 2006); however it is not apparent if the captured gene is expressed. In this report, we provide evidence that the maize CYP72A27 gene represents an almost intact cytochrome P450 monooxygenase (P450) gene recently captured by a Helitron and subsequently transposed into an Opie-2 retroposon. The four exons of the CYP72A27 within the element contain an open reading frame for 435 amino acid residues. We provide evidence that Helitron-captured CYP72A27 is transcribed and expressed in many tissues. To identify the progenitor, we discovered several maize P450 genes sharing high degree of sequence similarity with CYP72A27. These data suggest that capture of CYP72A27 happened recently or the captured gene is under strong evolutionary selection. These data provide further evidence that Helitrons can capture and mobilize functional genes.

P212

Creating Novel Allelic Variation at the *ps1* Locus Using Activator (Ac) Insertional Mutagenesis

(submitted by Ling Bai <lb226@cornell.edu>)
Full Author List: Bai, Ling[1]; Singh, Manjit[3]; Pitt, Lauren[2]; Sweeney, Meredith[2]; Brutnell, Thomas P.[2]
[1] Department of Plant Biology, Cornell University, Ithaca, New York 14853
[2] Boyce Thompson Institute, Cornell University, Ithaca, New York 14853
[3] Dept. of Horticulture, University of Georgia, Tifton, GA 31793

A large-scale insertional mutagenesis experiment was conducted targeting the pink scutellum1 locus. We selected 1092 Ac transposition events from a closely linked donor Ac resulting in the recovery of 17 novel ps1 alleles. Fourteen ps1 alleles carried an Ac insertion, whereas 3 "footprint" alleles were induced by Ac excision. Multiple phenotypic classes were identified corresponding to Ac insertions in the 5' UTR and coding region of the predicted Ps1 gene. To generate a stable allelic series, we employed genetic screens and identified 83 germinally-heritable ps1 excision alleles. Molecular characterization of these excision alleles revealed a position-dependent bias in excision allele frequencies and the predominance of 7 and 8 bp footprint products. In total, 19 unique ps1 excision alleles were generated in this study including several that resulted in weak mutant phenotypes due to in-frame amino acids insertion. These stable weak mutants are valuable in defining structural domains within the PS1 protein and can be applied to breeding programs to alter the carotenoid composition of the maize grain. The analysis of footprint alleles suggests a model of Ac excision in maize that is consistent with recent in vitro studies of hAT element excision. Importantly, the genetic and molecular methods developed in this study can be extended to generate novel allelic variation at any Ac-tagged gene in the genome.

P213

DLA: A Novel Approach for Amplifying Sequences Flanking Transposons

(submitted by Sanzhen Liu <liu3zhen@iastate.edu>)
Full Author List: Liu, Sanzhen[1]; Schnable, Patrick S.[1]
[1] Iowa State University, IA 50011-3650

Digestion-ligation-amplification (DLA) was developed to overcome limitations associated with existing methods for amplifying Mu transposon flanking sequences (e.g. Frey et al., 1998 and Settles et al., 2004). DLA can be used to amplify a large percentage of the Mu flanking sequences from a given genome. Alternatively, with suitable modifications, it can be used to selectively amplify subsets of Mu flanking sequences. Because this selectivity substantially reduces the numbers of sequences amplified, Mu flanking sequences that co-segregate with mutant phenotypes can be identified. DLA has been used to identify a sequence that is at least tightly linked with a Mu-tagged gene related to root hair development.

P214

Epigenetic Regulation of *Mu* Elements in Maize

(submitted by Damon Lisch <dlisch@berkeley.edu>)
Full Author List: Lisch, Damon[1]; Woodhouse, Margaret[2]; Singh, Jaswinder[1]; Erhard, Karl[1]
[1] U.C. Berkeley; Department of Plant and Microbial Biology; Berkeley, CA 94720
[2] U.C. Davis; U.C. Davis Genome Center; Davis, CA, 95616

MuDR transposons can be heritably silenced using a variant of MuDR called Mu killer, which encodes a hairpin RNA corresponding to mudrA, one of the two genes encoded by MuDR. Although not directly targeted by Mu killer, a second gene encoded by MuDR, mudrB is also silenced by Mu killer, but only when mudrB is in cis to mudrA. Here we provide an analysis of patterns of methylation within MuDR terminal inverted repeats following silencing by Mu killer. We demonstrate that although transposase from an active element can reverse methylation in the TIR of a silenced MuDR element, it does not result in heritable reactivation of that element. Further, we show that although the mop1/RDR2 mutation prevents some methylation of MuDR in the presence of Mu killer, it does not prevent silencing of that element. Finally, we provide evidence for an influence of chromosomal position on the heritability of MuDR silencing.

P215

Insertion of the Rice MITE mPing in Transgenic Arabidopsis

(submitted by Feng Zhang <fzhang@plantbio.uga.edu>)
Full Author List: Zhang, Feng[1]; Yang, Guojun[1]; Wessler, Susan R.[1]
[1] University of Georgia; Athens; GA; 30605

Despite the abundance of MITEs (Miniature Inverted-repeat Transposable Elements) in many eukaryotic genomes, their mechanism of transposition is poorly understood. The first active MITE, the rice mPing element, provides a unique opportunity to study the mechanism of Tourist MITE transposition. Unfortunately, mPing transposition behavior in rice is difficult to analyze because it is only active in very high copy number (~1000 copies) strains that contain numerous sources of transposase. In this study we report results using a heterologous assay system in Arabidopsis in conjunction with constructs containing the rice mPing and two sources of transposase encoded by the Ping and Pong elements. We find that transposase from either Ping or Pong can mobilize mPing. Analysis of excision sites in Arabidopsis indicate that mPing usually excises precisely in somatic tissue. ; Insertion sites into the Arabidopsis genome show an extended 9-bp target site preference, which is identical to that observed in rice. Of the 35 somatic mPing insertions isolated, 33 insertions are located in single copy regions or within 1kb of a coding sequence. In addition, examination of mPing insertions in a single-copy Ping/mPing transgenic Arabidopsis line indicated no preference for local transposition of mPing as 19 of 21 insertion events mapped to unlinked loci. Additional studies are underway to characterize germinal transposition of mPing in Arabidopsis.

P216

Isolation of McClintock's Original *Ds* Element in the Standard Position?

(submitted by Jianbo Zhang <jzhang@iastate.edu>)
Full Author List: Zhang, Jianbo[1]; Peterson, Thomas[1]
[1] Department of Genetics, Development and Cell Biology, Department of Agronomy, Iowa State University, Ames, IA 50011, USA

McClintock reported the first *Dissociation* (*Ds*) element in 1947 as a site of chromosome breakage located proximal to the *Waxy1* gene on maize chromosome 9S (the "Standard Position" of *Ds*). Efforts to isolate McClintock's original *Ds* are complicated by the fact that the Standard Position does not correspond to any known gene. From the Standard Position, *Ds* transposed to a new site (*Ds5245*, which has apparently been lost); *Ds5245* subsequently gave rise to the *sh1-m5933* allele. The *sh1-m5933* allele carries very complex rearrangements: a 30 kb insertion in *sh1* intron 7, and a duplication of the 5' portion of the *sh1* gene and upstream flanking sequence; the length and orientation of the duplication are unknown. We cloned the endpoint of the duplication by PCR, and mapped the sequence to a Bac clone (b0439M06) located ~50 kb distal to *sh1*. These results indicate that the distal duplication in *sh1-m5933* is from ~50 kb to ~200 kb. The distal-to-*sh1* duplications are in inverted orientation relative to each other, and they are separated by a half *Ds* corresponding to a 5' end of *doubleDs*. These structural features strongly suggest that *sh1-m5933* originated from *Ds5245* by sister chromatid transposition of a *doubleDs* element; and that *Ds* in the Standard Position is likely to be a *doubleDs* element. A similar conclusion was reached by Martinez-Farez and Dooner (1997). To clone the original *Ds* in the Standard Position, we used ligation-mediated PCR to clone a *doubleDs* element from McClintock's stocks. This *Ds* has the same *doubleDs* structure as that in *sh1-m5933* and *sh1-m6233*. However, the flanking sequences map to Bac clone c0326K21, located ~900 kb proximal to the *wx1* locus. Based on these predicted and observed structures and locations, we conclude that this *doubleDs* element is very likely McClintock's original *Ds* in the Standard Position.

P217

MaizeLTR: A Pipeline Designed for the Discovery and Annotation of LTR Retrotransposons in Maize Genome Sequence Data

(submitted by Regina Baucom <gbaucom@uga.edu>)
Full Author List: Baucom, Regina[1]; Bennetzen, Jeffrey L.[1]
[1] University of Georgia; Athens, GA, USA 30602

LTR retrotransposons comprise at least 70% of the maize nuclear genome and are commonly arranged as complexes of nested elements in the spaces between genes. Most of this repetitive DNA is represented by a few retrotransposon families that have amplified to high copy number. However, less is known about the element families that are present in low copy number. We designed an automated pipeline using Perl programs, MaizeLTR, to efficiently identify and annotate any intact LTR retrotransposon in the maize genome. This automated process first downloads the publicly available BACs from the Maize Genome Sequencing Consortium. Previously described maize repetitive elements are then masked and removed from the sequence. A de novo search for new LTR retrotransposons is then performed using structural criteria. This process should uncover previously undescribed elements, as well as elements that are highly nested. The results of applying this process to 5,786 maize BACs will be presented.

P218

Mapping Maize Centromeres using Chromatin Immunoprecipitation-Transposon Display

(submitted by Jinghua Shi <jshi@plantbio.uga.edu>)
Full Author List: Shi, Jinghua[1]; Dawe, R. Kelly[2]
[1] University of Georgia; Department of Plant Biology; Athens, GA, 30602
[2] University of Georgia; Departments of Plant Biology and Genetics; Athens, GA, 30602

Centromeres are typically composed of rapidly evolving repetitive DNA sequences. This repetitive feature makes it difficult to find single-copy markers that segregate during meiosis for genetic mapping. Previous studies showed that maize centromeric retroelements (CRM) insertions generate useful polymorphisms among inbred lines. We have now developed a high-throughput transposon display method for mapping CRM elements. By combining CENH3 Chromatin Imunoprecipitation (ChIP) with CRM display, we have precisely mapped all ten maize centromeres.

P219

Sample Sequence Analysis Uncovers the Nature of Genome Size Variation in Four *Zea* Accessions, Including the Teosintes *Z. luxurians* and *Z. diploperennis*

(submitted by Matt Estep <estepmc@uga.edu>)
Full Author List: Estep, Matt C.[1]; Bennetzen, Jeffrey L.[2]
[1] Department of Plant Biology; University of Georgia; Athens, GA 30607
[2] Department of Genetics; University of Georgia; Athens, GA 30607

Genome size varies almost 2-fold among the diploid species of the genus Zea. We investigated this variation by limited shotgun genomic sequencing of two teosinte species (Z. diploperennis & Z. luxurians) and Z. mays (B73). Sequence analysis was performed with an all-vs-all BLAST and by comparison to known repeats in maize. This analysis revealed that differential amplification of retrotransposons families and the Knob tandem repeat are responsible for the majority of variation in genome size between Z. diploperennis, Z. luxurians and Z. mays. However, each lineage has very different content of these mobile DNAs. The two Z. luxurians accessions analyzed were most similar to each other, and contained higher levels of Ji and PREM-1 LTR retrotransposons than the other two species. In contrast, Huck LTR retrotransposons were more abundant in Z. mays and Z. luxurians than in Z. diploperennis, while Cinful, Gyma and Zeon were more abundant in the two teosinte species than in B73 maize. The most dramatic difference in repeat content was the much higher level (more than 20X) of tandem knob repeats in Z. luxurians than in maize. The results indicate that the approximately two-fold larger genome of Z. luxurians compared to the other Zea species studies in this project is due to the greater accumulation of a number of different repeats.

P220

The Maize Mre11/Rad50/Nbs1 DNA Repair and Recombination Complex

(submitted by Cagla Altun <caltun@purdue.edu>)
Full Author List: Altun, Cagla[1]; Weil, Clifford F.[2]
[1] Plant Biology Program, Purdue University; West Lafayette, IN, 47907
[2] Department of Agronomy, Purdue University; West Lafayette, IN, 47907

We are only beginning to understand how plants protect and repair DNA, and find there are important and informative differences between plant and animal systems. For example, null mutations in most repair genes are early embryo lethal in mammals while Arabidopsis nulls deficient in the same genes are viable, even though the genes are single-copy. Recent findings connecting environmental stress and DNA repair, make plants an extremely valuable system for studying DNA repair.

