48th ANNUAL MAIZE GENETICS CONFERENCE

PROGRAM
&
ABSTRACTS

9 - 12 MARCH 2006

ASILOMAR CONFERENCE GROUNDS
PACIFIC GROVE, CALIFORNIA

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National Science Foundation

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Renessen

We thank these contributors for their generosity!

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General Information

Meals

All meals will be served family style in Crocker Dining Hall. Your first course, beverages and desert will be on the table, entrées are picked up from the kitchen. Breakfast is served from 7:00 to 8:00 AM. Lunch is available 12:30 to 1:30. If desired, a box lunch for Sunday can be individually requested up to 48 hours in advance. Dinner will be served from 6:00 PM to 7:00 PM on Thursday, Friday, and Saturday. Coffee, tea, bottled water and soft drinks are available at no charge during beverage breaks and the extended poster sessions.

Talks and Posters

All talks and community discussions will be in Merrill Hall. All posters will be presented in the tent structure south of Merrill Hall. Posters should be hung Thursday evening and stay up until Sunday, but must be removed by noon on Sunday.

Hospitality

After the evening session on Thursday and Friday there will be informal socializing in the poster tent. On Saturday evening there will be a complementary dessert reception from 7:30 to 11:00 at the nearby Monterey Bay Aquarium. Refreshments will be available via coupon redemption and/or cash both Thursday and Friday nights until midnight and additional refreshments will be available for purchase at the Monterey Bay Aquarium. Also, from 12:00 AM to 3:00 AM each evening, the Curlew Room will be available for informal socializing Asilomar maintains an in-house catering policy; food and beverage may not be brought on grounds.

Steering Committee

Please share your suggestions and comments about the meeting with the 2006 Steering Committee:

Jay Hollick, Chair (hollick@nature.berkeley.edu)
Anne Sylvester, co-Chair (annesyl@uwyo.edu)
Monika Frey (monika.frey@wzw.tum.de)
Marja Timmermans (timmerma@cshl.edu)
Richard Schneeberger (rschnee@ceres-inc.com)
Ed Buckler (esb33@cornell.edu)
Erin Irish (erin-irish@uiowa.edu)
Jorge Nieto-Sotelo (jorge@ibt.unam.mx)
Tom Brutnell (tpb8@cornell.edu)
Wesley Bruce (wes.bruce@pioneer.com)
Marty Sachs, local organizer for Midwest meetings, 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 Siegfried 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 Amy J. Sheets.

Next Maize Genetics Meeting

The 49th Annual Maize Genetics Conference will be held March 22-25, 2007 at the Pheasant Run Resort, St. Charles, IL (near Chicago). Local organizer is Marty Sachs (msachs@uiuc.edu).

Schedule of Events

Thursday, March 9

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: Monika Frey
7:15 PM	**Kathleen Newton, University of Missouri-Columbia** *Mitochondrial genomes in maize and its relatives*
8:05 PM	**Robert Pruitt, Purdue University** *Non-Mendelian inheritance of DNA sequence information in Arabidopsis*
9:00 PM – 12:00 AM	**INFORMAL POSTER VIEWING, & HOSPITALITY**

Friday, March 10

7:00 AM – 8:00 AM	**BREAKFAST**
8:00 AM – 8:15 AM	**ANNOUNCEMENTS**
8:15 AM – 10:15 AM	**SESSION 2 – DEVELOPMENT** Chair: Richard Schneeberger
8:15 AM	**Mike Muszynski** *The* delayed flowering1 *(*dlf1*) gene encodes a bZIP protein regulating floral signals at the shoot apical meristem in maize*
8:35 AM	**Ivan Acosta, Yale University** *Maize Sex Determination Gene* tasselseed1 *is a Lipoxygenase that Mediates Pistil Cell Death*
8:55 AM	**Susan Parkinson, University of California-Berkeley** *Restriction of* Silkless1 *action by a component required to maintain epigenetic paramutant states ensures monoecious development in maize*
9:15 AM	**Vicki Chandler, University of Arizona** *An RNA-Dependent RNA Polymerase is Required for Paramutation, an Allele Interaction that Establishes a Heritable Chromatin State*
9:35 AM	**Marja Timmermans, Cold Spring Harbor Laboratory** *Maize leaf polarity is specified through the opposing activity of two small regulatory RNAs*
9:55 AM	**Hector Candela, University of California-Berkeley** *Genetic and molecular analyses of* milkweed pod *mutants*

10:15 AM – 10:45 AM **BREAK W/ BEVERAGES**

10:45 AM – 12:25 PM **SESSION 3 – GENOMICS** Chair: Ed Buckler

10:45 AM **Rebecca Boston, North Carolina State University**
Gene Expression and Phylogenetic Relationships Reveal a Complex Protein Disulfide Isomerase Family

11:05 AM **Michael Scanlon, Cornell University**
Laser Microdissection-Microarray Analyses Of Narrow Sheath1*, A Gene Required For Maize Leaf Development*

11:25 AM **Brad Barbazuk, Donald Danforth Plant Science Center**
Gene discovery and annotation using 454 transcriptome sequencing

11:45 AM **Dan Rokhsar, US Department of Energy Joint Genome Institute**
Shotgunning the maize genome

12:05 PM **Richard Wilson, Washington University School of Medicine**
Sequencing the maize genome

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 – 6:00 PM **COMMUNITY FORUM** Chair: Tom Brutnell

5:00 PM **Post-Genomics Panel Discussion**
A community discussion of emerging technologies and techniques to fully exploit maize genome sequence data

6:00 PM – 7:15 PM **DINNER**

7:15 PM – 8:55 PM **SESSION 4 – PLENARY TALKS** Chair: Wes Bruce

7:15 PM **Joachim Messing, Waksman Institute, Rutgers University**
Structure and architecture of the maize genome

8:05 PM **Ottoline Leyser, University of York**
Calling long distance: Hormonal communication between meristems

9:00 PM **INFORMAL POSTER VIEWING & HOSPITALITY**

Saturday, March 11

7:00 AM – 8:15 AM **BREAKFAST**

8:15 AM – 10:15 AM **SESSION 5 –** Chair: Marja Timmermans
CELL BIOLOGY / BIOCHEMICAL GENETICS

8:15 AM **Mihaela-Luiza Marton, University of Hamburg**
Micropylar Pollen Tube Guidance in Maize by ZmEA1 and Discovery of Novel EA1-Box Protein Genes

8:35 AM **Rene Lorbiecke, University of Hamburg**
The plastid zinc ribbon factor AtZR1 from Arabidopsis is the functional ortholog of the maize Etched1 *protein and is required for plant viability*

8:55 AM **Martha James, Iowa State University**
Regulatory functions of the sugary1 *and* dull1 *genes in starch biosynthesis*

9:15 AM **David Braun, Pennsylvania State University**
Tie-dyed1 *encodes a novel gene controlling carbohydrate accumulation in leaves*

9:35 AM **Hank Bass, The Florida State University**
Identification of gene families encoding double-stranded telomere repeat DNA-binding and related proteins

9:55 AM **Jonathan Lamb, University of Missouri-Columbia**
Development of chromosome paints and single loci FISH probes for somatic chromosomes

10:15 AM – 10:45 AM **BREAK W/ BEVERAGES**

10:50 AM – 12:25 PM **SESSION 6 – GENOME STRUCTURE** Chair: Anne Sylvester

10:45 AM **Wolfgang Goettel, Waksman Institute, Rutgers University**
Allelic diversity of the p *locus by changes and replacements of regulatory sequences*

11:05 AM **Qinghua Wang, Waksman Institute, Rutgers University**
Unprecedented haplotype variability in the maize bz *genomic region*

11:25 AM **Georgia Davis, University of Missouri-Columbia**
Maize root transcripts are clustered in the genome on the basis of water stress-response and function

11:45 AM **Michael Zanis, Purdue University**
Fate and consequence of the Zag1 *and* Zmm2 *gene duplication in grasses*

12:05 PM **Michael Freeling, University of California-Berkeley**
Biased fractionation and intragenomic footprinting in plants

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.

6:00 PM – 7:00 PM **DINNER**

7:30 PM – 11:00 PM **DESSERT RECEPTION AT THE MONTEREY BAY AQUARIUM**

Sunday, March 12

7:00 AM – 8:00 AM **BREAKFAST**

8:00 AM – 8:15 AM **ANNOUNCEMENTS**

8:15 AM – 10:15 AM **SESSION 7 – QUANTITATIVE TRAITS / BREEDING** Chair: Jorge Nieto-Sotelo

8:15 AM **Robert Stupar, University of Minnesota**
Analysis of allelic variation in gene expression in B73 and Mo17 and implications for hybrid expression patterns

8:35 AM **William Briggs, University of Wisconsin-Madison**
QTL Analysis of Morphological Traits in a Large Maize-Teosinte Backcross Population

8:55 AM **Jianming Yu, Institute for Genomic Diversity, Cornell University**
Power analysis of an intergrated mapping strategy: Nested Association Mapping

9:15 AM **Gael Pressoir, Institute for Genomic Diversity, Cornell University**
Marker based polygene control and its application to the dissection of complex traits and estimation of variance components

9:35 AM **Owen Hoekenga, Boyce Thompson Institute for Plant Research**
Interdisciplinary analysis of aluminum tolerance in maize

9:55 AM **Narasimham Upadyayula, University of Illinois**
Enhanced Detection of Inflorescence Architecture QTL in Intermated B73 x Mo17 (IBM) RIL Population

10:15 AM – 10:35 AM **BREAK WITH BEVERAGES**

10:35 AM – 12:15 PM	**SESSION 8 – TRANSPOSONS**	Chair: Erin Irish
10:35 AM	**Margaret Woodhouse, University of California, Berkeley** *The Epigenetic Regulation of* Mutator *Transposons in Maize*	
10:55 AM	**Thomas Peterson, Iowa State University** *Alternative transposition of the maize* Ac/Ds *transposable element system induces chromosome breakage and major chromosomal rearrangements*	
11:15 AM	**Liza Conrad, Boyce Thompson Institute** *Characterization Ac-immobilized: a stabilized source of transposase and its use in a two component* Ac/Ds *gene tagging program in maize*	
11:35 AM	**Guojun Yang, University of Georgia** *The first active plant* Tc1/mariner*-like element: transposition of the rice* Osmar5 *in yeast*	
11:55 AM	**Matt Estep, University of Georgia** *Building new tools for the identification and characterization of repetitive DNA from incomplete data sets*	
12:15 PM – 12:25 PM	**ANNOUNCEMENTS**	
12:30 PM – 1:30 PM	**LUNCH & ADJOURNMENT**	

Posters

Biochemical Genetics

P1		*Withdrawn*
P2	**Leonardo Iniguez** <aliniquez@wisc.edu>	*Antisense expression analysis in the maize transcriptome and microarray cross platform comparisons*
P3	**Li Fan** <fanli@iastate.edu>	*Candidate gene cloning of glossy genes*
P4	**Andrea Skirpan** <als152@psu.edu>	*Characterization of BIF2 interacting proteins in maize axillary meristem development*
P5	**Wes Bruce** <wes.bruce@pioneer.com>	*Characterization of the orthologous WUSCHEL gene family in maize*
P6	**Lacey Strickler** <lace_j@yahoo.com>	*Characterization of the zebra lesion mimic (zl1) mutation in maize*
P7	**Deborah Groth** <dgroth@purdue.edu>	*Discovering Novel Starches for Fuel and Health: High-Throughout Screening of EMS Mutagenized Maize for Altered Starch Digestibility*
P8	**Xiquan Gao** <xgao@ag.tamu.edu>	*Disruption of a maize 9-lipoxygenase gene results in increased resistance to fungal pathogens and reduced levels of contamination with mycotoxin fumonisin*
P9	**Nikolaos Georgelis** <gnick@ufl.edu>	*Elucidation of the Differential Divergence Between the Two Subunits of ADP-glucose Pyrophosphorylase*
P10	**Andreas Fiesselmann** <a.fiesselmann@wzw.tum.de>	*Evolution of Indole-3-glycerol Phosphate Lyases in the Grasses*
P11	**Salvador Moguel** <smoguel2@unlnotes.unl.edu>	*Expression of Codon Optimized Fluorescent Marker Genes in Maize*
P12	**Klaas van Wijk** <kv35@cornell.edu>	*Functional differentiation of bundle sheath and mesophyll maize chloroplasts determined by comparative proteomics*
P13	**Feng Zhang** <fzhang@plantbio.uga.edu>	*Gene conversion between direct non-coding repeats promotes genetic and phenotypic diversity at a regulatory locus of Zea mays (L.)*
P14	**Jeffrey Church** <jbchurch@uiuc.edu>	*Genetic Control of the Carbon-Nitrogen Balance in Leaves of the Illinois Protein Strains*
P15	**Bryan Gibbon** <bgibbon@email.arizona.edu>	*Genetic analysis of opaque2 modifier genes*
P16	**Candice Hansey** <cnhansey@wisc.edu>	*Genetic and transcriptional analysis of a sugary enhancer allele*
P17	**Anne Britt** <abbritt@ucdavis.edu>	*Global Transcriptional Response to Ionizing Radiation (IR) is ATM-dependent, while IR-induced G2 arrest is ATR dependent*
P18	**Cristina Lopez** <clpgmm@cid.csic.es>	*Identification of new components of maize SnRK1 complex*
P19	**Tracie Hennen-Bierwagen** <tabier@iastate.edu>	*Identifying Protein-Protein Interactions Among Starch Biosynthetic Enzymes*
P20	**Farag Ibraheem** <fii100@psu.edu>	*Induction and Metabolic Engineering of Sorghum Antifungal Compounds in Maize*
P21	**Changfa Lin** <changfa@waksman.rutgers.edu>	*Isolation and characterization of maize sesquiterpene cyclase2 (stc2) gene involved in insect resistance*

P22 **Farooqahmed Kittur** <kitturf@vt.edu>
Maize Beta-Glucosidase Aggregating Factor (BGAF) is a Polyspecific Jacalin-related Chimeric Lectin and its Lectin Domain is involved in Beta-Glucosidase Aggregation

P23 **Yaqing Du** <yadu@plantbio.uga.edu>
Maize CENPC - a DNA and RNA binding protein

P24 **Mandeep Sharma** <mxs781@psu.edu>
Maize flavonoid 3'-hydroxylase encoded by red aleurone1 (pr1) is required for cyanidin and luteoforol biosynthesis

P25 **Linnea Bartling** <lkbartling@gmail.com>
Making, mapping and characterizing tassel seed mutants in maize

P26 **Mark Williams** <mark.e.williams@usa.dupont.com>
Map-based cloning of the nsf1 (nicosulfuron susceptible 1) gene of maize.