Expanding these studies to maize has revealed that, even among plants, there is informative diversity of responses to DNA damage. The highly conserved Mre11/Rad50/Nbs1 (MRN) DNA repair complex plays an important role in the processing of broken DNA ends and signaling to the cell that there has been damage. We have shown previously that maize is unusual in having two expressed Mre11 genes, Mre11A and Mre11B, which produce similar but distinct proteins. In addition, we have reported our discovery, cloning and characterization of the third, signaling component of the complex, the Nbs1 protein, in both maize and Arabidopsis, and its interaction with Mre11A but, interestingly, not with Mre11B. We have had antibodies raised against maize NBS1 and Mre11A and another antibody against a unique peptide sequence of Mre11B is underway. We are in the process of further verifying the interaction between NBS1 and Mre11A via pull-down assays and co-immunoprecipitation. We have a maize Nbs1 null allele that was identified by TILLING and also two Mre11B TUSC alleles. Homozygous maize Nbs1 nulls are viable and do not show any seedling phenotype. We are in the third backcross generation with the mutants and also looking into whether the mutants show any significant phenotype such as sterility in our winter nursery.

P221

Transposon-Induced Rearrangements in Natural and Transgenic Contexts

(submitted by Thomas Peterson <thomasp@iastate.edu>)
Full Author List: Peterson, Thomas[1]; Yu, Chuanhe[1]; Zhang, Jianbo[1]; Weber, David F.[2]
[1] Department of Genetics, Development and Cell Biology, Department of Agronomy, Iowa State University, Ames, Iowa, USA 50011
[2] Illinois State University, Normal, IL., USA, 61790-4120

Transposable elements are known to generate a variety of genome rearrangements, including deletions, direct and inverted duplications, and translocations. We have shown that in maize, rearrangements can occur by alternative transposition mechanisms that involve the termini of different copies of the Ac/Ds family. At the maize p1 locus, the presence of multiple copies of Ac termini leads to various rearrangements: Ac 3' and 5' termini in direct orientation can undergo Sister Chromatid Transposition, leading to the formation of flanking deletions and inverted duplications (Zhang and Peterson, 1999); while Ac termini in reversed orientation can undergo transposition reactions resulting in inversions, deletions and translocations (Zhang and Peterson, 2004; Zhang et al., in preparation). In each of these cases, the rearrangement breakpoints are bounded by the characteristic footprint or target site duplications typical of Ac transposition reactions. These results show how alternative transposition reactions could contribute significantly to genome evolution by generating chromosome rearrangements, and by creating new genes through shuffling of coding and regulatory sequences (Zhang, Zhang and Peterson, 2006). We are attempting to reproduce these alternative transposition pathways in transgenic plants. The system utilizes transgene constructs containing maize Ac termini in direct or reversed orientation. The action of Ac transposase on the Ac termini generates deletions, with one end anchored at the transgene locus and the other end at various flanking sites. The deletion-inducing segment is itself inserted within a second transposon (I/dSpm) for mobilization throughout the genome. The construct contains markers (maize c1 and p1 genes) for detection of both I/dSpm transposition and Ac-induced deletions. The current state of the project will be presented. To view an animation of the alternative transposition model, see http://jzhang.public.iastate.edu/Transposition.html.

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P222

Maize for Teaching Undergraduate Developmental Biology

(submitted by Dan Choffnes <dchoffnes@carthage.edu>)
Full Author List: Choffnes, Dan[1]
[1] Department of Biology; Carthage College; Kenosha, WI 53140

Undergraduate developmental biology curricula are becoming ever more challenging, as advances in cell biology, genomics, and the evolution of development, among others, find their way into textbooks. Within the course of a semester, students deal with classical fundamental concepts, such as syncytial development and chromatin remodeling, as well as more recent findings, such as small RNA biology and the genetic basis of morphological change. Maize can serve as an effective teaching tool for demonstrating numerous aspects of development related to plants and animals. Lessons using maize are interactive, macroscopic, and require less preparation time and expense than alternative demonstrations.

P223

Plant Genomics Research Experience for Teachers at the University of Missouri

(submitted by Susan Melia-Hancock <melia-hancocks@missouri.edu>)
Full Author List: Melia-Hancock, Susan[1]; Cone, Karen C.[2]; Davis, Georgia L.[1]
[1] Division of Plant Sciences, University of Missouri-Columbia, Columbia, Missouri 65211
[2] Division of Biological Sciences, University of Missouri-Columbia, Columbia, Missouri 65211

MU Plant Genomics Research Experience for Teachers (MUPGRET) completed its 3rd year of introducing high school and junior high science and agriculture teachers to the principles and techniques used in plant genomics laboratories at the University of Missouri. This year's teachers represented urban and rural school districts from 22 counties across the state. The 29 new teachers bring the total participants in this program to 71, potentially reaching 30,000 students over the next four years. Approximately 1700 classroom kits have been sent to participating teachers. Kits contain the same materials used in the laboratories the teachers performed themselves during the workshops. Many kits are re-useable and the lesson plans can be modified by the teachers to better fit into existing curricula. Participants in the MUPGRET program have expressed an appreciation for the hands on demonstrations that are possible when using classroom kits to teach genetic concepts. The corn seedling mutant kit tends to pique the interest of visual or tactile learners. It is often more difficult or time consuming for teachers to find or create laboratories aimed at this type of learner. Some students will request additional mutant seeds for science fair projects. The six two-day workshops are divided into three introductory workshops and three advanced workshops. The introductory workshops are offered every year to refresh basic knowledge and introduce "student friendly" laboratories into existing curricula. The week of advance workshops changes yearly and provides an opportunity for teachers to return and expand their plant genetics experiences. The "Chromosome Structure and Gene Expression" workshop presented by Karen Cone is an example of hot off the lab bench National Science Foundation (NSF) funded research channeled directly into classroom knowledge. Fifteen faculty members provided lectures, labs, and lab tours which are being made available to teachers and the community on the web at www.maizeinmissouri.org under the outreach section.

P224

Tribal College Outreach: Academic Partnerships and Educational Exchanges

(submitted by Mari Eggers <meggers@main.lbhc.cc.mt.us>)
Full Author List: Eggers, Mari[1]; Jackson, Dave[2]; Sylvester, Anne[3]
[1] Little Big Horn College, 1 Forest Lane, Crow Agency, MT 59022
[2] Cold Spring Harbor Laboratories, Cold Spring Harbor, NY 11724
[3] Department of Molecular Biology, University of Wyoming, Laramie WY 82071

Tribal Colleges (TC) serve Native American communities as education centers and are also central resources for broad community support. Frequently, the science curriculum at TCs focuses on organismal level and ecological learning important to the community. In an effort to enhance the molecular genetic curriculum, we are working together to design genetics workshops that exchange between tribal colleges and universities. Two workshops for students at Little Big Horn College (LBHC), Crow Agency MT have been conducted. The first workshop is held annually at LBHC and engages the students in learning about classical genetics through issues relevant to the Crow people. We focus on discussing horse coat color genetics, the genetics of human diseases such as diabetes, and current relevant issues such as transgenic crops. The second workshop is held annually at the University of Wyoming and initiates learning in the area of molecular genetics. We focus on learning about DNA and genes at the molecular level by using PCR for bacterial water quality testing. As part of these exchanges, we are working together to implement research opportunities both at the TC and the University for LBHC students and faculty. These two workshops allow for mutual learning between University faculty/students and Tribal College faculty/students. Several elements are essential for success. A critical component is to maintain ongoing interactions and continuous follow-up with the students and faculty at the TC. The concept of exchange is also critical for success. The partnership/exchange concept should be the foundation for all efforts at increasing diversity because it enhances cultural awareness as well as guides future teaching and learning plans. We will describe the details of our first round of workshops, what we learned and how we will enhance the program through bringing together other TC faculty and more PIs.

Author Index

Participant List

Participant	Address	Telephone	E-mail
Victor Abertondo	Iowa State University Dept of Agronomy Agronomy Hall 100 Osborn Dr Ames, IA 50010 USA	515-294-0498	vja@iastate.edu
Ivan Acosta	Yale University 165 Prospect St New Haven, CT 6520 USA	203-432-3894	ivan.acosta@yale.edu
Michael Adedotun	Agric-Link Multipurpose Cooperative Society Ltd PO Box 11611 Garki Federal Capital Abuja 234, Nigeria		
Kevin Ahern	Boyce Thompson Institute for Plant Research Cornell University Tower Rd Ithaca, NY 14853 USA	(607) 254-6747	ka38@cornell.edu
Marc Albertsen	Pioneer HiBred Intl. Inc. 7250 NW 62nd Avenue PO Box 552 Johnston, IA 501310552 USA	(515) 270-3648	Marc.Albertsen@pioneer.com
Jim Allen	University of Missouri 314 Tucker Hall Columbia, MO 65211 USA	573-884-2496	allenjo@missouri.edu
Cagla Altun	Purdue University 915 W State St West Lafayette, IN 47907 USA	(765) 496-3206	caltun@purdue.edu
Ina Amarillo	Florida State University Bio Unit I Chieftan Way Tallahassee, FL 32306 USA	(850) 644-8058	feamarillo@biol.fsu.edu
Jeppe Andersen	University of Aarhus Research Centre Flakkebjerg Forsoegsvej 1 Slagelse DK4200, Denmark	45 8999 3545	jepper.andersen@agrsci.dk
Richard Anderson	University of Toledo Biological Science Dept Mailstop 601 2801 W Bancroft St Toledo, OH 43606 USA	419-530-1538	randers4@utnet.utoledo.edu
John Arbuckle	Syngenta 317 330th St Stanton, MN 55018 USA	(507) 663-7699	john.arbuckle@syngenta.com
Ann Armenia	Michigan State University A247 Plant & Soil Science Bldg East Lansing, MI 48824 USA	517-231-7627	armeniaa@msu.edu
Charles Armstrong	Monsanto 700 Chesterfield Parkway West Chesterfield, MO 63017 USA	(636) 737-7229	clarms@monsanto.com
Kelsey Aurand	Truman State University Magruder Hall 3064 Kirksville, MO 63501 USA	660-785-0500	kma235@truman.edu

Ling Bai	Cornell University Boyce Thompson Institute 1 Tower Rd Ithaca, NY 14850 USA	(607) 254-6747	lb226@cornell.edu
Peter BalintKurti	USDAARS NC State 3418 Gardner Hall Dept. of Plant Pathology Raleigh, NC 276957616 USA	919 515 3516	peter_balintkurti@ncsu.edu
Solmaz Barazesh	Pennsylvania State University 208 Mueller Lab University Park, PA 16802 USA	(814) 863-4022	sxb944@psu.edu
Kelli Barr	University of Missouri-Columbia Division of Plant Sciences 1-31 Agriculture Building Columbia, MO 65211 USA	(573) 882-9228	klbxr6@mizzou.edu
Hank Bass	Florida State University Biology Unit I Dept. Biological Science Florida State University Tallahassee, FL 32306 USA	850 6449711	bass@bio.fsu.edu
Sutirtha Basu	Pioneer Hibred International Inc. 7200 NW 62nd Avenue Johsnton, IA 50131 USA	(515) 334-3543	sutirtha.basu@Pioneer.com
Regina Baucom	University of Georgia Genetics Department Athens, GA 30602 USA	706-542-9729	gbaucom@uga.edu
Philip Becraft	Iowa State University 2116 Molec Biol Bldg GDCB Dept. Ames, IA 50011 USA	515 2942903	becraft@iastate.edu
Stephanie Bellendir	University of Illinois - Urbana-Champaign NSRC 325 1101 W Peabody Dr Urbana, IL 61801 USA	217-265-6988	bellendi@uiuc.edu
Robert Bensen	Monsanto 62 Maritime Drive Mystic, CT 6355 USA	(860) 572-5277	robert.bensen@monsanto.com
James Birchler	University of Missouri 117 Tucker Hall Columbia, MO 65211 USA	(573) 882-4905	BirchlerJ@Missouri.edu
Anastasia Bodnar	Iowa State University Agronomy Hall G426 Ames, IA 50011 USA	515-294-1766	abodnar@iastate.edu
Martin Bohn	University of Illinois 302 W Florida Ave Urbana, IL 61801 USA	217 244 2536	mbohn@uiuc.edu
Courtney Bonney	Truman State University Division of Science 100 E Normal Kirksville, MO 63501 USA	660-785-4280	ceb005@truman.edu

Esteban Bortiri	USDAARS Plant Gene Expression Center PGEC 800 Buchanan Ave Albany, CA 94710 USA	510 5595919	ebortiri@berkeley.edu
Rebecca Boston	North Carolina State University 2614 Wells Ave Raleigh, NC 27608 USA	(919) 515-3390	boston@unity.ncsu.edu
David Bowen	Pioneer HiBred Intl 7200 NW 62nd Avenue Johnston, IA 50131 USA	(515) 270-3179	david.bowen@pioneer.com
Christopher Bozza	Cornell University Dept of Plant Biology 405 Bradfield Hall Ithaca, NY 14853 USA	(201) 919-3876	cgb25@cornell.edu
David Braun	Pennsylvania State University 208 Mueller Lab University Park, PA 16802 USA	814 8631108	dbraun@psu.edu
Peter Bretting	USDA/ARS National Program Staff Mailstop 5139, 5601 Sunnyside Ave Rm 2212 GWCC Beltsville, MD 20705 USA	(301) 504-5541	peter.bretting@ars.usda.gov
Patrick Brown	Cornell University 157 Biotechnology Bldg Ithaca, NY 14850 USA	(607) 254-4849	pjb34@cornell.edu
Katy Browning	Truman State University 416 W Dodson Apt 3 Kirksville, MO 63501 USA	(573) 268-2883	kate.browning@gmail.com
Wes Bruce	BASF Plant Science 26 Davis Dr Research Triangle Park, NC 27709 USA	(919) 547-2417	wes.bruce@basf.com
Thomas Brutnell	Boyce Thompson Institute Tower Road Ithaca, NY 14853 USA	(607) 254-8656	tpb8@cornell.edu
Edward Buckler	Cornell University 159 Biotechnology Ithaca, NY 14853 USA	(607) 255-3911	law14@cornell.edu
Brent Buckner	Truman State University 100 E Normal Street Kirksville, MO 63501 USA	660-785-4083	djb@truman.edu
Paul Bullock	Syngenta 2369 330th St Slater, IA 50244 USA	(515) 685-5116	paulbullock@syngenta.com
Brunilis Burgos	University of Georgia Dept of Genetics 4607 Plant Sciences Athens, GA 30607 USA	706-542-1010	brunilis@uga.edu
Andrew Burt	University of Guelph Dept of Plant Agriculture Crop Science Building Guelph, Ontario N1G 2W1, Canada	519-824-4120x58194	aburt@uguelph.ca

Dana Bush	University of Missouri - Columbia Div of Plant Sciences 1-31 Agriculture Bldg Columbia, MO 65211 USA	573-882-9228	dlw3f9@mizzou.edu
Marymar Butruille	Pioneer HiBred a DuPont Company 810 Sugar Grove Ave. Dallas Center, IA 50063 USA	515 334 4640	Marymar.Butruille@pioneer.com
Candida Cabral	University of Minnesota 411 Borlaug Hall 1991 Upper Buford Circle Saint Paul, MN 55108 USA	(612) 625-6223	cabr0024@umn.edu
Martin Calvino	Rutgers University Waksman Institute 190 Frelinghuysen Rd Piscataway, NJ 8854 USA	732-445-3801	mcalvino@eden.rutgers.edu
Darwin Campbell	USDAARS Iowa State University 526 Science II Ames, IA 50011 USA	(515) 294-4294	darwin@iastate.edu
Mark Campbell	Truman State University Division of Science 100 E Normal Kirksville, MO 63501 USA	660-785-4280	campbell@truman.edu
Matthew Campbell	The Institute for Genomic Research 9712 Medical Center Drive Rockville, MD 20850 USA	301 7957908	campbell@tigr.org
William Cande	Dept. Molecular ad Cell Biology University of Cal 1329 Walnut St. Berkeley, CA 947091408 USA	(51) 042-1669	Zcande@berkeley.edu
Hector Candela	PGECBerkeley 800 Buchanan Street Albany, CA 94710 USA	510 5595922	hcandela@nature.berkeley.edu
Jun Cao	Iowa State University 2049 Carver Co-Laboratory Ames, IA 50010 USA	(515) 294-1659	juncao@iastate.edu
Mario Carlone	Pioneer HiBred 13874 Lovers Lane Princeton, IL 61356 USA	(815) 875-6523	Mario.Carlone@pioneer.com
Nicholas Carpita	Purdue University 82 Limberlost Lane West Lafayette, IN 47906 USA	(765) 494-4653	carpita@purdue.edu
Nicola Carraro	University of Bologna University of Bologna DISTA viale Fanin 44 Bologna 40127, CA Italy	39 0512096648	nicola@sidoine.net
Kirstin Carroll	Oregon State University 2082 Cordley Hall Corvallis, OR 97331 USA	541-737-2322	Kirstin.carroll@oregonstate.edu
Terry Casstevens	Cornell University 402 Cameron Glen Dr. Apex, NC 27502 USA	(919) 303-6262	tmc46@cornell.edu

Andrea Chambers	University of Guelph Dept Plant Agriculture Guelph Ontario N1G 2WI, Canada		aarmst03@uuguelph.ca"
Michael Chandler	University of Wisconsin - Madison 1575 Linden Dr Madison, WI 53706 USA	(217) 840-7567	machandler@wisc.edu
Sadia Chaudhry	Oakland University 2711 Glouchester Rochester, MI 48309 USA	248-464-0183	sr2chaud@oakland.edu
Dahu Chen	DNA LandMarks 84 Richelieu StJeansurRichelieu J3B 6X3, Canada	(450) 358-2621	chend@dnalandmarks.ca
Jialiang Chen	Agreliant Genetics 4640 E State Road 32 Lebanon, IN 46052 USA	(765) 482-9833	jialiang.chen@agreliantgenetics.com
Tianle Chen	University of Georgia 2502 Miller Plant Sciences Building Athens, GA 30602 USA	(706) 542-1857	tchen@plantbio.uga.edu
Satya Chintamanani	Purdue University Lilly Hall of Life Sciences 915 West State Street West Lafayette, IN 47907 USA	(765) 494-9880	satya@purdue.edu
Eunyoung Cho	University of Georgia Plant Biology 2502 Miller Plant Science Building Athens, GA 30602 USA	(706) 542-1857	eycho@uga.edu
Eunsoo Choe	University of Illinois at Urbana-Champaign S122 Turner Hall 1102 S Goodwin Ave Urbana, IL 61801 USA	(217) 244-3388	echoe1@uiuc.edu
Dan Choffnes	Carthage College 2001 Alford Park Dr Kenosha, WI 53140 USA	(262) 551-2374	dchoffnes@carthage.edu
Paul Chomet	Monsanto Co. 62 Maritime Drive Mystic, CT 6355 USA	(860) 572-5224	Paul.Chomet@Monsanto.com
Surinder Chopra	Penn State University Dept of Crop and Soil Sciences 252 Ag Sci Ind Building University Park, PA 16803 USA	(814) 865-1159	SIC3@PSU.EDU
Prem Chourey	USDA ARS University of Florida Plant Pathology Department 1453 Fifield Hall Gainesville, FL 32611 USA	(352) 392-3631 345	pschourey@ifas.ufl.edu
George Chuck	Plant Gene Expression Center U.C. Berkeley 800 Buchanan St. Albany, CA 94710 USA	(510) 559-5922	gchuck@nature.berkeley.edu
Sivanandan Chudalayandi	University of Missouri 117 Tucker Hall Columbia, MO 65211 USA	(573) 882-4871	chudals@missouri.edu

Chia-Lin Chung	Cornell University 303G Plant Science Building Ithaca, NY 14853 USA	607-255-4783	cc435@cornell.edu
Jeffrey Church	University of Illinois Urbana-Champaign 1201 W Gregory Dr 389 ERML Urbana, IL 61801 USA	(217) 244-6146	jbchurch@uiuc.edu
Tom Clemente	University of Nebraska Center for Biotechnology N308 Beadle Center Lincoln, NE 685880665 USA	(402) 472-1428	tclemente1@unl.edu
Sandra Clifton	Washington University School of Medicine 4444 Forest Park Blvd Campus Box 8501 Saint Louis, MO 63108 USA	(314) 286-1800	sclifton@watson.wustl.edu
Suzy Cocciolone	BASF Plant Science 26 Davis Dr Research Triangle Park, NC 27709 USA	(919) 547-2793	suzy.cocciolone@basf.com
Ed Coe	UNIV OF MISSOURI 206 Heather Ln Columbia, MO 65203 USA	(573) 445-4172	coee@missouri.edu
Lisa Coffey	ISU Schnable Lab 2043 Roy J. Carver CoLab Apt. 1 Ames, IA 50011 USA	(515) 294-1659	lmcoffey@iastate.edu
Travis Coleman	University of Guelph Dept of Plant Ag Crop Science Bldg Guelph Ontario N1G 2W1, Canada		tcoleman@uoguelph.ca
Kristi Collura	University of Arizona Arizona Genomics Institute 303 Forbes Hall, 1140 E South Campus Dr Tucson, AZ 85721 USA	520-626-9594	kcollura@ag.arizona.edu
Eliette Combes	Limagrain Verneuil Holding Zac les Portes de Riom BP 173 Riom 63204, France	(3) 347-3671 750	sylvie.guillaume@limagrain.com
Karen Cone	University of MissouriColumbia 101 Tucker Hall Columbia, MO 65211 USA	(573) 882-2118	conek@missouri.edu
Liza Conrad	Cornell University 133 Boyce Thompson Institute 1 Tower Rd Ithaca, NY 14853 USA	(607) 254-6747	ljc28@cornell.edu
Gabriella Consonni	Universita Degli Studi Di Milano Via Celoria 2 Milan 20123, Italy	(5) 031-6524	gabriella.consonni@unimi.it
James Coors	Department of Agronomy University of Wisconsin 1218 Sweet Briar Rd. Madison, WI 53705 USA	(608) 262-7959	jgcoors@wisc.edu

Liliana Costa	University of Oxford Dept Plant Science South Parks Rd Oxford OX1 3RB, UK		Liliana.costa@plants.ox.ac.uk
Antonio Costa de Oliveira	University of Georgia Fred Davison Life Sciences Meeting Department of Genetics Athens, GA 30602 USA	(706) 542-9729	acostol@uga.edu
Denise Costich	Cornell University 175 Biotechnology Cornell University Ithaca, NY 14853 USA	(607) 255-3911	dc58@cornell.edu
Laura Courtney	Washington University School of Medicine 4444 Forest Park Ave Campus Box 8501 Saint Louis, MO 63108 USA	314-286-1800	lcourtne@watson.wustl.edu
William Courtney	Washington University School of Medicine 4444 Forest Park Ave Campus Box 8501 Saint Louis, MO 63108 USA	314-286-1800	wcourtne@watson.wustl.edu
Sarah Covshoff	Cornell University The Boyce Thompson Institute for Plant Research 1 Tower Rd Ithaca, NY 14853 USA	(607) 254-6747	sc349@cornell.edu
James Crowley	Monsanto Company 700 Chesterfield Pkwy West Chesterfield, MO 63017 USA	(636) 737-6302	jhcrow@monsanto.com
Jennifer Currie	University of Arizona 1140 E South Campus Dr 303 Forbes Hall Tucson, AZ 85721 USA	520-626-9594	jcurrie@ag.arizona.edu
Tatiana Danilova	University of Missouri Columbia 1609 Windsor Apt 3 Columbia, MO 65201 USA	(573) 882-4871	danilovat@missouri.edu
Teresa Davidson	Washington University School of Medicine 4444 Forest Park Ave Campus Box 8501 Saint Louis, MO 63108 USA	314-286-1800	tdavidso@watson.wustl.edu
Georgia Davis	University of Missouri - Columbia Division of Plant Sciences 1-31 Agriculture Building Columbia, MO 65211 USA	(573) 882-9224	davisge@missouri.edu
Kelly Dawe	University of Georgia 712 Cobb Street Athens, GA 30606 USA	706 2088051	kelly@plantbio.uga.edu
Natalia de Leon	University of Wisconsin Madison Moore Hall room 455 1575 Linden Dr Madison, WI 53706 USA	(608) 262-0193	ndeleongatti@wisc.edu
Sylvia de Sousa	University of Florida 2220 SW 34th st apt 46 Gainesville, FL 32608 USA	352 3286193	smsousa@ufl.edu

Jeremy DeBarry	University of Georgia Fred C Davison Life Sciences Dept of Genetics Athens, GA 30602 USA	706-542-9729	jdebarry@uga.edu
Prasit Deewatthanawong	Boyce Thompson Institute for Plant Research Cornell University Tower Rd Ithaca, NY 14853 USA	607-254-6747	pd72@cornell.edu
Joerg Degenhardt	Max Planck Institute for Chemical Ecology Hans Knoll Strasse 8 Jena D-07745, Germany	(493) 641-6436 52	degenhardt@ice.mpg.de
Diana Dembinsky	University of Tuebingen Auf Der Morgenstelle 28 Tuebingen 72076, Germany		diana.dembinsky@zmbp.uni-tuebingen.de
Braham Dhillon	Purdue University Dept of Botany & Plant Path, Lily Hall of Life Sci 915 W State St West Lafayette, IN 47907 USA	765-494-9880	bdhillon@purdue.edu
Thanda Dhliwayo	Iowa State University 100 Osborn Drive Dept of Agronomy Ames, IA 50011 USA	(515) 294-3517	tdhliwa@iastate.edu
Ines Dias de Carvalho	University of Illinois Urbana-Champaign 1102 S Goodwin Ave Urbana, IL 61801 USA	217-244-3388	ines@uiuc.edu
Hugo Dooner	Rutgers University Waksman Institute 190 Frelinghuysen Rd Piscataway, NJ 8855 USA	(732) 445-4684	dooner@waksman.rutgers.edu
Jane Dorweiler	Marquette University P.O. Box 1881 Milwaukee, WI 532011881 USA	(414) 288-5120	jane.dorweiler@marquette.edu
Ryan Douglas	Cornell University 228 Plant Science Ithaca, NY 14853 USA	(607) 254-1160	rnd4@cornell.edu
Limei Du	Wakesman Institute Rutergers University 190 Frelinghuysen Rd Piscataway, NJ 8854 USA	732-445-6247	limei@wakesman.rutergers.edu
Yaqing Du	University of Georgia 2502 Miller Plant Science Building Athens, GA 30602 USA	706-542-1010	yadu@plantbio.uga.edu
Patrice Dubois	Cornell University Boyce Thompson Institute 1 Tower Road Ithaca, NY 14853 USA	607-229-0486	pgd7@cornell.edu
Philippe DUFOUR	Limagrain Verneuil Holding ZAC LES PORTES DE RIOM BP 173 RIOM 63204, France	(3) 347-3671 754	sylvie.guillaume@limagrain.com