P27 **Hyun Young Yu** <yhy0922@vt.edu>
Mapping of BGAF Binding Regions on the Maize Beta-Glucosidase Isozyme Glu1

P28 **Matthew Meyer** <mrmwc2@mizzou.edu>
Photosystem II genes display a potential mechanism of Lepidopteran resistance.

P29 **Taijoon Chung** <taijoonc@email.arizona.edu>
Pre-mRNA Splicing And rRNA Processing Are Altered in the Endosperm of mto38, a Maize Opaque Mutant.

P30 **Alfons Gierl** <gierl@wzw.tum.de>
Rigidity of Carbohydrate Metabolism in Maize Kernels

P31 **Rita-Ann Monde** <rmonde@purdue.edu>
The Maize TILLING Project: Progress Report for Year 3.

P32 **Xiang Yang** <yangx@iastate.edu>
The RAMOSA Pathway in Maize Inflorescence Determinacy

P33 **Jorg Degenhardt** <degenhardt@ice.mpg.de>
The Role of the Maize Terpene Synthase Gene Family in Plant Defense

P34 **Montserrat Pages** <mptgmm@cid.csic.es>
The maize DIP1 (DBF1-interactor protein1) containing an R3H domain is a potential regulator of DBF1 activity in stress responses

P35 **Sylvia Sousa** <smsousa@unicamp.br>
The role of sorbitol and sorbitol dehydrogenase in maize kernel

P36 **Michael Held** <maheld@purdue.edu>
Viral-induced gene silencing of cellulose synthase (CESA) and cellulose synthase-like (CSL) genes in barley.

Bioinformatics

P37 **Yan Fu** <yfu@danforthcenter.org>
Ab Initio Protein-coding Gene Finding In Maize Genome

P38 **Chi-Ren Shyu** <ShyuC@missouri.edu>
A Computational Approach for Characterizing Standardized Phenotypic Images for Maize

P39 **Yujun Han** <yhan@plantbio.uga.edu>
A novel bioinformatics method for identifying candidate active transposable elements

P40 **Matthieu Falque** <falque@moulon.inra.fr>
A simulation approach to measure crossover interference in linkage maps

P41 **Shiran Pasternak** <shiran@cshl.edu>
Analyzing, Annotating, and Visualizing the Maize Genome Sequence

P42 **Mary Schaeffer** <SchaefferM@missouri.edu>
Applications of Plant Ontologies for describing and comparing phenotypes and gene expression data in plant databases.

P43 **Joerg Vandenhirtz** <joerg@lemnatec.de>
Automatic and 3 dimensional phenotyping of complete corn plants.

P44 **Bryan Penning** <bpenning@purdue.edu>
Cell wall genomics at Purdue: A website resource for cell wall related genes in maize, rice, and Arabidopsis

P45 **Ann Loraine** <aloraine@uab.edu>
Co-expression analysis of the metabolic network in Arabidopsis thaliana

P46 **Chengzhi Liang** <liang@cshl.edu>
Comparative Genome Analysis in Gramene

P47 **Carolyn Lawrence** <triffid@iastate.edu>
Contribute your data to MaizeGDB!

P48 **Olga Nikolova** <olia@truman.edu>
Gene Expression and Visualization Application (GENEVA): Development and Use in Shoot Apical Meristem Gene Expression Analysis

P49 **Matthieu Falque** <falque@moulon.inra.fr>
IRILmap: linkage map distance conversion software for intermated recombinant inbred lines

P50 **Steven Schroeder** <schroedersg@missouri.edu>
Integrated Software for SNP Discovery in Maize

P51 **Darwin Campbell** <darwin@iastate.edu>
MaizeGDB Standard Operating Procedures

P52 **Trent Seigfried** <devolver@iastate.edu>
MaizeGDB: The Maize Community Genetics and Genomics Database

P53 **Matt Scholz** <schmatthew@gmail.com>
Microarray analysis of maize chromatin mutants

P54 **Nigel Walker** <nigel@molbio.uoregon.edu>
Plant Orthologous Groups: A resource for comparative genomics with a focus on predicted RNA binding proteins.

P55 **Carolyn Lawrence** <triffid@iastate.edu>
Predicting Chromosomal Locations of Genetically Mapped Loci in Maize Using the Morgan2McClintock Translator

P56 **Alex Buerkle** <buerkle@uwyo.edu>
Statistical analysis of diverse sequences identifies putative functional domains in maize RABs and SODs

P57 **Zhiwu Zhang** <zz19@cornell.edu>
TASSEL 2.0: A Software Package for Association and Diversity Analyses in Plants and Animals

P58 **Brent Kronmiller** <bak@iastate.edu>
TE Nest: Automated chronological annotation and visualization of maize nested transposable elements

P59 **Matthew Campbell** <campbell@tigr.org>
The TIGR Rice Genome Annotation Database

Cell Biology

P60 **Amanda Wright** <ajwright@biomail.ucsd.edu>
discorcdia1 is required for asymmetric cell division and encodes a PP2A phosphatase regulatory subunit

P61 **Penny Kianian** <kiani002@umn.edu>
Analysis the MAD1 gene of Plants

P62 **Christian Ricks** <cbricks@gmail.com>
Characterization of pearl millet prolamins

P63 **Rebecca Boston** <boston@unity.ncsu.edu>
Differential Accumulation of ZmDerlin RNAs and Proteins during Prolonged ER Stress

P64 **Marina Dermastia** <marina.dermastia@bf.uni-lj.si>
Endophytic biological associations between bacteria and maize are genotype specific

P65 **Brenda Lowe** <brenda.lowe@monsanto.com>
Marker Assisted Breeding for Transformability in Maize

P66 **Mark Lubkowitz** <mlubkowitz@smcvt.edu>
Members of the Oligopeptide Transporter family function during germination in rice

P67 **Rachel Wang** <rachelcjw@berkeley.edu>
The distribution of AM1 during the meiotic cell cycle suggests that it functions both as a cell cycle switch and as a regulator of meiotic prophase progression

Cytogenetics

P68 **Ron Okagaki** <okaga002@umn.edu>
A Comparison of Centromere Mapping Techniques

P69 **Inna Golubovskaya** <innagol@uclink4.berkeley.edu>
Alleles of AFD1 uncouple axial element elongation and bouquet formation from RAD51 distribution and homologous pairing

P70 **William F. Sheridan** <bill_sheridan@und.edu>
Compound B-A Translocations: Creating B-A-A Translocations and Subdividing the Maize Chromosomes

P71 **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

P72 **Ina Amarillo** <feamarillo@bio.fsu.edu>
Construction of a High-Density Cytogenetic Map of Maize Chromosome 9 Using Sorghum BACs as FISH Probes

P73 **David Weber** <dfweber@ilstu.edu>
Cytogenetic Analysis of Transposon-Induced Chromosomal Rearrangements in Maize.

P74 **Tatiana Danilova** <danilovat@missouri.edu>
Development of PCR based FISH probes for identification of maize mitotic chromosomes.

P75 **Jennifer Holland** <jenniferjholland@mizzou.edu>
FISH as a means for detecting gene targets in mitotic Zea mays chromosomes

P76 **Kathleen Newton** <NewtonK@missouri.edu>
Organelle DNA Insertions into Maize Chromosomes

P77 **Jinghua Shi** <jshi@plantbio.uga.edu>
Partitioning of the maize epigenome by the number of methyl groups on histone H3 lysines 9 and 27

P78 **Fangpu Han** <hanf@missouri.edu>
Reactivation of nondisjunction for an inactive B centromere and minichromosomes derived from the B chromosome

P79 **Matthew Bauer** <mjbc4b@mizzou.edu>
The Effect of Interploidy Crosses on the Structure of Endoreduplicated Chromosomes

Developmental Genetics

P80 **Philippa Barrell** <barrellp@crop.cri.nz>
nrm2, a mutant defective in cytokinesis during meiosis

P81 **John Doebley** <jdoebley@wisc.edu>
A Distant Upstream Enhancer at the Maize Domestication Gene, tb1, has Pleiotropic Effects on Plant and Inflorescent Architecture

P82 **Daniel Koenig** <dpkoenig@ucdavis.edu>
A Genetic Basis for Variation of Complexity in Simple and Compound Leaves

P83 **Andrea Gallavotti** <agallavotti@ucsd.edu>
A Screening for Enhancers/Suppressors of the Ramosa1 Mutant Uncovered Two Novel Mutations Enhancing Branching in Maize

P84 **Diego Fajardo** <diegof@ufl.edu>
A potential role for the rough endosperm 3 (rgh3) mutant in endosperm-embryo interactions during seed development

P85 **Peter Bommert** <bommert@cshl.edu>
Analysis of the maize mutant compact plant2 (ct2)

P86 **Clinton Whipple** <cwhipple@biomail.ucsd.edu>
B class gene expression in a basal grass and non-grass outgroups confirms that the lodicule is homologous with the second whorl of monocot flowers

P87 **John Woodward** <jwoodward@plantbio.uga.edu>
Bladekiller_1 is Required for Shoot Meristem Maintenance and Development of the Maize Leaf Blade

P88	**Nils Muthreich** <nils.muthreich@zmbp.uni-tuebingen.de>	*Cell-type specific transcriptome profiling of shoot-borne root initiation in maize (Zea mays L.)*
P89	**R. Frank Baker** <rfb11@psu.edu>	*Clonal mosaic analysis indicates that Tie-dyed1 functions in the innermost layer of leaves*
P90	**Jiabing Ji** <jiabing@plantbio.uga.edu>	*Comparative genetic analyses of WOX4 function in the development of shoot lateral organs in Arabidopsis and tomato*
P91	**Eric Riedeman** <riedeman@wisc.edu>	*Divergent selection for vegetative phase change and its affects on resistance to common rust (Puccinia sorghi) and European corn borer (Ostrinia nubilalis)*
P92	**Jorge Nieto-Sotelo** <jorge@ibt.unam.mx>	*Effects of Hsp101 activity on adventitious root formation at the coleoptilar node in maize seedlings.*
P93	**Prem Chourey** <pschourey@ifas.ufl.edu>	*Evidence of possible cross-talk between sugar and hormone signaling in developing endosperm of maize*
P94	**Brandi Sigmon** <bsigmon@iastate.edu>	*Exploring the Role of ramosa1 in the Derivation of Domestic Maize*
P95	**Amanda Robinson** <arrobins@owu.edu>	*Expression Studies in Maize Homologs of Arabidopsis Floral Regulators.*
P96	**Javier Mendiola** <jmendiol@ira.cinvestav.mx>	*Functional role of ATRX and MOT-1 in female gametophyte development*
P97	**Alvar Carlson** <arcarlson@wisc.edu>	*Genetic Analysis of Somatic Embryogenesis in Maize Inbred A188*
P98	**Solmaz Barazesh** <sxb944@psu.edu>	*Genetic analysis of Bif1 demonstrates a role in axillary meristem initiation*
P99	**Nick Lauter** <nickl@iastate.edu>	*Gibberellic acid stimulates expression of microRNA172 to promote vegetative adulthood in maize*
P100	**Lionel Brooks** <lb259@cornell.edu>	*Global Expression Analysis of Early Events in Maize Leaf Initiation*
P101	**Brent Buckner** <bbuckner@truman.edu>	*Global Gene Expression Patterns in the Maize Shoot Apical Meristem*
P102	**Xianting Wu** <xzw104@psu.edu>	*Interaction of barren inflorescence 2 (bif2) and barren stalk 1 (ba1) in maize inflorescence development*
P103	**Byeong-Ha Lee** <leeb@cshl.edu>	*Interactions between ABPHYL1 and auxin polar transport in the shoot apical meristem*
P104	**Brad Townsley** <bttownsley@ucdavis.edu>	*KNOX evolution in monocots*
P105	**Magdalena Segura-Nieto** <msegura@ira.cinvestav.mx>	*Localization of three Actin Genes and their Proteins during Maize Seed Development*
P106	**Tiffany Langewisch** <langewit@purdue.edu>	*Maize Brittle Stalk2 encodes a COBRA-like protein expressed in early organ development and is necessary for development of tissue mechanical strength at maturity*
P107	**Erin Irish** <erin-irish@uiowa.edu>	*Microarray analysis of vegetative phase change in maize*
P108	**Mingshu Huang** <muh147@psu.edu>	*Molecular study of camouflage1, a gene involved in the chlorophyll biosynthesis pathway*
P109	**Nick Carpita** <carpita@purdue.edu>	*Neural network analyses of infrared spectra for classifying cell wall architectures*
P110	**Darren Hall** <hall.darren@gmail.com>	*New mutants affecting maize floret and spikelet development*

P111 **Ambika Chandra**
<auc135@psu.edu>
Parasitic castration by a stinking smut increases sexual reproductive allocation of its host buffalograss, in part by down-regulating a homologue of Tasselseed2 (Ts2)

P112 **Theresa Miller**
<theresa.miller@marquette.edu>
Photoperiod Regulation and Expression Profile of CONSTANS-like Genes in Maize

P113 **Andrea Eveland**
<aeveland@ufl.edu>
Regulation of C-Allocation in Developing Maize Florets: Implications for Seed Set and Grain Yield

P114 **Steven Runo**
<smruno@ucdavis.edu>
Striga KNOX1 (KNOTTED1-like homeobox) RNAi as a resistance mechanism in maize and sorghum

P115 **Masaharu Suzuki**
<msuzuki@mail.ifas.ufl.edu>
The Wpk1/Vp8 gene regulates aleurone differentiation in a region-specific pattern.