Badicean Dumitru	Institute of Genetics and Plant Physilogy ASM Padurii 20str Chisinau MD2002, Moldova		dbadicean@yahoo.com
Jon Duvick	Iowa State University 1707 38th Street Des Moines, IA 50310 USA	515 4805505	jduvick@iastate.edu
Jode Edwards	USDA ARS Department of Agronomy Iowa State University Ames, IA 500111010 USA	(515) 294-7607	jode@iastate.edu
Bill Eggleston	Virginia Commonwealth University 1000 W. Cary Richmond, VA 23284 USA	(804) 828-0503	weggles@vcu.edu
Robert Elshire	Cornell University 2665 Slaterville Road Slaterville Springs, NY 14881 USA	(607) 254-1160	rje22@cornell.edu
Scott Emrich	Iowa State University 3101 Coover Hall Ames, IA 50011 USA	(515) 451-9216	semrich@iastate.edu
Elhan Ersoz	Cornell University 175 Biotechnology Cornell University Ithaca, NY 14853 USA	(607) 255-3911	ee57@cornell.edu
Elisabeth Esch	Leibniz University of Hannover Institute of Plant Genetics Leibniz University Hannover Hannover 30419, Germany	49 511 762 3603	esch@genetik.uni-hannover.de
Matt Estep	University of Georgia Life Sciences Building Athens, GA 30602 USA	(706) 542-9729	estepmc@uga.edu
James Estill	University of Georgia Davison Life Sciences Dept of Genetics Athens, GA 30605 USA	706-542-9729	jestill@plantbio.uga.edu
Matthew Evans	Carnegie Institution of Washington Department of Plant 260 Panama St Stanford, CA 94305 USA	(650) 325-1521	mmsevans@stanford.edu
Andrea Eveland	University of Florida 1301 Fifield Hall Gainesville, FL 32611 USA	352-392-7911 x311	aeveland@ufl.edu
Diego Fajardo	University of Florida 1301 Fifield Hall Gainesville, FL 32611 USA	(352) 392-7574	diegof@ufl.edu
Jacqueline Farrell	Iowa State University 2282 Molecular Biology Building Ames, IA 50010 USA	515-294-0137	jdjax@iastate.edu
Maria Federico	University of Wisconsin-Madison Department of Agronomy 1575 Linden Dr Madison, WI 53706 USA	(608) 262-6521	mlfederico@wisc.edu

Antje Feller	The Ohio State University 1060 Carmack Rd Rm 218 Columbus, OH 43210 USA	614-688-4954	feller.ll@osu.edu
John Fernandes	Stanford University 385 Serra Mall Herrin Labs Walbot Stanford, CA 94305 USA	(650) 533-9376	jfernand@stanford.edu
Paulo Ferreira	Cold Spring Harbor Lab 1 Bungtown Rd Delbruck Cold Spring Harbor, NY 11724 USA	516 3678836	ferreira@cshl.org
Debbie Figueroa	Florida State University Biology Unit 1 Chieftan Way Tallahassee, FL 32306 USA	850-644-8058	figueroa@bio.fsu.edu
Pascal Flament	Limagrain Verneuil Holding Zac Les Portes de Riom BP 173 Riom 63204, France	(3) 347-3671 750	sylvie.guillaume@limagrain.com
Christina Fliege	University of Illinois - Urbana - Champaign NSRC 325 1101 Peabody Dr Urbana, IL 61801 USA	217-836-4199	cfliege2@uiuc.edu
Sherry Flint-Garcia	USDA ARS PGRU 205 Curtis Hall University of Missouri Columbia, MO 65211 USA	(573) 884-0116	flint-garcias@missouri.edu
Clark Ford	Iowa State University 2312 Food Science Building Ames, IA 50011 USA	515 2940343	cfford@iastate.edu
Cristian Forestan	University of Padova Dept of Envionmental Agronomy & Crop Production Viale dell'Universita 16 35020, Legnaro PD, Italy		cristian.forestan@unipd.it
David Foster	Syngenta Seeds 2369 330th St. Slater, IA 50244 USA	800 8316630	david.foster@syngenta.com
Michael Freeling	University of California - Berkeley 111 Koshland Hall Berkeley, CA 94720 USA	(510) 525-5276	freeling@nature.berkeley.edu
Monika Frey	Tu Muenchen LS Genetik Am Hochanger 8 Freising 85350 Bavaria, Germany		
Travis Frey	Monsanto 112 N. 2nd Ave. Huxley, IA 50124 USA	(515) 597-5839	travis.james.frey@monsanto.com
Ashleigh Fritz	Truman State University 416 W Dodson #3 Kirksville, MO 63501 USA	(816) 550-4344	aef138@truman.edu
Catrina Fronick	Washingotn University School of Medicine 4444 Forest Park Ave. Campus Box 8501 Saint Louis, MO 63108 USA	314-286-1800	cstrowman@watson.wustl.edu

Yan Fu	Donald Danforth Plant Science Center 975 N. Warson Rd St. Louis, MO 63132 USA	(314) 587-1495	yfu@danforthcenter.org
Scott Furbeck	Syngenta Seeds 906 Barbara Drive Heyworth, IL 61745 USA	(309) 473-2306	scott.furbeck@syngenta.com
Susan Gabay-Laughnan	University of Illinois 265 Morrill Hall Plant Biology 505 S. Goodwin Ave. Urbana, IL 61801 USA	217-333-2919	gabaylau@life.uiuc.edu
Antoine Gaillard	Maisadour Semences BP 27, Route de Saint Sever Haut Mauco 4000 Mont de Marsan, France	(335) 580-5845 4	gaillard@maisadour.com
Andrea Gallavotti	Cold Spring Harbor Laboratory 1 Bungtown rd Cold Spring Harbor, NY 11724 USA	(619) 892-4770	gallavot@cshl.edu
Martin Ganal	Traitgenetics GmbH Am Schwabeplan 1b Gatersleben D-06466, Germany		ganal@traitgenetics.de
Zhi Gao	University of Missouri 3811 Lyman Drive Columbia, MO 652035397 USA	(573) 882-4871	gaoz@missouri.edu
Zhifang Gao	DowAgroSciences LLC DowAgroSciences LLC Indianapolis, IN 46268 USA	(317) 337-3880	zgao@dow.com
Jack Gardiner	University of Arizona Dept of Pant Sciences 303 Forbes Building University of Arizona Tucson, AZ 85721 USA	(520) 621-8831	gardiner@ag.arizona.edu
Giuseppe Gavazzi	Universita Degli Studi Di Milano Via Celoria 2 Milan 20123, Italy		gabriella.consonni@unimi.it
Hartwig Geiger	University of Hohenheim Institute 350 D-70593 Stuttgart, Germany		
Nikolaos Georgelis	University of Florida 2209 Fifield Hall Gainesville, FL 32611 USA	3523921928 x 314	gnick@ufl.edu
Nathalie Geraldes	Syngenta Seeds Syngenta Seeds S.A.S. 12 chemin de l hobit BP 27 Saint Sauveur F31790 Saint Sauveur, France	(335) 627-9980 0	nathalie.geraldes@syngenta.com
Michael Gerau	University of Missouri - Columbia Division of Plant Sciences 1-31 Agriculture Building Columbia, MO 65211 USA	(573) 882-9228	mjgf36@mizzou.edu
Jeff Glaubitz	UW Genetics Panzea NSF project Genetics 425G Henry Malll Madison, WI 537061580 USA	608 2655804	glaubitz@wisc.edu

Jose Luis Godinez-Martinez	Cinvestav Campus Guanajuato Kim 9.6 Libramiento Norte Carretera Irapuato-Leon Apartado Postal 629 Irapuato Guanajuato 36500, Mexico		jgodinez@ira.cinvestav.mx
Shailendra Goel	IRD France 949 Rue Prof. Louis Rava Bat M1 Montpellier 34080, France	(334) 674-1625 0	goel@mpl.ird.fr
Inna Golubovskaya	University of California-Berkeley 1616 Berkeley Way Apt. D Berkeley, CA 947031275 USA	(510) 643-8277	innagol@berkeley.edu
Michael Gore	Cornell University Institute for Genomic Diversity 175 Biotechnology Building Ithaca, NY 14853 USA	(607) 255-1809	mag87@cornell.edu
John Gray	University of Toledo Biological Sci Dept Mailstop 601 2801 West Bancroft Street Toledo, OH 43606 USA	419 5301537	jgray5@uoft02.utoledo.edu
Steve Grier	Syngenta Seeds Inc. 217 Park Ln Apple Valley, MN 55124 USA	(507) 663-7662	steve.grier@syngenta.com
Daniel Grimanelli	IRD 911 Av Agropolis Montpellier 34394, France	(334) 674-1637 6	daniel.grimanelli@mpl.ird.fr
Erich Grotewold	Ohio State University 206 Rightmire Hall 1060 Carmack Road Columbus, OH 43210 USA	614/292-2483	grotewold.1@osu.edu
Edward Grow	University of Missouri 118 Crestmere Ave Columbia, MO 65201 USA	573-465-5679	ejgx93@mizzou.edu
Baozhu Guo	USDAARS 2747 Davis Road P.O.Box 748 Tifton, GA 317930748 USA	(229) 387-2334	bguo@tifton.usda.gov
Mei Guo	Pioneer HiBred Intl. Inc. 7300 NW 62nd Avenue P.O. Box 1004 Johnston, IA 501311004 USA	(515) 253-2146	Mei.Guo@pioneer.com
Jose Gutierrez-Marcos	University of Warwick Warwick - HRI Wellsbourne Warwick CV35 9EF, UK		J.F.Gutlerrez-Marcos@warwick.ac.uk
Frank Guzman Escudero	Graduate Research Fellowship-Intl Potato Ctr Mz L1 Lote 14 Coop Primavera Comas Lima 07, Peru		
Sarah Hake	UC Berkeley Plant Gene Expression Center Plant Gene Expression Center 800 Buchanan Street Albany, CA 94710 USA	(510) 559-5907	maizesh@nature.berkeley.edu

Christopher Hale	University of California - Berkeley Hollick Lab 111 Koshland Hall Berkeley, CA 94720 USA	(510) 643-1737	hale@berkeley.edu
Brad Hall	Iowa State University Iowa State University 2282 Molecular Biology Building Ames, IA 50011 USA	(515) 294-0137	bdhall@iastate.edu
Kasey Hames	University of Missouri - Columbia Division of Plant Sciences 1-31 Agriculture Building Columbia, MO 65211 USA	573-882-9228	kahcg2@mizzou.edu
Fangpu Han	University of Missouri 3811 Lyman Drive Columbia, MO 652035397 USA	(573) 882-4781	hanf@missouri.edu
Yujun Han	University of Georgia 4505 Miller Plant Sciences Athens, GA 30605 USA	(706) 389-6483	yhan@plantbio.uga.edu
Charles Hancock	University of Georgia 4505 Miller Plant Science Bldg Plant Biology Athens, GA 30602 USA	(706) 542-1857	cnhancock@plantbio.uga.edu
Lisa Haney	Iowa State University G426 Agronomy Hall Ames, IA 50011 USA	(515) 294-1766	lhaney@iastate.edu
L. Curtis Hannah	University of Florida 4400 NW 39th Avenue Apartment 434 Gainesville, FL 32606 USA	(352) 392-1928	Hannah@mail.ifas.ufl.edu
Candice Hansey	University of Wisconsin - Madison 1575 Linden Dr Madison, WI 53726 USA	(608) 469-0034	cnhansey@wisc.edu
Sarah Hargreaves	Iowa State University 2031 Roy J Carver Co-Lab Ames, IA 50014 USA	575-294-1659	sharg@iastate.edu
Frank Harmon	USDAARSPlant Gene Expression Center 1137 Cornell Avenue Albany, CA 94706 USA	(510) 559-5939	fharmon@nature.berkeley.edu
Carla Harper	Purdue University Agronomy Dept 915 W State St West Lafayette, IN 47907 USA	(765) 496-1917	charper@purdue.edu
Lisa Harper	USDAARS USDAARSPGEC 800 Buchanan Street Albany, CA 94710 USA	(510) 559-6111	ligule@nature.berkeley.edu
Ann Harris	Syngenta Seeds Inc 139 Finch St. Ames, IA 50010 USA	(515) 685-5145	ann.harris@syngenta.com
Megan Harvey	Iowa State University 100 Osborn Dr G418 Agronomy Hall Ames, IA 50011 USA	515-294-1766	meganh@iastate.edu

Marie Hasenstein	Iowa State University Agronomy Hall 100 Osborn Dr Ames, IA 50011 USA	515-294-1766	mahaas@iastate.edu
Andrew Hauck	University of Illinois Urbana-Champaign S-122 Turner Hall MC046 1102 S Goodwin Ave Urbana, IL 61820 USA	(540) 842-0668	ahauck@uiuc.edu
William Haun	University of Minnesota 250 Biosciences Center 1445 Gortner Ave St Paul, MN 55108 USA	(612) 624-6163	haunx003@umn.edu
James Hawk	University of Delaware 818 Hilltop Road Newark, DE 19711 USA	(302) 831-1379	jhawk@udel.edu
Ruifeng He	University of Arizona Arizona Genomics Institute Department of Plant Sciences Forbes 303 Tucson, AZ 85721 USA	520-626-9596	rthe@ag.arizona.edu
Michael Held	Purdue University Lily Hall 915 W State St West Lafayette, IN 47907 USA	(765) 494-4647	maheld@purdue.edu
Tracie Hennen-Bierwagen	Iowa State University 2182 Mol. Bio. Bldg Ames, IA 50011 USA	(515) 294-8208	tabier@iastate.edu
Evelyn Hiatt	Kentucky Wesleyan College 3000 Frederica Street Owensboro, KY 42301 USA	(270) 852-3158	ehiatt@kwc.edu
Frank Hochholdinger	University of Tuebingen Auf der Morgenstelle 28 Tuebingen 72074, Germany	(4) 970-7129 77024	hochhold@uni-tuebingen.de
Owen Hoekenga	USDAARS 230 Federal Nutrition Lab Cornell University Ithaca, NY 14853 USA	(607) 255-4502	owen.hoekenga@ars.usda.gov
David Holding	University of Arizona 6924 N Galaxy Place Tucson, AZ 85741 USA	520 621 9154	dholding@ag.arizona.edu
Jay Hollick	University of California - Berkeley Dept of Plant and Microbial Biology 111 Koshland Hall Berkeley, CA 94720 USA	510 643 1734	hollick@nature.berkeley.edu
Dawn Holligan-Nagel	University of Georgia Plant Biology 2502 Miller Plant Sciences Bldg Athens, GA 30602 USA	(706) 542-1857	dawn@plantbio.uga.edu
Eneda Hoxha	Truman State University 100 E Normal KB120 Kirksville, MO 63501 USA	(580) 458-1638	enedahoxha@gmail.com
AnPing Hsia	Iowa State University 706 Woodfield Way Rochester Hills, MI 48307 USA	(248) 844-0002	hsia@iastate.edu

Jun Huang	Waksman Institute Rutgers University 190 Frelinghuysen Rd Piscataway, NJ 8854 USA	(732) 445-2307	junhuang@waksman.rutgers.edu
Mingshu Huang	Pennsylvania State University Dept of Biology 616 Mueller Bldg State College, PA 16802 USA	(814) 863-5491	muh147@psu.edu
Matthew Hudson	University of Illinois 334 NSRC 1101 W Peabody Urbana, IL 61801 USA	217 244 8096	mhudson@uiuc.edu
Gregorio Hueros	Universidad de Alcala Dpto. Biologia Celular y Genetica Campus Universitario Alcala de Henares 28871, Spain	(349) 188-3921 9	gregorio.hueros@uah.es
Tyler David Huffman	University of Missouri - Columbia Division of Plant Sciences 1-21 Agriculture Building Columbia, MO 65211 USA	573-882-9228	thg7f@mizzou.edu
Alice Hui	University of Missouri-Columbia 324 Tucker Hall Columbia, MO 65211 USA	(573) 882-8033	ayhkq8@mizzou.edu
Judd Hultquist	Marquette University Dept of Biological Sciences PO Box 1881 Milwaukee, WI 53201 USA	(414) 702-7232	judd.hultquist@mu.edu
Charles Hunter	University of Florida 1301 Fifeld Hall Gainesville, FL 32611 USA	352-373-4687	ibe@ufl.edu
Tanveer Hussain	Pioneer Hi-Bred International 810 Sugar Grove Ave Dallas Center, IA 50063 USA	515-334-4469	Tanveer.Hussain@Pioneer.com
Laurie Hyrkas	Iowa State University 1301 Agronomy Hall Ames, IA 50011 USA	515-294-9369	
Farag Ibraheem	Pennsylvania State University 116 ASI Building Crop and Soil Sciences State College, PA 16802 USA	(814) 863-6172	fii100@psu.edu
Erin Irish	The University of Iowa 314 Hutchinson Ave. Iowa City, IA 52246 USA	(319) 335-2582	erin-irish@uiowa.edu
Dave Jackson	Cold Spring Harbor Lab 1 Bungtown Rd Cold Spring Harbor, NY 11724 USA	516 367 8467	jacksond@cshl.edu
Jennifer Jacobs	Monsanto 800 North Lindbergh Blvd Mail Zone Q4E St. Louis, MO 63167 USA	(314) 694-8138	Jennifer.L.Jacobs@monsanto.com
Natalie Jameson	Oakland University 3009 Helen Ct Royal Oak, MI 48073 USA	(248) 435-9111	nataliejameson@gmail.com

Diane Janick-Buckner	Truman State University 100 E Normal Street Kirksville, MO 63501 USA	660-785-4305	djb@truman.edu
Yi Jia	Iowa State University 2043 Roy J Carver Co-Laboratory Ames, IA 50010 USA	(515) 294-1659	jiayi@iastate.edu
Gurmuth Johal	Purdue University Dept of Botany 915 W State St West Lafayette, IN 47907 USA	(765) 494-4448	gjohal@purdue.edu
G. Johnson	University of Illinois 1004 E. Harding 301 Urbana, IL 61801 USA	(217) 333-4255	grjohnso@uiuc.edu
Elizabeth Jones	Pioneer HiBred 7300 NW 62nd Ave Johnston, IA 50131 USA	(515) 253-2493	liz.jones@pioneer.com
Rudolf Jung Jung	Pioneer HiBred International 7300 Nw 62nd Ave Johnston, IA 50131 USA	515 270 5934	rudolf.jung@pioneer.com
Shawn Kaeppler	University of Wisconsin 1575 Linden Dr Madison, WI 53705 USA	(608) 262-6521	smkaeppl@wisc.edu
Alexander Kahler	University of Minnesota 1991 Upper Buford Circle 411 Borlaug Hall St. Paul, MN 55108 USA	(612) 624-3749	kahl0041@umn.edu
Genichi Kakefuda	BASF Plant Science 26 Davis Drive Research Triangle Park, NC 277093528 USA	919 5472305	genichi.kakefuda@basf.com
Terry Kamps	University of Florida Horticultural Sciences Department PO Box 110690 Gainesville, FL 32611 USA	3523921928 326	kampstl@yahoo.com
Zhian Kamvar	Truman State University 100 E Normal Kirksville, MO 63501 USA	573-823-0762	
Catherine Kandianis	University of Illinois Urbana-Champaign S122 Turner Hall 1102 S Goodwin Ave Urbana, IL 61801 USA	774-487-8687	cbermude@uiuc.edu
Lisa Kanizay	University of Georgia 4608 Miller Plant Sciences Athens, GA 30602 USA	706-542-1010	lkanizay@plantbio.uga.edu
Etienne Kaszas	Syngenta Seeds 317 330th St Stanton, MN 55018 USA	507 663 7652	etienne.kaszas@syngenta.com
Toni Kazic	University of Missouri Dept. of Computer Science 201 Engineering Building West Columbia, MO 65211 USA	(573) 884-4549	toni@athe.rnet.missouri.edu
Kent Keim	BASF Plant Science Breeding 38W235 Toms Trail Drive St Charles, IL 60175 USA	(815) 895-9686	kent.keim@basf.com

Elizabeth Kellogg	University of MissouriSt Louis 61 Aberdeen Place Clayton, MO 63105 USA	(314) 516-6217	tkellogg@umsl.edu
Jerry Kermicle	University of Wisconsin Lab of Genetics 425G Henry Mall Madison, WI 53706 USA	(608) 262-1253	kermiclel@wisc.edu
Juthaporn Khampila	Khon Kaen University 123 Mitraparp Road Muang District Kohn Kaen Province 40002, Thailand	217-721-4398	kjuthapo@uiuc.edu
Raja Ram Khanal	University of Guelph Department of Plant Agriculture Crop Science Building Guelph Ontario N1G 2L1, Canada	519-341-1382	rkhanal@uoguelph.ca
Kazuhiro Kikuchi	Cornell University Boyce Thompson Institute for Plant Research 1 Tower Rd Ithaca, NY 14853 USA	(607) 254-6747	ee54@cornell.edu
Karen Koch	University of Florida 3120 NW 9th Place Gainesville, FL 32605 USA	3533921928 322	kekoch@ufl.edu
Judith Kolkman	Cornell University 4391 Jacksonville Rd. Trumansburg, NY 14886 USA	(607) 387-5583	jmk87@cornell.edu
Mai Komatsu	DuPont Route 141 and Henry Clay Road Dupont Experimental St Bldg 353 Room 106C Wilmington, DE 19880 USA	(302) 695-1299	mai.komatsu@usa.dupont.com
Nives Kovacevic	UWMadison 607 Eagle Heights Apt L Madison, WI 53705 USA	(608) 026-2652 1	nmkovacevic@wisc.edu
Richard Kowles	St Mary's University of Minnesota 700 Terrace Heights Winona, MN 55987 USA	(507) 457-1554	dkowles@smumn.edu
Allison Krill	Cornell University 175 Biotechnonogy Ithaca, NY 14853 USA	(607) 255-3911	amk72@cornell.edu
Ashlee Krisko	Oakland University 33215 Twickingham Dr Sterling Heights, MI 48310 USA	586-979-5559	aekrisko@oakland.edu
Alan Kriz	BASF 26 Davis Drive Durham, NC 27709 USA	973 519 4572	alan.kriz@basf.com
Brent Kronmiller	Iowa State University 409 Bessey, Plant Pathology Ames, IA 50011 USA	(515) 294-6843	bak@iastate.edu
Scott Kruchowski	Washington University School of Medicine 4444 Forest Park Ave Campus Box 8501 Saint Louis, MO 63108 USA	(314) 286-1800	skrychow@watson.wustl.edu

Paulette Krumpelman	Pioneer HiBred International 7300 NW 62nd Ave Johnston, IA 501312940 USA	(515) 270-3527	paulette.krumpelman@pioneer.com
Indrajit Kumar	Univesity of Illinois 325 NSRC 1101 Peabody Dr Urbana, IL 61801 USA	(217) 265-6988	ikumar2@uiuc.edu
Eleanor Kuntz	University of Georgia 4505 Miller Plant Sci Bldg Athens, GA 30609 USA	(706) 542-1857	ekuntz@uga.edu
Paul Ladipo	University of Missouri - Columbia 101 Tucker Hall Columbia, MO 65211 USA	(573) 882-1168	pble07@mizzou.edu
Shailesh Lal	Oakland University 346 Dodge Hall Rochester, MI 48309 USA	(248) 370-2785	lal@oakland.edu
Jane Langdale	University of Oxford Gunton Barn Charney bassett OX12 0EU, United Kingdom	00 44 1865 275099	jane.langdale@plants.ox.ac.uk
Tiffany Langewisch	University of Mo-Columbia 324 Tucker Hall Columbia, MO 65211 USA	(573) 882-8033	t11hw9@mizzou.edu
Laura Langton	Washington University Computer Science & Engineering 1 Brookings Dr Campus Box 1045 Saint Louis, MO 63130 USA	314-406-8493	langton@cst@wustl.edu
Brian Larkins	University of Arizona 2701 North Shannon Road Tucson, AZ 85745 USA	(520) 621-9958	Larkins@Ag.Arizona.edu
Nick Lauter	USDAARS and Iowa State University 415 Bessey Hall Iowa State University Ames, IA 50011 USA	515 294 8260	nickl@iastate.edu
Carolyn Lawrence	USDAARS Iowa State University 526 Science II Ames, IA 50011 USA	(515) 294-4294	triffid@iastate.edu
Kristen Leach	University of Missouri - Columbia Division of Plant Sciences 1-31 Agriculture Bldg Columbia, MO 65211 USA	(573) 882-9228	kalp55@mizzou.edu
Elizabeth Lee	University of Guelph 60 Fairweadow Dr Guelph N1H 7W5, Canada	5198244120 53360	lizlee@uoguelph.ca
Michael Lee	Iowa State University 100 Osborn Drive 1553 Agronomy Hall Dep of Agronomy Ames, IA 50011 USA	(515) 294-7951	mlee@iastate.edu
Guofu Li	Pioneer HiBred International Inc. 7300 NW 62nd Avenue Johnston, IA 501311004 USA	(515) 254-2804	Guofu.Li@pioneer.com