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QTL Analysis of Rice Tungro Disease

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Rapid and reliable purity identification of maize (Zea may L.) F1 hybrids using SSR marker

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P246 **Cesar Alvarez-Mejia** <calvarez@ira.cinvestav.mx>
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P248 **Wensheng Qin** <qinw@stanford.edu>
Function Characterization of MURB Protein in Maize

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Origin and diversification of maize White cap haplotypes

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P254 **Jin Li** <jinli@iastate.edu>
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P255 **Natalie Jameson** <nataliejameson@mac.com>
Recent Capture of Maize Cytochrome P450 Monooxygenase Gene by a Helitron

P256 **Ling Bai** <lb226@cornell.edu>
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P257 **George Rudenko** <rudenko@stanford.edu>
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P258 **Cagla Altun** <caltun@purdue.edu>
The Maize Mre11/Rad50/Nbs1 DNA repair and recombination complex

Plenary Abstracts

Plen1

Mitochondrial genomes in maize and its relatives

(presented by Kathleen Newton <NewtonK@missouri.edu>)
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Analyses of cytoplasmic mutations have shown that functional mitochondrial genomes are essential for plant growth and development, as well as for male fertility. To increase our understanding of the organization and evolution of plant mitochondrial genomes, mitochondrial DNAs (mtDNAs) have been sequenced and compared from maize NB, NA, CMS-C, CMS-S and CMS-T cytotypes, from the teosintes Zea mays ssp. parviglumis, Zea luxurians and Zea perennis and from the related grasses Tripsacum dactyloides and Sorghum bicolor. Extensive rearrangements distinguish maize mitochondrial genotypes and the variation in the sizes of the genomes is due mainly to large repeated regions. All of the Zea mtDNAs encode a common set of functional genes, but show variation in their "chimeric" open reading frames. Sequence similarity is very high in the coding regions and nearly as high in the non-coding regions. Phylogenetic relationships can be inferred from the percentage of shared mitochondrial sequence as well as from nucleotide substitutions. Both methods show that mtDNA from Z. mays ssp. parviglumis is more closely related to mtDNAs from the maize NB, NA and CMS-C cytotypes than to those from CMS-S and CMS-T. All of the Zea mtDNAs examined share greater than 88% of their sequences. With greater phylogenetic distance, nucleotide substitutions and small indels increase, as does the number of larger mitochondrial segments that have no apparent sequence similarity. In non-coding regions, mitochondrial genomes from Zea have essentially no sequences in common with those from dicots, and only very limited regions of similarity with those from rice. Analysis of plastid DNA sequences that have integrated into Zea mitochondrial genomes suggests that interorganellar DNA transfer is continuing. After integration, plastid DNA segments are dispersed and lost via mitochondrial recombination.

Plen2

Non-mendelian inheritance of DNA sequence information in Arabidopsis

(presented by Robert Pruitt <pruittr@purdue.edu>)
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Stable inheritance of allelic information represents one of the cornerstones of classical mendelian genetics. While various forms of genetic instability are well documented, none represent a general, genome-wide phenomenon that allows non-mendelian inheritance of specific genetic information. In contrast to this, progeny of hothead (hth) mutant plants are able to inherit allele specific DNA sequence information that was not present in the genomes of their parents, but was present in the genomes of their grandparents. All mutant alleles of the HTH locus share the unusual property of reverting to wild type with a frequency of approximately 10-1 revertants/chromosome/generation. All of the mutant alleles contain single nucleotide substitutions and in the revertants these nucleotides are specifically restored to the wild-type sequence. These restoration events are not the result of any previously characterized genetic process and similar genetic changes that restore ancestral genetic information at numerous loci scattered throughout the genome can be observed in the hothead mutant background. These data strongly suggest that, at least in these mutant plants, there is a cache of genetic information that is inherited outside the conventional DNA genome that can be utilized to guide subsequent genetic modifications.

Plen3

Structure and Architecture of the Maize Genome

(presented by Joachim Messing <messing@waksman.rutgers.edu>)
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Initial sequence analysis of the maize genome yielded important information prior to its complete sequencing. Maize arose by whole genome duplication (WGD) as recently 4.8 million years ago. About 70% of the 2,365-Mb B73 genome consists of repetitive sequences that are mainly retroelements (95%). A third of them come from just four retroelement families, Ji, Cinful, Opie, and Zeon. Most of these retroelements arose after WGD, making the maize genome a very "young" genome. About 22.5% is low-copy non-coding sequences and 7.5% coding sequences. Comparison of duplicated regions with rice shows an uneven expansion as well contraction of chromosomal regions. Birth and death of genes is widespread, indicating a very rapid life cycle of genes. Genes tend to be larger than in rice in part to larger average intron sizes. Differential chromosome expansion and gene amplification or loss does not create a bias in chromosomal methylation pattern. There is a good hit rate of genes by methyl-filtered and cot-derived sequences, but coverage in flanking regulatory sequences is much lower. However, based on the physical map, more than 90% of the genome is available in overlapping clones. Sequence assembly of clones indicates that a sequence collinear with the genetic map is now feasible.

Plen4

Calling long distance- hormonal communication between meristems

(presented by Ottoline Leyser <hmol1@york.ac.uk>)
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Balancing root and shoot growth, and primary and secondary shoot growth necessarily requires long-distance signalling between the root and the shoot, and between primary and secondary shoot apical meristems. Classically both shoot-derived auxin and root-derived cytokinin have been implicated in these processes. More recently, additional components of the network of signals that regulate shoot branching have been identified. Mutants at 4 loci, called MAX1-MAX4, with increased shoot branching appear to define an additional branch-inhibiting pathway that interacts with auxin to mediate branch inhibition. Grafting studies have demonstrated that three of these loci are involved in the production of a long-range graft transmissible signal that inhibits bud growth, and this is consistent with the molecular identities of these genes. Progress in identifying this new hormone and in unravelling its interactions with auxin and cytokinin will be presented.

Short Talk Abstracts

T1

The delayed flowering1 (dlf1) gene encodes a bZIP protein regulating floral signals at the shoot apical meristem in maize

(presented by Mike Muszynski <mgmuszynski@gmail.com>)
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The transition from vegetative to reproductive development is a critical point of plant development, ensuring the reproductive success of the species. Relatively few genes regulating this important agronomic trait have been identified for monocots. The sole maize flowering time gene cloned is indeterminate1 (id1) which regulates a leaf-generated signal required for the transition to flowering. We have cloned the delayed flowering1 (dlf1) gene which also promotes the floral transition in maize. dlf1 mutants are late flowering, having an extended vegetative stage of growth & producing more leaves compared to wild type plants. A standard Mutator transposon tagging strategy was used to generate six new Mu-tagged dlf1 alleles. A modified PCR-based cloning method (Selected Amplification of Insertion Flanking Fragments, SAIFF) was used to isolate dfl1 gene-specific sequences. dlf1 encodes a protein with homology to bZIP transcription factors with a basic domain, leucine zippers & several putative phosphorylation sites. 3-D protein modeling of a DLF1 missense mutation revealed a sensitive amino acid position that is predicted to affect DNA binding affinity. The dlf1 gene is most highly expressed in the shoot apical meristem. Transcript accumulates during vegetative growth, peaks prior to the floral transition & then diminishes in early reproductive growth. This pattern of expression of dlf1 is partially dependent on id1 functions, as dlf1 transcript does accumulate in id1-m1 mutants but is static. Homologous proteins were identified from the rice genome & wheat EST databases. The monocot DLF proteins & Arabidopsis AtbZIP14 & AtbZIP27 define a distinct DLF-like bZIP subgroup. Loss-of-function mutations in the Arabidopsis AtbZIP14 gene (recently identified as the floral activator FD) cause a delay in flowering time, confirming that the DLF subgroup A of bZIP proteins share a role in regulating the floral transition in both monocots & dicots.

T2

Maize Sex Determination Gene tasselseed1 is a Lipoxygenase that Mediates Pistil Cell Death

(presented by Ivan Acosta <ivan.acosta@yale.edu>)
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The sex determination (SD) pathway of maize, a developmental cascade leading to the formation of unisexual flowers, provides a unique non-animal genetic system to study cell death and cell arrest. Distinct processes have been associated with this SD pathway, including tasselseed-mediated pistil cell death in staminate florets and gibberellin (GA)-mediated stamen cell arrest in pistillate florets. Here, we report the isolation by positional cloning of the tasselseed1 gene, which encodes a putative lipoxygenase (lox_ts1), a nonheme iron containing fatty acid dioxygenase. Characterization of the lox gene in nine ts1 mutant lines showed that all contain different mutations thus confirming unequivocally that the lox gene is ts1. Expression analysis by real-time RT-PCR analysis demonstrate that lox_ts1 is mainly transcribed in developing tassel inflorescences in what it seems an oscillating pattern and in situ hybridizations on the same tissue show that lox_ts1 is expressed in aborting pistil primordia. The LOX_TS1 protein is predicted to belong to the type 2 LOX subfamily, whose members carry a putative chloroplast transit peptide and specifically oxygenate the fatty acid at carbon atom 13 (13-LOX) of the hydrocarbon backbone. Assays to test the subcellular localization of LOX_TS1 are under way. A recombinant LOX_TS1 heterologously expressed in Escherichia coli is currently being tested for activity with the most common lipoxygenase substrates. A discussion will be presented about how LOX_TS1 leads to pistil cell death and how it may act along with another maize sex determination protein, the short-chain alcohol dehydrogenase TS2.

T3

Restriction of *Silkless1* action by a component required to maintain epigenetic paramutant states ensures monoecious development in maize

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From analysis of maize *silkless1 (sk1)*, tasselseed- and anther ear-type mutations we know monoecious inflorescence architecture develops through selective organ abortion or arrest in initially perfect flowers (1). In tassel flower primordia, pistils abort, allowing stamen maturation. Conversely, in ears, pistils develop while stamen initials arrest. Tissue-specific function of the factor encoded from the *sk1* locus is hypothesized to be the key difference between these two alternate fates as pistil development is always aborted in *sk1* mutant plants (2). Calderon-Urrea Dellaporta (1) showed that *tasselseed1 (ts1)* and *tasselseed2 (ts2)* work in the same pathway leading to pistil cell death; however, as *ts2* is expressed in all pistil primordia, they proposed that *Sk1* alleles act to protect primary ear pistils from *ts2*-mediated cell death. This model requires that *Sk1* be restricted from acting in primary tassel florets. Our observation of tassel feminization in plants homozygous for mutant alleles of *required to maintain repression6 (rmr6)*, a genetic component required to establish and maintain epigenetic paramutant states at multiple loci (3), suggested that *Rmr6* might be responsible for this regulation of *Sk1* action. Double mutant analyses show that *sk1* is epistatic to *rmr6*; therefore, *Sk1* is ectopically expressed in primary tassel florets of *rmr6* mutant plants. Thus, *Rmr6* functions to ensure the proper tissue-specific expression of *Sk1* required for monoecious inflorescence development. This role is consistent with analyses showing that *Rmr6* maintains transcriptional repression of alleles subject to paramutation (3). The role of epigenetic repression in maintaining maize monoecy demonstrates that such plastic regulatory mechanisms provide a system for rapid morphological evolution, which, in the ancestors of maize provided an agronomically important trait.

1. Calderon-Urrea, A. and S. L. Dellaporta (1999) Development 126: 435-441.
2. Dellaporta S. L. and A. Calderon-Urrea (1994) Science 266: 1501-1505.
3. Hollick, J. B., J. Kermicle, and S.E. Parkinson, (2005). Genetics 171: 725-740.