Li Li	Iowa State University 2049 Roy J. Carver Co-Laboratory Ames, IA 50010 USA	(515) 294-1659	lilsunny@iastate.edu
Wei Li	Iowa State University 2282 Molecular Biology Building Pammel Dr Ames, IA 50010 USA	515-294-0137	wli@iastate.edu
Xuexian Li	University of Georgia Room 2502 Miller Plant Sciences Bldg Athens, GA 30602 USA	706-542-1010	xli@plantbio.uga.edu
Ying Li	University of Illinois - Urbana-Champaign NSRC 325 1101 W Peabody Dr Urbana, IL 61801 USA	217-265-6988	yingli3@uiuc.edu
Yubin Li	Rutgers University Waksman Institute of Microbiology 190 Frelinghuysen Rd Piscataway, NJ 8854 USA	732-445-2307	yubin@waksman.rutgers.edu
Yun Li	University of Georgia 4608 Miller Plant Sciences Athens, GA 30602 USA	706 5421010	yli@plantbio.uga.edu
Chengzhi Liang	Cold Spring Harbor Lab 1 Bungtown Rd Cold Spring Harbor, NY 11724 USA	(516) 367-8328	liang@cshl.edu
Guy Lima	Oakland University 2200 N Squirrel Rd Rochester, MI 48309 USA	(248) 895-5315	galima@oakland.edu
Damon Lisch	U.C. Berkeley 1104 Ordway St Albany, CA 94706 USA	(510) 708-9491	dlisch@berkeley.edu
Jia Liu	The Institute for Genomic Research 9712 Medical Center Dr Rockville, MD 20850 USA	(301) 795-7836	jliu@tigr.org
Friedrich Longin	University of Hohenheim Institute of Plant Breeding 350a Fruwirthstr 21 D-70599 Stutttgart, Germany		flongin@uni-hohenheim.de
Robenzon Lorenzana	University of Minnesota 1991 Upper Buford Circle Room 411 Borlaug Hall St. Paul, MN 55108 USA	(612) 625-6794	loren138@umn.edu
Ashley Lough	University of Missouri-Columbia 324 Tucker Hall Columbia, MO 65211 USA	(573) 882-8033	anl6d9@mizzou.edu
Aaron Lovenz	University of Wisconsin 1575 Linden Dr Madison, WI 53706 USA	608-852-7327	alorenz@uisc.edu
Brenda Lowe	Monsanto 62 Maritime Drive Mystic, CT 6255 USA	(860) 572-5216	brenda.lowe@monsanto.com
Lewis Lukens	University of Guelph 123 Dulbin St N Guelph N1H4N5, Canada	519 7632811	llukens@uoguelph.ca

China Lunde	UC Berkeley Plant Gene Expression Center Plant Gene Expression Center 800 Buchanan Street Albany, CA 94710 USA	(510) 559-5710	lundec@berkeley.edu
Song Luo	Chromatin Inc 2201 W Campbell Park Dr Ste 10 Chicago, IL 60612 USA	312-455-1935	sluo@chromatininc.com
Jianxin Ma	Purdue University 1057 Marwyck St West Lafayette, IN 47906 USA	(765) 463-4089	maj@purdue.edu
Yi Ma	Penn State University 208 Mueller Lab University Park, PA 16802 USA	(814) 863-5491	yum105@psu.edu
Anthony Mahama	Iowa State University 1004 Pinon Drive Apt. 6 Ames, IA 50014 USA	(515) 294-0948	aassibi@iastate.edu
Sara Martens	Oakland University 5980 Oakland Valley Dr Rochester, MI 48306 USA	248-882-3122	chops10blop@yahoo.com
Robert Martienssen	Cold Spring Harbor Laboratory 1 Bungtown Road Cold Spring Harbor, NY 117242212 USA	(516) 367-8322	martiens@cshl.edu
Jerome Martin	IRD France 157 bd Charles warnery Montpellier 34000, France	(3) 346-7416 250	jerome.martin@mpl.ird.fr
Rick Masonbrink	University of Missouri - Columbia 117 Tucker Hall Columbia, MO 65211 USA	660-541-2073	Remkv6@mizzou.edu
Maria Laura Mayor	Iowa State University 1555 Agronomy Hall Ames, IA 50011 USA	(515) 294-3517	mlmayor@iastate.edu
Patricio Mayor	University of Minnesota Department of Agronomy and Plant Genetics 411 Bourlaug Hall; 1991 Buford Circle Saint Paul, MN 55108 USA	651-786-9323	mayor006@umn.edu
Yumsie Andre Mbom	Rufosad Kumba Cameroon 156 C C C Kumba South West Province West Africa, Cameroon		xoadbi@yahoo.ie
Donald McCarty	University of Florida 3120 NW 9th Place Gainesville, FL 32605 USA	3533921928 322	drm@ufl.edu
Amy McCaskill	BASF Plant Sciences 26 Davis Dr Research Triangle Park, NC 27709 USA		
William McCombie	Cold Spring Harbor Laboratory One Bungtown Road Cold Spring Harbor, NY 11724 USA	(516) 422-4083	mccombie@cshl.edu

Annie McGill	University of Wisconsin-Madison Dept of Agronomy; 439 Plant Sciences 1575 Linden Drive Madison, WI 53706 USA	(608) 262-6521	mamcgill@wisc.edu
Karen McGinnis	University of AZ Dept of Plant Sciences 303 Forbes Hall Tucson, AZ 85721 USA	(520) 621-1695	mcginnis@ag.arizona.edu
Michael McMullen	USDAARSPGRU University of Missouri 205 Curtis Hall Columbia, MO 65211 USA	(573) 882-7606	mcmullenm@missouri.edu
Paula McSteen	Penn State University 208 Mueller Lab University Park, PA 16802 USA	(814) 863-1112	pcm11@psu.edu
Kendra Meade	Iowa State University 1541 Agronomy Hall Ames, IA 50011 USA	(515) 294-9429	kameade@iastate.edu
Robert Meeley	Pioneer A DuPont Company 3214 Edwards Ave Des Moines, IA 50312 USA	(515) 270-3770	bob.meeley@pioneer.com
Susan Melia-Hancock	University of Missouri - Columbia Division of Plant Sciences 1-31 Agriculture Bldg Columbia, MO 65211 USA	(573) 882-9228	melia-hancocks@missouri.edu
Xin Meng	Pioneer HiBred 7250 NW 62nd Avenue Johnston, IA 50131 USA	(515) 270-3661	xin.meng@pioneer.com
Joachim Messing	Rutgers University 190 Frelinghuysen Rd Piscataway, NJ 8854 USA	(732) 445-4256	messing@waksman.rutgers.edu
Julie Meyer	Truman State University 100 E Normal Kirksville, MO 63501 USA	660-785-7138	JMM130@truman.edu
Louis Meyer	University of Missouri - Columbia 324 Tucker Hall Columbia, MO 65211 USA	(573) 882-8033	ljmr29@mizzou.edu
Suzanne Mickelson	Pioneer HiBred International 7200 NW 62nd Ave Johnston, IA 50131 USA	(515) 254-2803	suzanne.mickelson@pioneer.com
Mihai Miclaus	Rutgers University Waksman Institute of Microbiology 190 Frelinghuysen Rd Piscataway, NJ 8854 USA	(732) 445-3801	mihai@waksman.rutgers.edu
Mark Millard	USDAARS Plant Introduction Station G212 Agronomy Hall Iowa State University Ames, IA 50011 USA	(515) 294-3715	mjmillar@iastate.edu
Theresa Miller	Marquette University Department of Biological Sciences PO Box 1881 Milwaukee, WI 53201 USA	414-288-1419	Theresa.miller@mu.edu

Patrick Minx	Washington University School of Medicine 4444 Forest Park Ave Campus Box 8501 St Louis, MO 63108 USA	(314) 286-1800	pminx@watson.wustl.edu
Deborah Moeller	Washington University School of Medicine 4444 Forest Park Ave Campus 8501 Saint Louis, MO 63108 USA	314-286-1800	dmoeller@watson.wustl.edu
Lorena Moeller	Iowa State University B421 Agronomy Hall Ames, IA 50011 USA	515-294-5940	lorenam@iastate.edu
Amitabh Mohanty	Cold Spring Harbor laboratory 1 Bungtown Road Cold Spring Harbor, NY 11724 USA	(516) 367-8827	Mohanty@cshl.edu
RitaAnn Monde	Purdue University Department of Agronomy 915 West State St West Lafayette, IN 47907 USA	(765) 494-4787	rmonde@purdue.edu
Stephen Moose	University of Illinois Department of Crop Sciences 1201 W. Gregory Drive Urbana, IL 61801 USA	217 2446308	smoose@uiuc.edu
Dilbag Multani	Pioneer Hi-Bred International Inc A DuPont Co 7300 NW 62nd Ave PO Box 1004 Johnston, IA 50131 USA	(515) 334-4618	dilbag.multani@pioneer.com
Nick Murphy	University of Arizona 496 S. Douglas Wash Rd. Vail, AZ 85641 USA	(520) 488-8151	nick@ag.arizona.edu
Shaun Murphy	Florida State University Biology Unit I Chieftan Way Tallahassee, FL 32306 USA	(850) 644-8058	murphy@sb.fsu.edu
Theresa Musket	University of Missouri - Columbia Division of Plant Sciences 1-31 Agriculture Bldg Columbia, MO 65211 USA	(573) 882-9228	muskett@missouri.edu
Michael Muszynski	Syngenta Seeds 2369 330th St. Slater, IA 50244 USA	(515) 685-5203	michael.muszynski@syngenta.com
Nils Muthreich	University of Tuebingen Center/ZMBP Department of General Genetics Aul der Morgenstelle 28 Tuebingen 72076, Germany		nills.muthreich@zmbp.uni-luebingen.de
Alan Myers	Iowa State University Department of BBMB 1210 Molecular Biology Building Ames, IA 50011 USA	(515) 294-6116	ammyers@iastate.edu

Ervin Nagy	University of Georgia Genetics Department Life Sciences Building Athens, GA 30602 USA	(706) 542-9729	dnagye@uga.edu
Ramesh Nair	Plant Sciences Institute Iowa State University 2505 NW Parkridge Dr. Ankeny, IA 50023 USA	(515) 294-3541	rnair@iastate.edu
Apurva Narechania	Cold Sping Harbor Laboratory Williams Building One Bungtown Road Cold Spring Harbor, NY 11724 USA	(646) 522-8888	apurva@cshl.edu
Myron Neuffer	University of Missouri 109 Curtis Hall Columbia, MO 65211 USA	(573) 882-7735	gneuffer@aol.com
Kathleen Newton	University of Missouri-Columbia 105 Tucker Hall Columbia, MO 65211 USA	(573) 882-4049	newtonk@missouri.edu
Junjian Ni	Cornell University Plant Breeding Dept Cornell University Ithaca, NY 14853 USA	(607) 255-3103	jn66@cornell.edu
David Nicholl	Syngenta 3054 Cornwallis Rd RTP, NC 27709 USA	(919) 541-8680	david.nicholl@syngenta.com
Devin Nichols	University of Illinois 389 ERML 1201 W Gregory Dr Urbana, IL 61801 USA	(217) 244-6146	dmnichol@uiuc.edu
Jorge Nieto-Sotelo	Instituto de Biotecnologia de la UNAM Av. Universidad #2001 Col. Chamilpa Cuernavaca Mor. 62250, Mexico	52-777 329-1614	jorge@ibt.unam.mx
Joana Novais	University of Illinois at Urbana-Champaign 1102 S Godwin Ave Urbana, IL 61801 USA	217-244-3388	jnovais@uiuc.edu
Brent O'Brien	University of Florida 1301 Fifield Hall Gainesville, FL 32601 USA	3523924711x311	bob2373@ufl.edu
Devin O'Connor	University of California - Berkeley Plant and Microbial Biology 800 Buchanan St Berkeley, CA 94710 USA	(510) 559-5922	devo@nautre.berkeley.edu
Kazuhiro Ohtsu	Iowa State University 2049 Roy J. Carver CoLaboratory Ames, IA 50011 USA	(515) 294-1659	kazohtsu@iastate.edu
Milena Ouzunova	KWS SAAT AG Grimmsehlstr. 31 Einbeck 37555, Germany	49 5561 311 352	m.ouzunova@kws.com
Saranyan Palaniswamy	The Ohio State University 420 W 12th Ave 570 TMRF The Ohio State University Columbus, OH 43210 USA	(614) 218-3553	Saranyan.Palaniswamy@osumc.edu