T4

An RNA-Dependent RNA Polymerase is Required for Paramutation, an Allele Interaction that Establishes a Heritable Chromatin State

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The mop1 gene (mediator of paramutation 1) is required for the establishment of paramutation at three maize genes (b1, pl1, and r1) and to maintain the transcriptionally silenced state at two genes (b1 and pl1). In addition, mop1 mutations reactivate silenced Mutator elements and a subset of transcriptionally silenced transgenes. Plants carrying mutations in the mop1 gene also stochastically exhibit pleiotropic developmental phenotypes. Taken together the mop1 mutant phenotypes demonstrate that the gene plays a critical role in establishing and maintaining specific silent chromatin states. The mop1 gene has been cloned using a map-based and candidate gene approach. Large scale mapping positioned the gene to 0.64 cM on chromosome 2S; within that region very tightly linked markers were identified that showed no recombination with mop1 in a large mapping population (>10,000 individuals). Comparison with the syntenous region in rice and sequences from the 250 kbp of BAC sequence spanning the tightly linked markers identified the RDR101 gene as a strong candidate locus for mop1. The candidate locus was sequenced in two mop1 mutations, mop1-1 (pre-existing in a Mutator population) and mop1-2 (EMS-induced); both mutations contained lesions in RDR101, confirming that mop1 encodes a RNA Dependent RNA polymerase. This maize RDRP is most similar to RDR2 in Arabidopsis thaliana, which has been implicated in the production of trans-acting siRNA molecules. The above data as well as models for how the mop1 gene mediates paramutation will be presented.

T5

Maize leaf polarity is specified through the opposing activity of two small regulatory RNAs

(presented by Marja Timmermans <timmerma@cshl.edu>)
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Adaxial/abaxial polarity directs the flattened outgrowth and patterning of the leaf. In maize, this asymmetry is specified in part through the polarized expression of rolled leaf1 (rld1), a class III homeodomain leucine zipper transcription factor that specifies adaxial/upper fate. Through in situ hybridization analyses we were able to show that the adaxial specific expression of rld1 is set up by the expression pattern of a 21-nucleotide microRNA, miR166. The peak of miR166 expression occurs immediately below the incipient leaf and thus below the domain in which miR166 acts on rld1, but an expression gradient of miR166 is established in developing leaves. These observations led us to propose that miRNA166 may form a movable signal that emanates from a signaling center below the incipient leaf to specify the abaxial domain. Specification of adaxial fate also requires the activity of leafbladeless1 (lbl1). Double mutant and expression analyses indicate that lbl1 acts upstream of rld1 and is required for the accumulation of rld1 transcripts in the developing leaf. We have cloned lbl1 and found that this gene encodes a key component in the biogenesis pathway of a second small regulatory RNA, the trans-acting siRNA, ta-siR2142. Loss of lbl1 function leads to misexpression of miR166 throughout the initiating leaf indicating a role for lbl1 and ta-siR2142 in the spatiotemporal regulation of miR166. These observations present the intriguing possibility that adaxial/abaxial polarity is specified through the opposing action of two small regulatory RNAs, ta-siR2142 and miR166.

T6

Genetic and molecular analyses of *milkweed pod* mutants

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The leaves of maize exhibit polarity along the abaxial-adaxial axis. Our characterization of *milkweed pod-1* (*mwp-1*), a recessive mutation found by Oliver Nelson, indicates that this gene is required for normal abaxial identity in sheath tissue. A second allele, *mwp-2*, was identified among the progeny of crosses between *mwp-1* mutants and plants carrying active *Mu* transposons. Loss of *mwp* function conditions a sectored phenotype that includes extra intermediate veins, absence of the abaxial hypodermal schlerenchyma and overproliferation of abaxial mesophyll cells. In addition, an ectopic ligule is present at the sheath-auricle boundary on the abaxial face of vegetative leaves, a phenotype reported previously only for dominant *Rld1* alleles that escape miRNA regulation. The synergistic phenotype that we observe in *Rld1 mwp* double mutants points to both genes being functionally related. A map-based approach has allowed us to identify a candidate gene within a small genetic interval. The identification of molecular lesions in both alleles, a deletion in *mwp-2* and an insertion in *mwp-1*, supports the idea that we have identified the right gene.

T7

Gene Expression and Phylogenetic Relationships Reveal a Complex Protein Disulfide Isomerase Family

(presented by Rebecca Boston <boston@unity.ncsu.edu>)
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Protein disulfide isomerases (PDIs) catalyze the formation of proper disulfide bonds and participate in endoplasmic reticulum (ER) quality control as part of the cellular ER-stress response. To identify plant PDI-like (PDIL) proteins, we performed a genome-wide search of Arabidopsis thaliana and identified 104 thioredoxin (TRX) domain containing proteins, 22 of which group with orthologs of known plant PDIs in a well-supported clade. Using the Arabidopsis PDIL sequences in iterative BLAST searches, we identified orthologous sets of plant PDIL sequences in rice (19) and maize (22). Comparison of these three plant species representing the two major evolutionary groups in plants provided an explicit view of the sequential evolutionary events that resulted in the diversity observed for this protein family. The phylogenetic analysis resolved the phylogeny into 10 classes. Five classes (I-V) had the two TRX domains typically found in PDIL proteins in other higher eukaryotes while the remaining five classes (VI-X) had a single TRX domain. RNA profiling and quantitative RT-PCR analyses of the maize PDILs (ZmPDILs) showed marked differences in expression within and among classes. The major PDI (ZmPDIL1-1), the class V PDIL (ZmPDIL2-3), and the class VI maize PDIL (ZmPDIL5-1) were up-regulated by ER stress yet ZmPDIL5-1 was not localized to the intracellular membrane fractions when assayed after subcellular fractionation. This study provides a model for effective use of high throughput and genomic sequencing information as a foundation for experiment-based analysis that leads to complete, accurate and permanently useful datasets.

T8

Laser Microdissection-Microarray Analyses Of *narrow sheath1*, A Gene Required For Maize Leaf Development.

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Loss of function mutations in the narrow sheath (ns) duplicate genes (ns1 and ns2) cause the deletion of a mediolateral domain from maize leaves. The ns genes encode duplicate WUSCHEL1-like homeobox (WOX) putative transcription factors that function redundantly and non-cell autonomously to direct recruitment of leaf founder cell-initials in a lateral domain of the shoot apex. In order to investigate the genetic and biochemical mechanisms of NS1 gene function, laser microdissection-microarray analyses (LMM) were used to identify differentially expressed genes in ns mutant meristems. LMM is a powerful technique that permits the isolation of RNA from specific cells and tissues within fixed plant tissues. RNA collected from approximately 10,000 meristematic cells was used in microarray analyses of NS1 function; the relatively large size of the maize vegetative meristem renders this plant especially tractable for LMM procedures. Microarray analyses of over 45,000 maize cDNAs (enriched for meristem-derived cDNAs) identified candidate genes related to NS1 function in the SAM, including genes predicted to be involved in auxin signaling and transport, jasmonate-induced biochemical-genetic pathways, cell division/growth, and chromatin remodeling. Quantitative RT-PCR and in situ hybridizations of novel maize genes are used to verify candidate transcripts differentially expressed in ns mutant apices. These experiments promise to microdissect genetic pathways and networks in maize meristems, and provide novel insight into the mechanisms of lateral organ development.

T9

Gene discovery and annotation using 454 transcriptome sequencing

(presented by Brad Barbazuk <bbarbazuk@danforthcenter.org>)
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454 DNA sequencing technology achieves significant throughput and cost savings relative to traditional Sanger sequencing. Using a combination of wet lab experiments, bioinformatic analyses and simulations we demonstrate that 454 sequencing is an efficient tool for gene discovery and genome annotation in maize. Simulations suggest that 454 sequencing can efficiently capture the bulk of a transcriptome. Experimentally, over 261,000 ESTs were generated from cDNA isolated using laser capture microdissection (LCM) from the developmentally important shoot apical meristem (SAM) of maize. This single sequencing run annotated over 25,000 genomic sequences (MAGIs). 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 contains SAMs). In addition, 30% do not align to any of the over 648,000 extant maize ESTs. Furthermore, this analysis captured ~400 transcripts for which homologous sequences have not been identified in other species. We also observed alternative splicing variants for >20% of assayed transcripts. In combination, these results indicate that the combination of LCM and the deep sequencing possible with 454 technology enriches for SAM-expressed transcripts, including both potentially rare transcripts and rare transcript isoforms.

T10

Shotgunning the maize genome

(presented by Daniel Rokhsar <dsrokhsar@lbl.gov>)
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Conventional wisdom holds that the maize genome, with its high density of long repetitive elements, is not a good candidate for whole genome shotgun sequencing. To explore the structure of the maize genome and the feasibility of various sequencing strategies, we sequenced over one million paired shotgun reads from the B73 inbred line. Analysis of this million-read sample (~0.37X coverage of the genome) in the context of other public maize sequences provides an emerging picture of the repetitive structure and history of the Zea mays genome. The recent tetraploidization is evident, and the pattern of repetitive activity over the past ~10 million years can be analysed to suggest that a whole genome shotgun strategy could successfully capture ~90% of the maize genome. We will present an overview of our newly funded project to test this strategy through "whole chromosome shotgun" sequencing of chromosome 10 of the Missouri 17 inbred line from flow-sorted material.

T11

Sequencing the maize genome.

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We recently have initiated a program to generate a near complete sequence of the genome of maize cultivar B73. This effort - expected to require three years of work - will utilize a minimal tiling path of approximately 19,000 mapped BAC clones, and will focus on producing high-quality sequence coverage of all identifiable gene-containing regions of the maize genome. These regions will be ordered, oriented and, along with all of the intergenic sequences, anchored to the extant physical and genetic maps of the maize genome. Important features of the project include immediate release of preliminary and high-quality sequence assemblies, and the development of a genome browser that will facilitate user interaction with sequence and map data. At this conference, we will further discuss the ongoing effort and our results to date.

T12

Micropylar Pollen Tube Guidance in Maize by ZmEA1 and Discovery of Novel EA1-Box Protein Genes

(presented by Mihaela-Luiza Marton <mihaela_marton@botanik.uni-hamburg.de>)
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In contrast to most animal and many lower plant species, sperm cells of flowering plants are nonmotile and are transported from the stigma to the female gametophyte (embryo sac) via the pollen tube to allow double fertilization. Up to now, little is known about the molecules produced by the female gametophyte that control pollen tube guidance. Using a transcriptomics based approach with dissected egg cells of unfertilized maize embryo sacs, we have identified the first molecule involved in short-range signaling required for pollen tube attraction by the female gametophyte during the fertilization process. The egg apparatus-specific gene *ZmEA1* encodes a small protein of 94 amino acids that is likely to be further modified after translation (Marton *et al*., Science 307, 2005). Genomic Southern blots and databases analysis revealed the presence of a number of genes encoding small proteins with homology to the C-terminal part of ZmEA1. The encoded proteins were clustered in at least three different classes. Class 1 and 2 ZmEA1-like proteins (EAL) are cereal-specific and contain three distinct boxes that were distinguished as WP-, EA1- and A-rich box. Less related EAC proteins (EA1-box containing proteins) were identified in dicotyledonous plant species, which showed a week homology only within the EA1-box in the C-terminal region. The progress on the identification of the mature ZmEA1 peptide sequence and molecular as well as cellular analyses of the different novel EA1-box protein genes will be presented. Finally, we will discuss the role of ZmEA1 and related proteins in self-incompatibility within the grasses.

T13

The plastid zinc ribbon factor AtZR1 from Arabidopsis is the functional ortholog of the maize Etched1 protein and is required for plant viability

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In maize, loss of Etched1 (Et1) function conditions a pleiotropic mutant phenotype with fissured and cracked kernels and virescent seedlings due to impaired plastid development. The Et1 gene was cloned by transposon tagging and encodes a plastid protein with similarity to the zinc ribbon (ZR) domain of the nuclear transcription elongation factor TFIIS. ET1 was found to be associated with the transcriptionally active chromosome (TAC) of plastids. Both, relative transcriptional and ribonuclease activity of TAC were significantly reduced in an Et1-deficient mutant supporting ET1 to act in transcript elongation in plastids. One putative Et1 homolog was identified in Arabidopsis which we named AtZr1. Seeds of a homozygote AtZr1 T-DNA knockout mutant were wrinkled and unable to germinate in soil. However plants could be recovered on medium and showed a severe pleiotropic phenotype with altered morphology and impaired chloroplast development. To study Et1/AtZr1 gene orthology AtZr1-KO complementation experiments were carried out. Et1 is the first cloned member of a conserved family of ZR proteins in eukaryotes. Current analyses of additional ZR proteins from maize and Arabidopsis imply that these proteins could play a role in other stages of development and in different tissues. The presented data further emphasize the necessity of Et1/AtZr1 factors for proper plastid development and hypothesize ZR proteins being conserved components of transcription in organelles per se.

T14

Regulatory functions of the *sugary1* and *dull1* genes in starch biosynthesis

(presented by Martha James <mgjames@iastate.edu>)
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Production of starch is fundamental to the plant life cycle, providing an energy source for growth and development in present and future generations. Synthesis of stored and transient starch forms requires control of the activities of many different enzymes, possibly by the association of starch enzyme complexes. Starch consists of the glucose homopolymers amylose (Am) and amylopectin (Ap). Glucose monomers are joined via alpha-1,4-linkages by starch synthases (SS) to form linear chains in both molecules, and Ap also has ~5% branch linkages in which glucosyl units are joined by alpha-1,6 bonds. The clustered positioning of these branches provides defined structural organization to Ap. Selective trimming activities of the starch debranching enzymes (DBE) are believed to be important determinants of Ap structure, possibly underlying the process of starch crystallization. The maize *sugary1* (*su1*) gene codes for the isoamylase-type DBE ISA1. Two additional isoamylase-type DBEs, ISA2 and ISA3, are known, as well as one pullulanase-type DBE. Double mutant analysis shows both Zpu1 and Zmisa2 are genetic modifiers of *su1*. Purification of native DBE activities from wild type and mutant endosperm reveals the existence of distinct isoamylase-containing DBE assembly states. One appears to be homomeric for ISA1, while two higher molecular mass forms contain both ISA1 and ISA2. Activities of the DBE complexes differ during endosperm development, correlating with changes in transcript expression patterns for the *su1* and Zmisa2 genes. In vitro, the relative activity of each DBE assembly state varies according to the structure of the glucan substrate. Thus, homo- and heteromeric ISA complexes likely have distinct temporal- and substrate-specific functions in starch biosynthesis. The maize *dull1* (*du1*) gene, which codes for SSIII, also was identified as a genetic modifier of *su1* in 1947. Our analysis of native SS activities in *su1* mutants reveals that loss of ISA1 results in deficiency of both SSIII protein and activity, and deficiency of SSI activity (but not protein). In contrast, analysis of starch metabolizing enzyme activities in *du1* mutants shows increases in both ISA1 and ISA1/ISA2 complex activities. Thus, *du1* appears to negatively regulate isoamylase-type DBE activity, while *su1* provides a positive regulatory function for SSIII, as well as other SSs.

T15

Tie-dyed1 encodes a novel gene controlling carbohydrate accumulation in leaves

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My lab is interested in the signals and genetic programs coordinating leaf differentiation and development. Clonal analyses of maize leaf development have shown that clonal (mother to daughter cell) lineages are arranged longitudinally along the long axis of the leaf. To identify genes coordinating regional leaf identity we screened for mutants with sectors that extend laterally to cells beyond the clonal lineages. A recessive mutant, tie-dyed1 (tdy1), was identified with yellow and green pigmented leaf sectors that violate clonal lineage relationships. We determined that tdy1 sectoring requires high light, is restricted to a narrow developmental time and results in the yellow tissue hyperaccumulating sugars and starch. A clonal mosaic analysis of tdy1 revealed that a mobile signal, possibly sucrose, produced in the tdy1 mutant tissue is able to transform the phenotype of wild type cells to mutant. From these and other data we propose that TDY1 functions to upregulate the sugar export pathway within leaves. To characterize tdy1 at the molecular level we isolated additional alleles via a directed Mutator(Mu) transposon tag. A tightly linked Mu1 element was cloned, and two additional alleles containing Mu insertions into the same gene verified that we had identified the correct gene. The Mu insertions are all located in the 5'UTR of a novel gene. Expression studies are underway to test our proposed model for TDY1 function.

T16

Identification of gene families encoding double-stranded telomere repeat DNA-binding and related proteins

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We are investigating the functional significance of meiotic telomere behavior in maize. As part of this effort, we have screened for genes encoding homologs of the human telomeric proteins TRF1 and TRF2. These proteins, and their counterparts in other species all contain a conserved myb-like domain (sometimes called the telobox) that confers sequence-specific binding to double-stranded telomere repeat DNA. Three different families of TRF-like proteins have been identified, and each family has a unique protein motif composition or arrangement. Two of these families were discovered as a direct result of our screens (Marian et al., Plant Physiol, 2003; Marian & Bass, BBA-GSE, 2005). The first of these, the single myb histone (SMH-type) family, is represented by six genes in maize (Smh1-Smh6). The SMH-type genes encode small, basic proteins with a unique triple motif structures of (a) an N-terminal myb-like domain of the homeodomain-like superfamily, (b) a central region with homology to the conserved H1 globular domain found in the linker histones H1/H5, and (c) a coiled-coil domain near the C terminus. The second of these, the Terminal acidic SANT (TACS-type) family, is represented by a full-length cDNA encoding a 45-kDa protein with a C-terminal myb/SANT-like domain. Gene expression and protein homology modeling data indicate that the TACS1 protein may function in chromatin remodeling within shoot primordia or related tissues. In addition, a third family of TRF-like proteins has been found in many plant species. In maize, these were first described as being encoded by the initiator-binding protein genes, IBP1 and IBP2 (Lugert & Werr, Plant Mol Biol, 1994). We will summarize the differences between these three major families and present new immunolocalization data for the SMH proteins.

T17

Development of chromosome paints and single loci FISH probes for somatic chromosomes

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Any large segment of genomic DNA from maize is likely to contain repetitive elements. These repetitive elements have expanded differently among different evolutionary lineages. As a result, retroelements can be used as FISH probes to paint the maize genome distinguishing it in interspecies hybrids. Retroelement paints were used to visualize Tripsacum dactyloides chromatin introgressed into maize lines. The abundance of repetitive elements in the maize genome complicates the use of large genomic clones, e.g. BACs, as FISH probes to detect single loci. Improving the sensitivity of FISH resulted in routine detection of 3-4 kb genomic targets on somatic chromosomes, which allows the problem of repetitive elements to be circumvented by using small probes free of retrotransposon sequence. A collection of single loci probes were prepared from genes clusters, very long cDNAs, genomic clones of genes, and pools of sequences selected from the unique portions of BACs. These probes were used to mark each chromosome in maize and a number of relatives in mitotic spreads. This collection will serve as a core probe set for unambiguous identification of each chromosome in the mitotic karyotype. FISH analysis of mitotic chromosomes will aid in the rapid cytological mapping of other genes and transgenes as well as the recognition of chromosomal aberrations. The physical location of individual RescuMu and Activator elements, transgenes, and previously unmapped genes were determined cytologically. The methodology will allow single loci FISH probes to be developed for most genetic and physical locations.

T18

Allelic diversity of the p locus by changes and replacements of regulatory sequences

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Phlobaphenes are reddish flavenoid pigments that are frequently found in male and female maize floral organs. Phlobaphene accumulation is controlled by the p1 gene, which encodes an R2R3 MYB-like transcriptional activator. In contrast, the p1-paralogous gene p2 does not induce visible phlobaphene synthesis in maize tissues, although p2 and p1 gene products are virtually identical. Both p1 and p2 are thought to have arisen from a tandem duplication event from an ancestral p gene. Various allelic forms of p1 with distinct tissue-specific and time-specific expression patterns have been collected and investigated. The epigenetically regulated P1-wr allele is characterized by colorless pericarp and red glumes. P1-wr consists of multiple P1-wr repeats that are arranged in a tandem head-to-tail array. While the sequence of one P1-wr unit isolated from inbred W23 has been reported previously, the structure of the entire locus remained unknown. To understand the molecular basis of the allelic diversity at the p locus we sequenced and analyzed a contiguous 330 kb region from inbred line B73 that contain all P1-wr repeats, p2 and flanking genes. Although all P1-wr repeats are structurally identical, the presence of few polymorphic sites (including transposons insertions) among P1-wr repeats suggests a rather recent P1-wr amplification. Interestingly, the P1-wr cluster is flanked by sequences that resemble p2 5' and p2 3' ends. In addition, the 3' end of the distal p2-like sequence is displaced by retrotransposon insertions. Based on our analysis at P1-wr, we present a model of the genesis of the P1-wr cluster from a single myb-homolog to the present multigene complex. We extended our comparative analysis to other p1 alleles as well. These studies suggest that changes or replacements of regulatory sequences and not coding sequences have created the allelic diversity at the p locus.

T19

Unprecedented haplotype variability in the maize bz genomic region

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Maize is probably the most diverse of all crop species. Large and unexpected differences among haplotypes were first revealed in a comparison of about 100 kb of sequence from the bz genomic regions of two different Corn Belt inbred lines. First, the retrotransposon clusters, which comprise most of the repetitive DNA in maize, differed markedly in makeup & location relative to the genes in the region. Thus, the two allelic chromosomal regions can pair only at the genes that they have in common. Second, the composition of genes in the bz region appeared to differ between the two inbreds. Four adjacent gene sequences were present in one line, but absent in the other, suggesting a violation of genetic microcolinearity within the same species. Recently, we showed that this apparent intraspecific violation of colinearity arises from the movement of gene fragments by *Helitrons*, a newly discovered class of eukaryotic transposons. *Helitrons* lack the strong predictive terminal features of other transposons, so the definition of their ends is greatly facilitated by the identification of their vacant sites in *Helitron*-minus lines. To investigate further the extent of occurrence of variation induced by Helitrons and, possibly, other novel transposons, we are carrying out an analysis of the genomic organization of the *bz* genomic region in a group of 12 carefully selected inbred lines, races, and relatives of maize. Size-fractionated, *Not*I BAC libraries are constructed and *bz* clones are isolated and sequenced from each library. This vertical comparison has revealed the existence of several new *Helitrons*, new LTR- and nonLTR-retrotransposons, solo-LTRs, DNA transposons of every superfamily, and a novel type of insertion that we call *TAFT* (for TA-flanked transposon) because it is flanked at either end by (TA) repeats. Sequencing of the *bz* genomic region from different inbred lines & land races has identified chimeric haplotypes that combine retrotransposon clusters found in different haplotypes. Recombination in the gene space shared by different haplotypes shuffles retrotransposon clusters, creating huge heterogeneity in genome organization among modern maize lines.

T20

Maize root transcripts are clustered in the genome on the basis of water stress-response and function.

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The timing and extent of root development are principle determinants of the ability of a plant to withstand inadequate soil moisture. The chromosomal distribution of maize root transcripts representing varying degrees of water stress was characterized by determining the genetic and/or genomic locations of maize root EST derived from cDNA libraries and from EST identified by unique alignment of SAGE tags to EST. Starting with 22,532 maize root EST, map locations were determined electronically ("e-mapping") for 6,058 EST (1237 genetically + 4937 physical - 116 common to both maps) via queries of maize genomic sequence databases. The mapped EST came from maize seedling root cDNA libraries produced under varying durations of -1.6 MPa water stress [5 hours (WS5) and 48 hours (WS48)], a well-watered control (WW), and a subtractive library containing water-stressed sequences. Root segment-specific information for clones in each library was provided by molecular bar codes introduced during cloning of transcripts. The five most highly expressed SAGE transcripts were located on chromosomes 8, 9, 3, 1, and 1 respectively. Significant expression differences along a chromosome were observed with several contigs representing higher than average levels of well-watered transcript expression. The Kolmogornov-Smirnof test was used to identify significant differences in the distribution of well-watered vs. water-stressed transcripts in the maize genome. Significant differences were observed in the distal region of chromosomes 1S, 2S, 5S, 7L, and 9L. The long arm of chromosomes 1 and 8 displayed the least differences between well-watered and water-stressed transcripts. Using the GO Slim annotation in relation to the kinematic information enabled us to produce an annotated physical map where root segment and treatment are displayed. This data can be coupled with data from DNA microarray, SAGE, QTL or mutant position, or in comparison to other genome maps to further our understanding of maize root response to drought.

T21

Fate and consequence of the Zag1 and Zmm2 gene duplication in grasses

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The genes regulating flower development have been well characterized, but the evolutionary changes associated with the diversification of genes in developmental pathways remain poorly understood. Gene duplications play an important role in providing the raw material necessary for genetic/developmental pathway diversification. We present data on two developmentally important duplicate genes, Zag1 and Zmm2, which are key determinants of stamen and carpel specification in grasses. Zag1/Zmm2 are maize C-function organ identity genes as defined in the ABC model of floral development. In grasses, Zag1 and Zmm2 have partitioned C-function activity through the evolution of subfunctionalized cis-regulatory regions and coding regions of the genes. Using phylogenetic methods, we identified the origin of the Zag1/Zmm2 duplication. Our data indicate the duplication occurred prior to the diversification of grasses, with non-grass lineages having a single Zag1/Zmm2 gene. Both genes have been preserved across the grasses, suggesting selection for retention of both gene activities early in the history of this duplication event. Using bioinformatics approaches and the rice genome, we show that the Zag1/Zmm2 gene duplication event was part of a major genome duplication event that occurred early in the evolution of the grasses. We have examined the expression patterns of Zag1 and Zmm2 using in situ hybridizations and RT-PCR in post-duplication and pre-duplication species. Our results indicate that the differential expression patterns occurred soon after the duplication event of Zag1/Zmm2. Postduplication species such as Avena and Sorghum show Zag1 expression is primarily in the carpel and ovule whereas Zmm2 expression is in the stamen filaments and within the ovule. The pre-duplication species Joinvillea shows expression in stamens and carpels. Lastly, we present work on coding region subfunctionalization using Arabidopsis as a heterologous system. In addition to our work on Zag1/Zmm2, we are examining a more ancient duplication in the D-Class genes Zmm25/Zmm1/Zag2.