Shiran Pasternak	Cold Spring Harbor Laboratory 500 Kappock Street Apartment 1E Bronx, NY 10463 USA	(516) 367-6977	shiran@cshl.edu
Wojtek Pawlowski	Cornell University 401 Bradfield Hall Ithaca, NY 13052 USA	607 2548745	wp45@cornell.edu
Bryan Penning	Purdue University 915 W State St West Lafayette, IN 47907 USA	(765) 494-7924	bpenning@purdue.edu
Thomas Peterson	Iowa State University 2208 Molecular Biology Building Ames, IA 50011 USA	(515) 294-6345	thomasp@iastate.edu
Kimberly Phillips	Pennsylvania State University 607 Mueller Lab University Park, PA 16802 USA	(814) 880-2547	kap262@psu.edu
Jessica Ponder	Truman State University 100 E Normal Kirksville, MO 63501 USA	660-665-8862	JNP465@gmail.com
Richard Pratt	Ohio State Univ. Dept. of Hort. and Crop Science OARDC 1680 Madison Ave. Wooster, OH 44691 USA	(330) 263-3972	pratt.3@osu.edu
Gael Pressoir	IGD Cornell University 716 N Tioga St Ithaca, NY 14850 USA	607 255 1809	ghp5@cornell.edu
Gernot Presting	University of Hawaii 1955 EastWest Road Ag Sciences Rm 218 Honolulu, HI 96822 USA	808 9568861	gernot@hawaii.edu
Jill Preston	University of Missouri - St Louis Dept of Biology R223 One University Blvd Saint Louis, MO 63121 USA	(316) 361-3150	jcpxt8@studentmail.umsl.edu
Ian Prust	University of Wisconsin-Madison 1575 Linden Dr Madison, WI 53706 USA	(608) 262-6521	prust@wisc.edu
Vinay Pulletikurti	Illinois State University Dept of Biological Science Normal, IL 61790 USA	309-438-2685	
Pablo Rabinowicz	The Institute for Genomic Research div of JCVI 10338 Procera Dr. Rockville, MD 20850 USA	301 762 4704	prabinowicz@yahoo.com
Kellie Reimann	Pioneer HiBred International Inc 7300 NW 62nd Ave Johnston, IA 50131 USA	(515) 254-2871	kellie.reimann@pioneer.com
Henry Richbourg	UNCW 3108 Ervins Place Drive Castle Hayne, NC 28429 USA	(919) 601-5055	hlr7003@uncw.edu

Eric Riedeman	University of Wisconsin-Madison Agronomy/Plant Breeding and Plant Genetics 1575 Linden Dr Madison, WI 53706 USA	(310) 977-3177	riedeman@wisc.edu
Joan Rigau-Lloveras	Consorci CSIC-IRTA Laboratori de Genetica Molecular Vegetal Consorci CSIC-IRTA Jordi Girona 18-26 08034 Barcelona, Spain		
Michael Robbins	Pennsylvania State University 116 ASI Bldg Curtin Rd University Park, PA 16802 USA	(814) 404-1919	mlr263@psu.edu
Leilani Robertson-Hoyt	University of Wisconsin 5210 Genetics/Biotech Madison, WI 53706 USA	(608) 265-5804	robertsonhoy@wisc.edu
Peter Rogowsky	ENSLyon 46 allee Italie Lyon 69364, AK France	334 7272 8607	peter.rogowsky@ens-lyon.fr
Arnaud RONCERET	Cornell University Department of Plant Breeding 418 Bradfield Hall Ithaca, NY 14853 USA	(607) 342-1123	ar346@cornell.edu
Jacques Rouster	Biogemma 8, rue des freres Lumiere 63100 Clermont-Ferrand, France		jacques.rouster@biogemma.com
Ken Russell	University of Nebraska Dept of Agronomy PO Box 830915 Lincoln, NE 68583 USA	(402) 472-1562	krussell3@unl.edu
Paolo Sabelli	University of Arizona Dept of Plant Sciences 303 Forbes Building Tucson, AZ 85721 USA	520-621-9154	BSabelli@ag.arizona.edu
Marty Sachs	USDAARS S108 Turner Hall 1102 S. Goodwin Ave. Urbana, IL 61801 USA	(217) 244-0864	msachs@uiuc.edu
Ayna Salas	University of Illinois 389 ERML 1201 W Gregory Dr Urbana, IL 61801 USA	217-244-6146	asalas@uiuc.edu
Muhammad Saleem	University of Tuebingen ZMBP / Department of General Genetics Auf der Morgenstelle 28 72076 Tuebingen, Germany		
Phillip San Miguel	Purdue University WSLR 170 South University Street West Lafayette, IN 47907 USA	(765) 496-6328	pmiguel@purdue.edu

Liu Sanzhen	Iowa State University 2043 Roy J Carver Co-Laboratory Ames, IA 50010 USA	515-294-1659	liu3zhen@iastate.edu
Ananda K. Sarkar	Cold Spring Harbor Lab 1 Bungtown Road Cold Spring Harbor, NY 11724 USA	1 516 367 6818	sarkara@cshl.edu
Michael Scanlon	Cornell University 195 Hillcrest Road Ithaca, NY 14853 USA	607 2541156	mjs298@cornell.edu
Mary Schaeffer	USDAARSPGRU USDAARSPGRU 205 Curtis HallUMC Columbia, MO 65211 USA	573 8847873	schaefferm@missouri.edu
Justin Schares	Iowa State University 2102 Molecular Biology Ames, IA 50011 USA	(314) 269-5531	ragnarok@iastate.edu
Robert Schmidt	University of Calif. San Diego Division of Biological Sciences UCSD 9500 Gilman Dr. La Jolla, CA 92093 USA	858 5341636	rschmidt@ucsd.edu
Patrick Schnable	Iowa State University 2035 B Roy J Carver Co-Laboratory Ames, IA 50011 USA	(515) 294-0975	schnable@iastate.edu
David Schwartz	University of Wisconsin Madison Biotechnology Center 425 Henry Mall Madison, WI 53706 USA	(608) 265-0546	dcschwartz@wisc.edu
Marvin Scott	USDA ARS Department of Agronomy Iowa State University Ames, IA 500111010 USA	(515) 294-7825	pscott@iastate.edu
Magdalena SeguraNieto	Cinvestav Campus Guanajuato Km 9.6 Libramiento Norte carretera Irapuato Leon Irapuato 36500, Mexico	52 462 623 9665	msegura@ira.cinvestav.mx
Trent Seigfried	USDAARS Iowa State University 526 Science II Ames, IA 50011 USA	(515) 294-4294	devolver@iastate.edu
Rajandeep Sekhon	Pennsylvania State University 116 ASI Building University Park, PA 16803 USA	814-883-6533	rss222@psu.edu
David Selinger	Pioneer HiBred 7200 NW 62nd Ave PO Box 184 Johnston, IA 50131 USA	515 2542646	david.selinger@pioneer.com
Mary Senior	Syngenta Seeds Inc. 3054 East Cornwallis Rd RTP, NC 27709 USA	(919) 597-3041	lynn.senior@syngenta.com
A. Mark Settles	University of Florida PO Box 110690 Horticultural Sciences Gainesville, FL 326110690 USA	(352) 392-7574	settles@ufl.edu

Mandeep Sharma	Pennsylvania State Univesity 116 ASI Building University Park, PA 16802 USA	(814) 441-9874	mxs781@psu.edu
Moira Sheehan	Cornell University 201 East Jay St. Ithaca, NY 148503619 USA	607 2790267	mjs224@cornell.edu
Josh Shendelman	Iowa State University 1112 SW 47th Circle Ankeny, IA 50023 USA	(515) 229-3851	jshendel@iastate.edu
William Sheridan	University of North Dakota 5010 Copper Gate Drive Grand Forks, ND 58203 USA	(808) 553-5510	bill.sheridan@und.edu
Jinghua Shi	University of Georgia Dept of Plant Biology 2502 Miller Plant Sciences Bldg Athens, GA 30605 USA	(706) 542-1010	jshi@plantbio.uga.edu
Kyungju Shin	University of Missouri 101 Tucker Hall Columbia, MO 65211 USA	(573) 882-1168	ksgw3@mizzou.edu
Arthur Shockley	University of Georgia 4505 Miller Plant Sci Bldg Athens, GA 30609 USA	(706) 542-1857	ashockley@plantbio.uga.edu
Brandi Sigmon	Iowa State University 2282 Molecular Biology Bldg Ames, IA 50011 USA	(515) 294-0137	bsigmon@iastate.edu
Sofia Silva	University of Illinois at Urbana-Champaign AW-101 Turner Hall 1102 S Goodwin Ave Urbana, IL 61801 USA	217-244-3388	ssilva@uiuc.edu
Carl Simmons	Pioneer HiBred International 7300 N.W. 62nd Avenue Johnston, IA 50131 USA	(515) 270-5949	carl.simmons@pioneer.com
Andrea Skirpan	Penn State University 208 Mueller Lab University Park, PA 16802 USA	814 8634022	als152@psu.edu
Thomas Slewinski	Pennsylvania State University 616 Mueller Lab University Park, PA 16802 USA	814-863-5491	TLS315@psu.edu
Matthew Smid	University of Guelph Dept. Plant Agriculture 50 Stone Rd. W. Guelph ON N1G 2W1, Canada	(519) 824-4120 52509	msmid@uoguelph.ca
Heath Smith	Truman State Univesity Magruder Hall 3064 Kirksville, MO 63501 USA	(660) 665-2136	has039@truman.edu
Brian Smith-White	NCBI 8600 Rockville Pike Bethesda, MD 20894 USA	301-402-4047	smtwhite@ncbi.nlm.nih.gov
Gertraud Spielbauer	University of Florida 1143 Fifield Hall PO Box 110690 Gainesville, FL 32611-0690 USA	(11) 498-9218 93497	g_spielbauer@web.de

William Spooner	CSHL 51 Humberstone Road Cambridge CB4 9LL, United Kingdom	44 7899 654373	whs@ebi.ac.uk
Nathan Springer	University of Minnesota 250 Biological Sciences Center 1445 Gortner Ave Saint Paul, MN 55108 USA	(612) 624-6241	springer@umn.edu
Ann Stapleton	University of North Carolina Wilmington 601 S. College Wilmington, NC 28403 USA	910 9627267	stapletona@uncw.edu
Joshua Stein	CSHL 152 Nagog Hill Road Acton, MA 1720 USA	978 2644338	steinj@cshl.edu
David Stern	Boyce Thompson Institute for Plant Research 5051 Tuttle Road Burdett, NY 14818 USA	(607) 254-1306	DS28@cornell.edu
Robyn Stevens	University of Illinois Urbana-Champaign 1102 S Goodwin Ave Urbana, IL 61801 USA	(217) 244-3388	allschei@uiuc.edu
Philip Stinard	Maize Genetics Stock Center USDAARS S123 Turner Hall 1102 S. Goodwin Ave. Urbana, IL 61801 USA	(217) 333-6631	pstinard@uiuc.edu
Jennifer Stonaker	University of California - Berkeley 111 Koshland Hall MC 3102 Berkeley, CA 94720 USA	(510) 632-1737	jenne@berkeley.edu
Joshua Strable	University of Iowa Dept of Biological Sciences 143 Biology Building Iowa City, IA 52242 USA	319-335-2582	joshua-strable@uiowa.edu
Anthony Studer	University of Wisconsin - Madison 425 Henry Mall Genetics Biotech Building #5210 Madison, WI 53706 USA	608-265-5804	studer@wisc.edu
Robert Stupar	University of Minnesota 1445 Gortner Ave. St. Paul, MN 55108 USA	(612) 624-5415	stup0004@umn.edu
Yuejin Sun	Dow AgroSciences 9330 Zionsville Rd Indianapolis, IN 46268 USA	(317) 337-3630	ysun@dow.com
Sergei Svitashev	Pioneer 7300 NW 62nd Avenue Johnston, IA 501311004 USA	(515) 270-4020	sergei.svitashev@pioneer.com
Kayleigh Swaggart	Truman State Univesity Magruder Hall 3064 Kirksville, MO 63501 USA	(816) 694-0722	kas935@truman.edu
Kankshita Swaminathan	University of Illinois 818 West Hill Street Urbana, IL 61801 USA	217 265 6988	kank@uiuc.edu
Ruth Swanson-Wagner	Iowa State University 2049 Roy J Carver Co-Laboratory Ames, IA 50011 USA	(515) 294-1659	swansonr@iastate.edu