T22

Biased fractionation and intragenomic footprinting in plants

(presented by Michael Freeling <freeling@nature.berkeley.edu>)
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We have found evidence that the genomes going into the most recent tetraploidy in the Arabidopsis lineage were immediately marked epigenetically, and that one of them has thereafter been 1.6X a better target for chromosomal mutation. Our novel blast Viewer aligns any region of Arabidopsis with any other (http://synteny.cnr.berkeley.edu/AtCNS/). Using this Viewer, we proofed the A. Paterson group's alpha pairs (2003), included our additional pairs, making a total of 3197 pairs, and then analyzed the sequence patterns of fractionation and conservation for coding and noncoding DNA. Alpha pairs are remnants from a fractionated tetraploid. We conclude that fractionation from the tetraploid was biased to one or the other of the original parental chromosomes, implying that epigenetic marking can calibrate the rate of chromosomal mutation. Fractionation has left clusters of retained genes, and these clusters are rich in regulatory genes and genes encoding proteins that interact, as predicted by the gene balance hypothesis (see Birchler et al., 2005. Trends Genet. 21: 219). We hypothesize that biased fractionation duplicates functional modules composed of dose-sensitive genes and the regulatory sites that drive them. We have studied some of these sites as conserved noncoding sequences; most carry G-box core sequences or other believable protein-binding motifs, and have been purified of many over-abundant sequences. Small RNA binding sites exist, but are rare. We are applying what we have learned from the Arabidopsis alpha tetraploidy to the grasses, where tetraploidy has also been important. Preliminary results will be presented. Our Bl2seq Viewer web application should be a useful tool for all who use evolutionary conservation as an indicator of sequence function.

T23

Analysis of allelic variation in gene expression in B73 and Mo17 and implications for hybrid expression patterns

(presented by Robert Stupar <stup0004@umn.edu>)
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A combination of Affymetrix microarray and allele-specific expression analyses were applied to investigate the differential regulation of maize alleles, and the regulatory alterations that occur following F1 hybridization. Seedling, immature ear and embryo tissues were profiled in the inbred lines B73, Mo17, and reciprocal hybrids B73xMo17 and Mo17xB73. Using microarray analyses, we identified many genes that are differentially expressed between inbreds B73 and Mo17. For a subset of the differentially expressed genes expression is only detected in one of the two inbreds. Further analysis of these genes shows that they are present in the genomes of both inbreds, but differ in their regulation. We proceeded to profile the expression patterns in the reciprocal hybrids. There was no evidence for parental affects in the tissues that we studied as no genes were differentially expressed in B73xMo17 relative to Mo17xB73. The genes that were not differentially expressed in B73 relative to Mo17 were also not altered in their hybrid expression state. The vast majority of the B73 versus Mo17 differentially expressed genes were expressed at mid-parent, or additive, levels in the hybrid. Almost no genes displayed expression levels in the hybrid that were outside the range of the inbred parents. We further studied a set of 27 genes that were differentially expressed in the two inbred lines by using allele-specific expression analysis; we investigated the respective transcriptional contribution of the inbred alleles in the hybrid. This analysis provided evidence that the majority of differential expression in B73 and Mo17 is due to cis-regulatory variation, not differences in trans-acting regulatory factors. This may explain the predominance of additive expression and relative lack of epistatic effects observed in the microarray data as genes subject to cis-regulatory variation are expected to be expressed at mid-parent, or additive, levels in the hybrids.

T24

QTL Analysis of Morphological Traits in a Large Maize-Teosinte Backcross Population

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Domesticated maize (Zea mays ssp. mays) and its wild progenitor, teosinte (Z. mays ssp. parviglumis), differ dramatically in their overall plant architecture and the morphology of their female inflorescences. A few key loci controlling these differences were initially identified by QTL analyses of maize-teosinte intercross progeny. These earlier studies utilized low-density genetic maps and relatively few progeny, reducing the power to detect QTL and accurately estimate their location and effect. Subsequent advancements in the physical mapping of ESTs and SSRs have enabled the construction of genetic maps of higher density with more uniform genomic distribution and coverage. Furthermore, the development of inexpensive high throughput SNP and SSR assays has permitted the genotyping of greater numbers of progeny. In this study, a BC1 population derived from a cross between the maize inbred W22 and teosinte was grown in two environments for phenotypic analysis. A total of 1723 progenies were genotyped for >300 SNP, SSR, and candidate gene markers and phenotyped for 22 morphological traits. A genetic map and QTL analyses will be presented. Seed of the BC1 plants is archived and 1000 BC2S6 families are being developed from this population. These materials should enable positional cloning the identified QTL without the need to collect additional cross-overs surrounding the QTL.

T25

Power analysis of an intergrated mapping strategy: Nested Association Mapping

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Attempts to dissect complex trait at molecular level have been hindered by the limits of the current methods, with low resolution and narrow reference population for linkage analysis and concerns about population structure and high density marker coverage for association mapping. Here, we examine the power of an integrated mapping approach, Nested Association Mapping (NAM), which exploits simultaneously the advantages of linkage analysis and association mapping. We demonstrated the power of NAM for genome-wide quantitative trait locus (QTL) mapping through computer simulation with varied number of QTL and trait heritability. The study followed closely the procedures undertaken by an ongoing maize QTL mapping project. Twenty-five maize populations, each of which has 200 recombinant inbred lines (RIL), are being derived from the cross of 25 diverse inbred lines to a common inbred. With a dense coverage (2.6 cM) of common-parent-specific (CPS) markers, the genome information for 5000 RIL can be inferred based on the parental genome information. Essentially, the linkage information was captured by the CPS markers and the linkage disequilibrium information among locus resided between CPS markers was then projected to RIL based on parental information, ultimately allowing for the genome-wide high-resolution mapping. The power of NAM with 5000 RIL allowed 30% to 79% of the simulated QTL to be precisely identified. With the ongoing genome sequencing projects, NAM would greatly facilitate complex traits dissection in many species in which a similar strategy can be readily applied.

T26

Marker based polygene control and its application to the dissection of complex traits and estimation of variance components

(presented by Gael Pressoir <ghp5@cornell.edu>)
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We will describe the applications of a new method that allows for an improved estimation and control of polygenic additive variation in maize. Special emphasis will be given to association mapping and germplasm evaluation for complex traits.
Current association mapping methodologies do not account effectively and simultaneously for complex pedigrees, families, founding effects and population structure. As population structure can result in spurious associations, it has constrained the use of association studies in plant genetics. Association mapping, however, holds great promise if true signals of functional association can be separated from the vast number of false signals generated by population structure. We have developed a unified mixed-model approach to simultaneously account for multiple levels of relatedness detected by random genetic markers. We applied this new approach to a sample of 277 maize diverse inbred lines with complex familial relationship and population structure for quantitative trait dissection. Our method showed an improved control on both Type I and Type II error rates over other methods. As this new method crosses the boundary between family-based and structured association samples, it provides a powerful complement to the current methods for association mapping. We will also describe other applications of this method as it also allows accurate estimation of the additive genetic variance(s) component(s) and heritabilities in any germplasm collection.

T27

Interdisciplinary analysis of aluminum tolerance in maize

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Aluminum (Al) toxicity is a profound limitation to crop production worldwide, reducing yields on up to 50% of potentially arable lands. Breeding for Al tolerance and agronomic practices aimed at ameliorating soil acidity have historically been productive avenues for improved crop production. However, it is widely recognized that additional improvements in crop Al tolerance will depend on biotechnology. We undertook an experimental plan integrating statistical genetic, genomic and proteomic approaches, building upon our previous work on the genetic and physiological bases for Al tolerance in maize. We identified 5 Al tolerance QTL in the Intermated B73 x Mo17 (IBM) recombinant inbred population, which together explain nearly 60% of the variance observed. Physiological profiling is underway, but we hypothesize that Al activated citrate release explains at least part of the differences in Al tolerance observed. We used microarray analysis to characterize the root tip transcriptome, together with a limited use of proteomics to validate the gene expression work. The results of the microarray experiments have been analyzed with respect to the physical and genetic maps of maize and in a comparative sense with the rice genome. Based on all of these experiments, we selected candidate Al tolerance genes to evaluate using association analysis. We identified several single nucleotide polymorphisms in multiple genes that are associated with Al tolerance. NSF Plant Genome Award DBI #0419435, a CGIAR Generation Challenge grant, a McKnight Foundation Collaborative Crop Research Program and USDA-ARS base funds support this work.

T28

Enhanced Detection of Inflorescence Architecture QTL in Intermated B73 x Mo17 (IBM) RIL Population

(presented by Narasimham Upadyayula <upadyayu@uiuc.edu>)
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Genetic control of maize inflorescence architecture is of interest to developmental and evolutionary biologists as well as crop scientists. A number of maize inflorescence mutants have been identified and genes cloned, providing useful resources for developmental, evolutionary and breeding related studies in maize and across the cereals. QTL analysis is complementary and is identifying many more loci that influence inflorescence architecture. We summarize a series of QTL studies, four in standard F2:3 or BC1:S1 populations, and one in a population that was random mated for four cycles and recombinant inbred lines derived. We measured several traits on tassel and ear, and also calculate traits such as ratio of branch number to spikelet number. In standard QTL mapping populations we detect a couple to several QTL for each trait, with total numbers approximately 30 to 50. In contrast, in the IBM population, depending on permutation generated LOD threshold used, we detect approximately 300 - 500 total QTL for 13 different tassel infloresence architecture traits. This demonstrates the power of IBM population. In some cases two QTL are detected in a region where a single QTL was detected in a non-random mated population. Due to the highly correlated nature of inflorescence architecture traits, we performed multivariate principal components analysis (PCA) on the phenotypic data, and also used the PC values as traits and performed QTL analysis on them. This resulted in detection of fewer QTL, 125 in IBM, which may be relatively important in quantitative control of overall inflorescence architecture variation. We identified QTL that map to relevant mutant loci such as ts4, lg3, fea2, td1, ra1. However, the vast majority of QTL are not near inflorescence mutants and genes, which provides initial information for new gene discovery approaches. Our results demonstrate greatly enhanced detection of QTL on the higher resolution IBM mapping population, creating mapping information that will become increasingly useful with sequencing of the maize genome.

T29

The Epigenetic Regulation of Mutator Transposons in Maize

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Transposons make up a large proportion of the plant genome, particularly in maize. Given the mutagenic potential of transposable elements, it is not surprising that plants evolved a system by which to silence them. It has been shown previously that Mu killer, an inverted repeat of one of the two Mutator autonomous genes, silences Mutator transposons in maize. Here two mutants are discussed that are involved in this silencing process: mop1, which gradually reverses the silenced state of one of the two genes encoded by the autonomous element; and the nfa family of genes, which prevent full silencing by Mu Killer. These mutants, together with Mu killer, suggest a pathway through which Mutator transposon silencing is initiated and maintained.

T30

Alternative transposition of the maize Ac/Ds transposable element system induces chromosome breakage and major chromosomal rearrangements.

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McClintock reported that maize stocks containing active Ac/Ds elements exhibit a high frequency of chromosomal aberrations, including direct and inverted duplications, and translocations. Here, we show that such rearrangements can be generated by alternative transposition reactions that involve the termini of different Ac/Ds elements in close proximity. We studied a series of p1 alleles that carry a fAc (fractured Ac) insertion in the p1 gene intron 2, and a second Ac element nearby, in different sites and orientations. Each of these alleles was tested for its ability to induce chromosome breakage as measured by loss of the linked Dek1 gene. From these stocks, we isolated a number of putative translocations in which the p1 locus on chromosome 1 is joined to a site from a different chromosome. Candidate translocations were first identified 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, University of Minnesota) to identify the chromosome involved in the translocation. In this way, 6 new putative translocation stocks were identified. Candidate translocation stocks were also confirmed by cytogenetic analysis at meiotic diakinesis of sporocytes; see abstract by Weber et al. In addition to translocations, we have shown that alternative transposition reactions can generate deletions, inversions, and duplications. These results demonstrate that alternative transposition reactions can be a major force in shaping genome structure. Although these studies were limited to the maize Ac/Ds transposable element system, it is possible that similar reactions could occur with other transposable element systems as well. 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, J. Zhang, and D. Weber.

T31

Characterization *Ac-immobilized*: a stabilized source of transposase and its use in a two component *Ac/Ds* gene tagging program in maize

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We have identified and characterized a novel *Activator (Ac)* transposable element that is incapable of excision yet contributes to the canonical negative dosage effect of *Ac*. Cloning and sequence analysis of this immobilized *Ac (Ac-im)* revealed that it is identical to *Ac* with the exception of a 10 bp deletion of sequences at one end of the element. In screens of approximately 6800 seeds, no germinal transpositions of *Ac-im* were detected. Importantly, *Ac-im* catalyzes germinal excisions of a *Dissociation (Ds)* element resident at the *r1* locus resulting in the recovery of independent transposed *Ds* insertions in approximately 4.5% of progeny kernels. Furthermore, *Ac-im* transactivates multiple *Ds* insertions both germinally and in somatic tissues including those in reporter alleles at *bronze1, anthocyaninless1* and *anthocyaninless2*. We propose a model for the generation of *Ac-im* as an aberrant transposition event that failed to generate an 8 bp target site duplication and resulted in the deletion of *Ac* end sequences. I will discuss our recent progress on defining regulatory elements using EMS mutagenesis and a broader program to distribute *Ds* insertions throughout the genome using *Ac-im* as the source of transposase.

T32

The first active plant *Tc1/mariner*-like element: transposition of the rice *Osmar5* in yeast

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Elements of the *Tc1/mariner* transposon superfamily are widely distributed in eukaryotic genomes. Both active *Tc1/mariner* elements isolated from insect genomes and phylogenetically-reconstructed elements from fish genomes have been widely used for animal transgenesis and transposon tagging. However, while *mariner*-like elements have recently been found to be widespread in the genomes of flowering plants, up until this study, no active plant element has been identified. A previous study demonstrated that a *mariner*-like element in the rice genome (*Osmar*5) encoded a transposase that specificially bound to the *Osmar5* ends. In this study, we demonstrate that when expressed in the yeast *Sachromyces cerevisae*, the *Osmar5* transposase is able to excise a nonautonomous derivative of *Osmar*5 from the 5' untranslated region of an *ade*2 gene, resulting in *ade*2 revertants. The sequences of footprints suggest a model for how the element is cut from the donor site. In addition, the excised elements inserted either to linked sites in the donor plasmid or to unlinked sites in the yeast chromosomes. For all characterized new insertion events, the dinucleotide "TA" was duplicated upon *Osmar*5 integration. Our identification of the first active plant *Tc1/mariner* element may provide a new wide-host range transposon for plant gene tagging.

T33

Building new tools for the identification and characterization of repetitive DNA from incomplete data sets

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Eukaryotic genomes vary tremendously in nuclear genome DNA content and organization. In order to unravel the molecular basis of this variation, a key step is to describe and compare the genomic structure and content of many organisms with known phylogenetic relatedness. Repetitive DNAs, especially transposable elements, are exceptionally variable components of genomes, and their investigation can serve as a preliminary tool for characterizing the properties of an entire nuclear genome. With the advent of whole genome sequencing, increasing amounts of sequence data offer the opportunity for genome characterization. Much effort has been made to find and describe genes in genome sequence data. However, methods to efficiently characterize repeats still need to be developed. A great quantity of BAC, whole genome shotgun (WGS) and BAC-end sequence (BES) data are available for numerous genomes. If appropriately analyzed, these sample sequences could provide an efficient and cost-effective method for comparing genomes. We have developed several novel algorithms, as well as improved some extant applications, to address these issues using fully sequenced BACs, WGS or BES data. These approaches utilize structural characteristics of the repeats being investigated and collectively allow for the characterization of all high-copy-number repeats in a genome. Bac-Breaker, an improved version of LTR_Struc, now has the ability to resolve nested LTR-retrotransposon structures from large sequence contigs. Assisted Automated Assembler of Repeat Families (AAARF) is under construction to 'walk out' pseudomolecules representing families of repeats from WGS or BES data. To prove the utility of this sample sequencing approach, random shotgun libraries were produced and analyzed for several plant species, including Zea luxurians and Zea diploperennis. Using these datasets, we were able to characterize the nature and history of genome growth and transposon evolution in these plant species.

Poster Abstracts

P1

Withdrawn

P2

Antisense expression analysis in the maize transcriptome and microarray cross platform comparisons

(submitted by Leonardo Iniguez <aliniquez@wisc.edu>)

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The Maize Oligonucleotide Array Project developed the first generation of low cost 70mer oligonucleotide spotted microarrays for the maize community. At onset of this project, 57,452 70mer oligonucleotides were designed to represent 25,969 EST assemblies, 20,206 singleton ESTs, 9707 Assambled Zea mays (AZM), 804 non redundant repeat elements, 467 organelles, 288 maize community favorites and 11 transgenes. Empirical optimization of these oligos was conducted using NimbleGen arrays. The use of these oligos in both platforms has shown high level of congruency under specific matching condition. In this work, we report two main areas of investigation. One is the use NimbleGen arrays to investigate antisense expression. We assessed the 57,452 oligonucleotide sequences from the spotted arrays in both the sense and antisense orientation using NimbleGen arrays. These NimbleGen arrays were interrogated with RNA derived from maize tissues of plants exposed to different environmental conditions. The second aim of this work is to compare interpretation of gene expression using the 70mer spotted arrays and the Affymetrixs GeneChip Maize Genome Array. Using two parameters of identity (100 and 95%) 4,939 and 7,079 70mer probes respectively, matched the sequences used by Affymetrix to design their probe sets. We report here the relationship between both platforms in their ability to detect of gene expression when interrogated with the same maize RNA samples.

P3

Candidate gene cloning of glossy genes

(submitted by Li Fan <fanli@iastate.edu>)
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The epicuticular waxes that coat terrestrial plants and protect against both abiotic and biotic stresses are composed of long-chain alcohols, aldehydes, alkanes, esters, ketones and hydroxy-beta-diketones. All of these compounds are derived from very long chain fatty acids. VLCFAs are generated via the cyclical activities of ketoacyl-CoA synthase, 3-ketoacyl reductase, 3-hydroxyacyl dehydratase and enoyl-CoA reductase, which elongate the 18C products of de novo fatty acid biosynthesis 2C units per cycle. Maize glossy mutants exhibit reduced accumulation of epicuticular waxes on the surfaces of seedling leaves. A near-saturated screen for glossy mutants identified a total of 26 gl loci, many of which are represented by multiple Mu- and EMS-induced alleles (Dietrich et al., in prep).

This unique genetic resource is being used to clone gene involved in the biosynthesis of epicuticular waxes. Maize homologs were identified for the yeast tsc13, elo2, elo3 and Arabidopsis cer6 genes, all of which are involved in wax metabolism. Using IDP markers the four corresponding genomic sequence contigs (MAGIs) were genetically mapped to maize chromosomes 8, 4, 6, and 8. Further analyses established that gl26 (which maps to chromosome 8) is a homolog of tsc13, which encodes enoyl-CoA reductase, one of the four enzymes that catalyze the elongation of VLCFAs.

P4

Characterization of BIF2 interacting proteins in maize axillary meristem development

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The maize inflorescence is a highly branched structure produced by an inflorescence meristem and four types of axillary meristems; the branch meristem, spikelet pair meristem, spikelet meristem, and the floral meristem. The "barren inflorescence" class of maize mutants have defects in axillary meristem initiation and/or development and are characterized by a reduced number of branches and spikelets in the inflorescence and floral defects. barren inflorescence2, bif2, encodes a cytoplasmic protein kinase. bif2 is expressed in branch, spikelet pair, spikelet and floral meristems, floral organs and vegetative axillary meristems and is required for the initiation of all axillary meristems.

Proper inflorescence development requires complex signaling events. Axillary meristem initiation in maize is controlled, in part, by BIF2. To study the signaling partway mediated by BIF2, we sought to identify interacting proteins using yeast two-hybrid screens of tassel (male inflorescence) and ear (female inflorescence) libraries. Several classes of interacting proteins were identified and we will show confirmation of interaction via in vitro binding assays and the results of in vitro phosphorylation assays.

P5

Characterization of the orthologous WUSCHEL gene family in maize

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The Arabidopsis homeodomain transcription factor, WUSCHEL (WUS) plays an important role in the identity and maintenance of the shoot apical and inflorescence meristems as well as ovule development. We have been investigating the role of the maize orthologs to AtWUS in inflorescence development in maize. We identified 16 genomic contigs by searching the GSS database with the 66 amino acid homeodomain of the AtWUS gene and clustered these into eight paralogous groups using PHYLIP. Two contigs clustered with the AtWUS gene while the remaining seven groups showed similarities to AtWOX genes. Six of the eight paralogous groups also encoded the WUS box while only two have a second downstream partially-conserved motif designated as the "LEL-box". This LEL-box was reminiscent of the repressive EAR domain found in certain zinc finger transcription factors. RNA levels for 14 of the 16 maize contigs were detected using the Lynx MPSS analysis across 225 RNA libraries and showed similar expression patterns within paralogous families but not across families. We also detected evidence of altered splicing for one member each of the WUS and WOX5 clusters. Ectopic expression of the maize WUS ortholog introduced into embryo tissues by particle bombardment produced embryoid-like outgrowths in a non-cell autonomous fashion. These data also suggest that the maize WUS ortholog can stimulate embryogenesis in older non-embryogenic tissues from recalcitrant inbreds. Constitutive overexpression of the maize WUS ortholog in transgenic maize leads to increased leaf and stem width, stunted height, and knotted-like outgrowths on leaves.

P6

Characterization of the zebra lesion mimic (zl1) mutation in maize

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The zl1 phenotype is characterized by cross-bands of necrotic tissue on the leaves. This mutation was one of many that were identified in a mutator active population of corn. Through allelic testing it was found to be a novel mutation distinct from the associated phenotypic mutants zn1 and zn2. Phenotypic analysis of zl1 has shown that this mutant has an environmentally dependent phenotype in response to diurnal cycle. zl1 mutants grown under continuous light show suppression of the necrotic banding. zl1 plants grown in four-hour diurnal cycles show an increase in band number and width. When zl1 mutants are grown under etiolated conditions and then rapidly exposed to light they exhibit rapid death of the developmentally older leaves. Combining these observations suggests that light is the factor that causes these necrotic bands and they also suggest that zl1 function is required in a circadian fashion. Through Southern Blot analysis a 4kb band was observed co-segregating with a mutator 7 element. This band was cloned and sequenced. Analysis of zl1 using bioinformatics has revealed that zl1 shows a high degree of similarity to a family of genes in Arabidopsis thaliana named KCO1 through KCO6. Published evidence shows that these genes encode a type of novel potassium channel protein in plants. The homology that zl1 has to these genes combined with the phenotypic analysis allows one to hypothesize that zl1 encodes a potassium channel and that it is controlled in a circadian fashion. We have identified new zl1-like mutations in EMS and RescueMu lines. The results of allelism tests and progress on confirming the zl1 locus will be presented.

P7

Discovering Novel Starches for Fuel and Health: High-Throughout Screening of EMS Mutagenized Maize for Altered Starch Digestibility

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Carbohydrate research is increasingly focused on changing the biochemical nature of starch to create more efficient substrates for biofuel production and healthier foods for human consumption. A key factor in both these processes is starch digestion: a cost-limiting step in producing fuel ethanol is the conversion of cornstarch to glucose by a thermostable alpha-amylase and, in the human gut, starch is broken down into smaller sugars by a pancreatic alpha-amylase. Rates of starch digestion are influenced heavily by starch granule structure and composition, therefore changing these genetically should create more reliable and consistent sources of starch than the currently used chemical modification. Our goal is to isolate maize mutants with altered starch digestibility and develop new varieties with these value-added traits.

To identify such mutants among segregating M3 families of EMS mutagenized maize, we have developed a miniaturized, high-throughput kernel processing and starch digestion assay that can process up to 600 samples per day (ten times more than other, current methods). Samples of cleaned flour are prepared from individual, lyophilized endosperms, organized in microtiter plates and both raw and cooked forms are then digested using pancreatic alpha-amylase. Digestion rate is determined by measuring the amount of reducing sugars released at 0, 20 and 120 min with a colorimetric assay. Data are presented for several mutant families segregating putative changes in digestibility. Starch from these mutants will be subjected to more detailed chemical analysis and we hope to correlate these results with diffrences in the reflective NIR spectra of the mutant kernels.

P8

Disruption of a maize 9-lipoxygenase gene results in increased resistance to fungal pathogens and reduced levels of contamination with mycotoxin fumonisin

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In this study we tested the hypothesis that 9-lipoxygenases (9-LOXs) and their metabolites are mycotoxin susceptibility factors in corn that are induced and utilized by Fusarium verticillioides and other phytopathogenic fungi to increase fungal sporulation and mycotoxin production in seeds. This hypothesis is based on three key observations; (1) oxylipins produced from linoleic and other free fatty acids in Aspergillus spp., so called psi-factors, are potent regulators of sporogenesis and mycotoxins synthesis; (2) the primary products of plant 9-LOX reactions, fatty acid hydroperoxides 9S-HPOT(D)E, which are structurally similar to psi-factors, strongly induce both Aspergillus conidiation and mycotoxin production in vitro; (3) transcript levels of a maize 9-LOX gene, ZmLOX3, are induced in corn lines that are susceptible but not resistant to aflatoxin contamination. To test our hypothesis, we generated maize near-isogenic lines (NILs) that are either Mutator-insertional mutant or wild type at the ZmLOX3 locus. Currently, mutants and wild type NILs are at the BC4F4 stage in B73 genetic background which is susceptible to fumonisin contamination. Oxylipin profiling suggested that germinating lox3 mutant seed are devoid of most 9-LOX derived fatty acid hydroperoxides. Fumonisin B1 production and conidiation of F. verticillioides were drastically reduced when the fungus was grown on mutant lox3 kernels providing a strong support of our hypothesis. Moreover, conidiation of a distantly related fungal species, Colletotrichum graminicola, a causal agent of anthracnose leaf blight, was significantly reduced on lox3 mutant leaves as compared to wild type leaves. Importantly, fungal biomass of both pathogens was not affected by the lox3 mutation. These data strongly support our hypothesis that 9-LOX-derived metabolites positively regulate both fungal conidiation and mycotoxin production and are susceptibility factors in maize.