Anne Sylvester	University of Wyoming Dept of Molecular Biology 1000 East University Ave Laramie, WY 82071 USA	(307) 766-4993	annesyl@uwyo.edu
Tim Symanietz	Syngenta 2369 330th st. Slater, IA 50014 USA	(515) 685-5265	tim.symanietz@syngenta.com
Elizabeth Takacs	Cornell University 228 Plant Sciences Ithaca, NY 14850 USA	607-254-1160	emt32@cornell.edu
Graziana Taramino	Dupont Crop Genetics Experimental Station E353/106A Rt 141 & Henry Clay Wilmington, DE 19880 USA	302 695 8854	graziana.taramino@cgr.dupont.com
Reuben Tayengwa	University of Florida Cancer & Genetics Research Complex 1376 Mowry Rd Rm 235 Gainesville, FL 32610 USA	352-273-8073	reubent@ufl.edu
Carla Taylor	Pioneer HiBred International 7200 NW 62nd Ave Johnston, IA 50131 USA	(515) 334-6756	Carla.Taylor@pioneer.com
Beth Thompson	University of California Berkeley PGEC 800 Buchanan Street Albany, CA 94710 USA	(510) 559-5922	bethompson@berkeley.edu
Jeffry Thornsberry	Northwest Missouri State University Northwest Missouri State University 800 University Dr. Maryville, MO 64468 USA	(660) 562-1812	jthorns@nwmissouri.edu
Marja Timmermans	Cold Spring Harbor Lab 1 Bungtown Road Cold Spring Harbor, NY 11724 USA	516 3678835	timmerma@cshl.edu
Joshua Tolbert	Maize Genetics Cooperation Stock Center 1102 S. Goodwin Ave. S11 Turner Hall Urbana, IL 61802 USA	217 3339743	jtolbert@uiuc.edu
Chad Tomlinson	Washington University School of Medicine 4444 Forest Park Ave Campus Box 8501 Saint Louis, MO 63108 USA	314-286-1800	ctomlins@watson.wustl.edu
Christopher Topp	University of Georgia 2502 Miller Plant Sciences Athens, GA 30602 USA	(706) 542-1010	ctopp@plantbio.uga.edu
William Tracy	University of WisconsinMadison 1575 Linden Dr. Madison, WI 53706 USA	(608) 669-5052	wftracy@wisc.edu
Dorothy Tuthill	University of Wyoming Department of Molecular Biology 1000 E. University Ave. Laramie, WY 82071 USA	(307) 766-4994	dtuthill@uwyo.edu

Narasimham Upadyayula	University of Illinois at Urbana-Champaign S-115 Turner Hall 1102 S Goodwin Ave Urbana, IL 61801 USA	(217) 244-3388	upadyayu@uiuc.edu
James Uphaus	AgReliant Genetics 4640 East St Rd 32 Lebanon, IN 46052 USA	(765) 482-9833	jim.uphaus@agreliantgenetics.com
Veronica Vallejo	Michigan State University 247 Plant & Soil Sciences Bldg East Lansing, MI 48826 USA		
Joerg Vandenhirtz	LemnaTec Germany LemnaTec 18 Schumanstr. Wuerselen 52146, Germany	01149 2405 412612	joerg@lemnatec.de
Kranthi Varala	University of Illinois 1101 W Peabody Dr NSRC #190 Urbana, IL 61801 USA	217-265-6988	kvarala2@uiuc.edu
Serena Varotto	University of Padova Departmentof Environmental Agronomy and Crop Produ Agripolis Legnaro 35020, Italy	(390) 498-272858	serena.varotto@unipd.it
Leah Viesselmann	University of Wisconsin - Madison 1575 Linden Madison, WI 53706 USA	417-704-5413	lviesselmann@wisc.edu
Erik Vollbrecht	Iowa State University 2206 Molecular Biology Ames, IA 50010 USA	515 2949009	vollbrec@iastate.edu
Ramon Wahl	Max-Planchk-Institute for terrestial Microbiology Karl von Frisch StraBe 35043 Marburg Hessen, Germany		wahlra@mpi-marburg.mpg.de
VIRGINIA WALBOT	STANFORD UNIVERSITY STANFORD UNIVERSITY DEPARTMENT OF BIOLOGICAL SCIENCES STANFORD, CA 943055020 USA	650 7232227	walbot@stanford.edu
Matt Walch	University of Minnesota 411 Borlaug Hall 191 Upper Buford Circle Saint Paul, MN 55108 USA	612-625-6223	Walch023@umn.edu
Tina Wambach	University of Guelph Crop Science Building Guelph Ontario N1G 2W1, Canada	5198244120x58164	twambach@uoguelph.ca
Huai Wang	University of WisconsinMadison 804 Eagle Hts Apt F Madison, WI 53705 USA	(608) 265-5804	wang10@wisc.edu
Kan Wang	Iowa State University G405 Agronomy Hall Ames, IA 50011 USA	(515) 294-4429	kanwang@iastate.edu

PoHao Wang	Pennsylvania State University 116 ASI Building University Park, PA 16802 USA	814-441-9440	puw116@psu.edu
Qinghua Wang	Rutgers University Waksman Institute 190 Frelinghuysen Rd Piscataway, NJ 8854 USA	732-445-2307	ginghua@waksman.rutgers.edu
Rachel Wang	University of California - Berkeley 345 Life Sciences Addition Berkeley, CA 94720 USA	(510) 643-8277	rachelcjw@berkeley.edu
Doreen Ware	USDAARS 8 Melview Ct. Melville, NY 11747 USA	(516) 367-6979	ware@cshl.edu
Allison Weber	University of Wisconsin - Madison 5210 Genetics Biotechnology Ctr Bldg Doebley Lab 425 Henry Mall Madison, WI 53706 USA	(608) 265-5804	allisonweber@wisc.edu
David Weber	Illinois State University Dept of Biological Sciences Campus Box 4120 Normal, IL 61790 USA	(309) 438-2685	dfweber@ilstu.edu
Gerd Weber	University of Hohenheim Institute for Plant Breeding Fruwirthstr. 21 Stuttgart 70599, Germany	49 711 459 22341	weberg@uni-hohenheim.de
Becky Weeks	Iowa State University 2282 Molecular Biology Ames, IA 50011 USA	515-294-0137	rlmauton@iastate.edu
Fusheng Wei	University of Arizona Arizona Genomics Institute 303 Forbes Building PO Box 210036 Tucson, AZ 85721 USA	(520) 626-9585	fushengw@ag.arizona.edu
Clifford Weil	Purdue University Agronomy Department 915 W State St West Lafayette, IN 47907 USA	(765) 496-1917	cweil@purdue.edu
Susan Wessler	University of Georgia 186 Ashbrook Dr. Athens, GA 30605 USA	706 542 1870	sue@plantbio.uga.edu
Rick Westerman	Purdue University WSLR Building West Lafayette, IN 47907 USA	(765) 494-0505	westerman@purdue.edu
Leah Westgate	University of Missouri - Columbia 2251 W. Botner Rd. Columbia, MO 65202 USA	573 8828033	westgatel@missouri.edu
Clinton Whipple	Cold Spring Harbor Laboratory 1 Bungtown Rd Cold Srping Harbor, NY 11724 USA	516 3678827	whipple@cshl.edu
Mark Williams	DuPont Crop Genetics Research Experimental Station Bldg 353 Rm 118E Rt. 141 Henry Clay Wilmington, DE 19803 USA	(302) 540-6023	mark.e.williams@cgr.dupont.com

Hlaing Win	University of Illinois Urbana-Champaign 325 NSRC 1101 W Peabody Dr Urbana, IL 61801 USA	217-891-5643	hwin2@uiuc.edu
Roger Wise	USDAARS Iowa State University Department of Plant Pathology Ames, IA 50011-1020 USA	(515) 294-9756	rpwise@iastate.edu
Katrin Woll	KWS SAAT AG KWS SAAT AG Grimsehlstr. 31 Einbeck 37574, IL Germany	(55) 613-1183 2	k.woll@kws.com
Cheryl Wong	Truman State University Magruder Hall 3064 Kirksville, MO 63501 USA	660-665-8910	ccw133@truman.edu
John Woodward	Cornell University 228 Plant Sciences Ithaca, NY 14852 USA	(607) 255-1160	jbw46@cornell.ed
Jian Wu	North Carolina State University Plant Biology Department Box 7649 851 Main Campus Dr Partner III Room 205A Raleigh, NC 27695 USA	919-515-3570	jwu3@ncsu.edu
Xianting Wu	Pennsylvania State University 607 Mueller Lab University Park, PA 16802 USA	(814) 863-4022	xzw104@psu.edu
Yusheng Wu	South Dakota State University Dept of Biology and Microbiology, Box 2140D Northern Plains Biostress Lab 252 Brookings, SD 57007 USA	660-688-5493	yshmh2@yahoo.com
Eleanore Wurtzel	Lehman College The City University of New York	(718) 960-8643	wurtzel@lehman.cuny.edu
Jian Hong Xu	Rutgers University 190 Frelinghuysen Rd Piscataway, NJ 8854 USA	(732) 445-3801	jianhong@waksman.rutgers.edu
Masanori Yamasaki	Kobe University Food Resources Education & Research Ctr 1348 Uzurano Kasai Hyogo 675-2103, Japan		yamasakim@tiger.kobe-u.ac.jp
Jianbing Yan	CIMMYT Apdo Postal 6 641 Mexico Mexico 06600, Mexico	(595) 952-1900 1384	J.Yan@cgiar.org
Xiang Yang	Iowa State University 2282 Molecular Biology Building Pammel Dr Ames, IA 50011 USA	(515) 294-0137	yangx@iastate.edu
Hong Yao	University of Missouri - Columbia Dept of Biological Sciences 117 Tucker Hall Columbia, MO 65211 USA	(573) 882-4871	yaoho@missouri.edu

Immanuel Yap	Gramene 259 Emerson Hall Cornell University Ithaca, NY 14853 USA	607 2552089	ivy1@cornell.edu
Kai Ying	Iowa State University Schnable Lab Carver Co-Lab 2043 ISU Ames, IA 50010 USA	515-294-1659	yingk@iastate.edu
Ken Yourstone	Pioneer HiBred 9704 Monroe Ct Urbandlae, IA 50322 USA	(515) 270-5919	kenneth.yourstone@pioneer.com
Jianming Yu	Kansas State University 2004 Throckmorton Hall Department of Agronomy Manhattan, KS 665065501 USA	(785) 532-3397	jyu@ksu.edu
JuKyung Yu	Syngenta Seed 317 330th St Stanton, MN 55018 USA	(507) 663-7681	ju-kyung.yu@syngenta.com
Weichang Yu	University of Missouri 117 Tucker Hall Columbia, MO 65211 USA	(573) 882-4556	wy593@mizzou.edu
Yeisoo Yu	University of Arizona Arizona Genomics Institute 303 Forbes Building POBox 210036 Tucson, AZ 85721 USA	(520) 626-9585	yeisooyu@ag.arizona.edu
Michael Zanis	Purdue University 915 W. State St. BTNYPurdue University West Lafayette, IN 47907 USA	(765) 494-6606	mzanis@purdue.edu
Feng Zhang	University of Georgia Rm 4505 Miller Plant Sciences Bldg Athens, GA 30603 USA	(706) 542-1857	tzhangf@plantbio.uga.edu
Han Zhang	University of Georgia 2502 Miller Plant Sciences Building Room 4608 Athens, GA 30605 USA	706-542-1010	imphan@uga.edu
Hongbin Zhang	Texas AM University Department of Soil and Crop Sciences 2474 TAMU College Station, TX 778432474 USA	(979) 862-2244	hbz7049@tamu.edu
Jianbo Zhang	Iowa State University 2288 Molecular Biology Building Department of Genetics Development and Cell Biolog Ames, IA 50011 USA	515 294 5054	jzhang@iastate.edu
Lifang Zhang	Cold Spring Harbor Laboratory 1 Bungtown Road Cold Spring Harbor Laboratory Cold Spring Harbor, NY 11724 USA	(516) 367-8330	zhangl@cshl.edu
Wei Zhang	University of Illinois Urbana-Champaign 389 ERML 1201 W Gregory Dr Urbana, IL 61801 USA	217-417-9005	wzhang25@uiuc.edu

Xiaolan Zhang	University of Georgia Miller Plant Sciences Building Room 2502 Athens, GA 30602 USA	(706) 542-1010	xzhang@plantbio.uga.edu
Zhiwu Zhang	Cornell University 175 Biotechnology Cornell University Ithaca, NY 14853 USA	(607) 255-3911	zz19@cornell.edu
Han Zhao	University of Illinois 389 ERML 1201 W Gregory Dr Urbana, IL 61801 USA	(217) 244-6146	zhaohan@uiuc.edu
Yonglian Zheng	Huazhong Agri. Univ. State Key Laboratory of Crop Genetic Improvement Huazhong Agri. Univeristy Wuhan Hubei 430070, China	(862) 787-286870	yonglianzheng@gmail.com
Qing Zhou	BASF Plant Science LLC. 26 Davis Dr. Research Triangle Park, NC 27709 USA	(919) 547-2395	qing.zhou@basf.com
Shiguo Zhou	University of Wisconsin Madison Biotechnology Center 425 Henry Mall Madison, WI 53706 USA	(608) 265-7930	szhou@wisc.edu
Shane Zimmerman	USDAARS S123 Turner Hall University of Illinois Urbana, IL 61801 USA	(217) 333-6631	sazimmer@uiuc.edu
Robert Zimmermann	Washington University Center for Geno Sciences 4444 Forest Park Ave Box 8510 Saint Louis, MO 63108 USA	314-413-8797	RPZ@CSE.WUSH.edu
Roman Zimmermann	ZMBP Tuebingen Germany ZMBP Center for Plant Molecular Biology Dept Auf der Morgenstelle 28 72076 Tuebingen, Germany	(497) 071-2974608	roman.zimmermann@zmbp.uni-tuebingen.de
Jared Zyskowski	University of Wisconsin-Madison Agronomy/Plant Breeding and Plant Genetics 1575 Linden Dr Madison, WI 53706 USA	310-977-3177	zyskowski@wisc.edu