P9

Elucidation of the Differential Divergence Between the Two Subunits of ADP-glucose Pyrophosphorylase

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ADP-glucose pyrophosphorylase (AGP) is an allosteric enzyme that catalyzes a rate-limiting step in starch synthesis. AGP is heterotetrameric consisting of two identical small and two identical large subunits. In maize endosperm, the large subunit is encoded by Shrunken-2 (Sh2) and the small subunit is encoded by Brittle-2 (Bt2). The two subunits show considerable identity pointing to a common origin. Even though the two subunits are not functionally interchangeable, the small subunit is more conserved among species than is the large subunit. The most publicized hypothesis states that the small subunit is more important in AGP activity than is the large subunit because it is catalytic whereas the large subunit is allosteric. To test this hypothesis, both Sh2 and Bt2 were mutagenized by error-prone PCR and expressed in E.coli strain AC70R1 that lacked endogenous AGP activity (glgc). Active maize endosperm AGP complemented the glgc mutant leading to glycogen production. Glycogen production was scored by iodine staining and verified by phenol reaction. The probability that a missense mutation in either gene led to inactivation of AGP and consequently no obvious production of glycogen was 33% for Sh2 and 34.5% for Bt2 . This result points to little to no difference in robustness to missense mutations between the two AGP subunits with respect to AGP activity. It also suggests that other reasons account for the differential divergence between the large and the small subunit of AGP.

P10

Evolution of Indole-3-glycerol Phosphate Lyases in the Grasses

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Gene duplication plays an important role in the evolution of secondary metabolic pathways. In maize, duplicated tryptophan synthase alpha genes from primary metabolism are recruited for the production of free indole. Indole is either converted to a defense chemical (DIMBOA) or used directly for signaling. The wound and elictor inducible gene Igl is responsible for the production of volatile indole. Indole is part of the volatile cocktail that attracts carnivorous insects after the plant has been damaged by caterpillars. Genes homologous to Igl are found in Oryza sativa (Ehrhartoideae), Hordeum ssp (Pooideae), and Sorghum bicolor (Panicoideae). Preliminary data indicate that these homologues are induced by wounding and have catalytic activities similar to maize IGL. The production of volatile indole might be an ancient feature of the grasses.

P11

Expression of Codon Optimized Fluorescent Marker Genes in Maize

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Green fluorescent protein (GFP) and derivatives thereof with shifted excitation/emission variants including blue (BFP), and yellow (YFP) provide a means to study protein trafficking in plants. Introduction into maize via Agrobacterium -mediated transformation of mGFP5 under the control of the rice actin1 promoter coupled with its first intron resulted in poor cellular fluorescence across a number of independent transgenic maize lines. RNA gel blot analysis of the maize mGFP5 transgenic lines revealed strong mGFP5 transcript production. However, immunoblot analysis of the same lines indicated poor accumulation of mGFP5 protein. These data taken together suggested translational efficiency of mGFP5 in maize is low. In order to increase the putative low translational efficiency of fluorescent marker genes in maize, we synthesized codon-optimized versions of GFP, BFP, and YFP. Plant expression cassettes harboring the respective codon-optimized fluorescence genes fused to a plastid transit peptide have been assembled under the control of the maize ubiquitin promoter coupled with its first intron. The fluorescence cassettes were subcloned into the binary plasmid pZP212 and introduced into immature maize embryos by Agrobacterium-mediated transformation. Primary transformants (T0) were regenerated under antibiotic selection and screened by Southern blot analysis and fluorescence in leaf tissue. Comparative analysis of fluorescent proteins, BFP, GFP and YFP in T1 plants between these resulting transgenic events and maize mGFP5 transgenic events revealed that expression of fluorescent proteins was several times higher in transgenic events transformed with codon optimized fluorescent proteins. UV-confocal microscopy observations showed fluorescence in several transgenic events transformed with codon optimized fluorescent proteins, YFP and GFP, but in none of the maize mGFP5 transgenic lines. In BFP events, significant background fluorescence from cell walls made it difficult to visualize expression. These results shows the utility of the maize codon optimized GFP, and variants thereof, as a means to observe in vivo protein trafficking in maize.

P12

Functional differentiation of bundle sheath and mesophyll maize chloroplasts determined by comparative proteomics

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Chloroplasts of maize leaves differentiate into specific bundle sheath (BS) and the mesophyll (M) types to accommodate C4 photosynthesis. Consequences for other plastid functions and plastid gene expression are not well understood and are the focus of this presentation. Denaturing and non-denaturing 2-Dimensional gels, as well as stable isotope labeling techniques (cICAT and iTRAQ), are used to obtain a quantitative comparative proteome analysis of purified M and BS chloroplast stroma, as well as thylakoid membranes. The stromal analysis identified 400 proteins by mass spectrometry and differential BS and M expression was determined for 125, covering a wide range of plastid functions [1]. Enzymes involved in lipid biosynthesis, nitrogen import, tetrapyrrole and isoprenoid biosynthesis are preferentially located in the M chloroplasts. In contrast, enzymes involved in starch synthesis and sulfur import preferentially accumulate in BS chloroplasts. The different soluble anti-oxidative systems accumulate at higher levels in M chloroplasts, indicative of higher rates of ROS production. We also observed differential accumulation of several proteins involved in expression of plastid-encoded proteins. Enzymes involved in triose-phosphate reduction are primarily located in the M chloroplasts, indicating that the M localized triose phosphate shuttle should be viewed as part of the BS localized Calvin cycle, rather than a parallel pathway. Preliminary data regarding differential expression of the M and BS thylakoid membranes will be presented. In addition, we present unpublished data on the consequences of nuclear gene disruption of a homologue of Arabidopsis HCF136 involved in assembly of Photosystem II. These data are made available via the Plastid Proteome Data Base, PPDB (http://ppdb.tc.cornell.edu/), also allowing an instant comparison with Arabidopsis chloroplast proteome information.

1 Majeran, W., Cai, Y., Sun, Q., van Wijk, K.J. (2005) The Plant Cell 17(11) 3111-40

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P13

Gene conversion between direct non-coding repeats promotes genetic and phenotypic diversity at a regulatory locus of Zea mays (L.)

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While evolution of coding sequences has been intensively studied, diversification of non-coding regulatory regions remains poorly understood. In this study, we investigated the molecular evolution of an enhancer region located 5 kb upstream of the transcription start site of the maize pericarp color1 (p1) gene. The p1 gene encodes an R2R3 Myb-like transcription factor that regulates flavonoid biosynthetic pathway in maize floral organs. Distinct p1 alleles exhibit organ-specific expression patterns on kernel pericarp and cob glumes. A cob glume-specific regulatory region has been identified in the distal enhancer. Further characterization of 6 single-copy p1 alleles, including P1-rr (red pericarp/red cob) and P1-rw (red pericarp and white cob), reveals 3 distinct enhancer types. Sequence variations in the enhancer are correlated with the p1 gene expression patterns in cob glume. Structural comparisons and phylogenetic analyses suggest that evolution of the enhancer region is likely driven by gene conversion between long direct non-coding repeats (~ 6 kb in length). Given tandem and segmental duplication are common in both animal and plant genomes, our studies suggest that recombination between non-coding duplicated sequences could play an important role in creating genetic and phenotypic variations.

P14

Genetic Control of the Carbon-Nitrogen Balance in Leaves of the Illinois Protein Strains

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Carbon catabolism and nitrogen assimilation are two fundamentally crucial and often competitive processes taking place within maize vegetative tissues. Previous studies have shown regulation of carbon fixation by nitrate and ammonium, as well as regulation of nitrogen assimilation by sugars, yet little is known about the interface between carbon and nitrogen pathways and the gene families controlling these junctions. We utilized the Illinois High Protein (IHP) and Illinois Low Protein (ILP) lines of maize, two products of divergent selection that differ widely in their respective abilities to sequester and utilize nitrogen, as a system for exposing altered control points for C and N metabolism. Preliminary global gene expression profiles were analyzed to determine possible patterns of metabolic coordination in response to environmental factors (light/N) and among genotypes. Hypotheses formulated from the expression profiles will be presented in conjunction with data from real-time qRT-PCR assays on candidate genes and putative housekeeping genes. Progress made towards characterizing differences in candidate gene structure and family densities among genotypes will also be reported in terms of the development of fosmid genomic libraries from IHP and ILP. Insights gained from these studies will be discussed in the context of future experiments that will examine carbon exchange, metabolite abundance and protein accumulation.

P15

Genetic analysis of opaque2 modifier genes

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opaque2 modifier (mo2) genes convert the soft, starchy endosperm of maize opaque2 (o2) mutants to a hard vitreous phenotype. By systematically introgressing mo2 genes into o2 germplasm, plant breeders in South Africa and CIMMYT were able to develop several hard endosperm o2 mutants that they designated "Quality Protein Maize", or QPM. Genetic mapping of modifier genes could accelerate their transfer to commercially valuable germplasm and would facilitate their isolation and molecular characterization. We have begun to map genetic loci linked to the modified phenotype by bulked segregant analysis of vitreous and opaque seed from a cross of a South African QPM (K0326Y) and a soft o2 inbred (W64Ao2). There are two loci clearly linked to the modified phenotype. The first was found in bin 7.02 and is near the 27-kD gamma-zein locus, consistent with prior results from RFLP mapping. The second was found in bin 9.02 and may be associated with starch synthesis genes. Prior studies of modifier action indicated that starch synthesis is altered in mo2 genotypes. Sequencing of starch synthesis genes in isogenic backgrounds showed that 4 of these genes have sequence differences in a mo2 compared to o2 or normal. Ongoing work is being done to determine if these genes are genetically linked to o2 modifiers.

P16

Genetic and transcriptional analysis of a sugary enhancer allele

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A sugary-enhancer allele was isolated in a near-isogenic background, allowing Mendelian segregation to be visualized. The gene was mapped to chromosome bin 2.09 using microsattelite markers. Affymetrix and long-oligo arrays were used to assess transcriptional profiles at 16 dap and 24 dap. Analysis of the transcriptional profiles will be presented.

P17

Global Transcriptional Response to Ionizing Radiation (IR) is ATM-dependent, while IR-induced G2 arrest is ATR dependent

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The protein kinases ATM and ATR are involved in a wide variety of damage responses in mammals. The Arabidopsis genome encodes both ATM and ATR homologs; plants with knockouts of these genes are viable. Here we perform a global transcriptional analysis of short-term response to ionizing radiation, and suggest that the IR-hypersensitivity of ATM knockouts may be due, to a failure of short-term transcriptional response to IR. To determine whether ATM and/or ATR play a role in response to IR-induced damage, we compared the transcriptional response of wild-type, atr, and atm seedlings to gamma radiation, harvesting the seedlings 1.5 hr after treatment. Using whole-genome Affymetrix chips, we found that approximately 400 genes were upregulated by gamma radiation. Of those genes with an identifiable function, 17% appear to be involved in DNA repair, DNA replication, and/or cell cycle regulation. ATM, but not ATR, was required for the induction of virtually all of these.
One of the more robustly induced transcripts is that encoding CycB1;1. CyclinBs normally promote the transition into M phase, an event which could prove disastrous in the presence of double strand breaks. In mammalian cells, the expression of CyclinB is strongly repressed, rather than induced, by radiation. The immediate ATM-dependent induction of the CycB1;1 transcript stands in contrast to our observation of ATM-independent CycB1;1:GUS protein expression at later times after irradiation. We believe we are observing two independent responses to radiation that are regulated by two different signal transduction kinases. ATM induces the immediate transcriptional upregulation of Cyclin B1;1 in response to IR. ATR, in contrast, induces a late S/G2 arrest response to replication blocks. This arrest precludes that active degradation of CyclinB that naturally occurs at anaphase, leading to hyperexpression of the CycB1;1:GUS protein in the absence of its transcriptional induction. Why CycB1;1, unlike other CycB1s or CycBs, should be regulated in this manner remains a mystery, but it is possible that this particular CycB1;1 might, if overexpressed, act to inhibit progression into M phase.

P18

Identification of new components of maize SnRK1 complex

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Plants have developed different mechanisms to integrate external environmental situations and coordinate the production and metabolism of the carbohydrates required for growth or to adapt to environmental stress.

The SNF1/AMP-activated protein kinase (AMPK) protein family regulate biological responses to environmental and nutritional stress in eukaryotes. In plants, this complex plays a central role in carbohydrate metabolism and as a regulator of key enzymes involved in the production of sugars or nitrate assimilation. Yeast SNF1 and animal AMPKs form a ternary complex with two additional types of proteins: SNF4/AMPKg and SIP1/ SIP2/GAL83/AMPKb. The SNF4/g-subunit plays a regulatory role in the complex, whereas the b-subunit functions as a target selective adaptor that anchors the catalytic kinase and the regulator. In maize plant SnRKs interact with a unique adaptor-regulator protein, AKINgb, which contains a N-terminal KIS domain.We are studying the regulation of this complex under stress conditions. Our results indicate that AKINgb, expression is highly induced by osmotic stress suggesting a role in abiotic stress responses. In addition, we have isolated a new catalytic subunit described only in cereals, ZmSnRK1b. Complementation of yeast snf1 or snf4 mutants by ZmSnRK1b demonstrate a functional relationship. The deduced aminoacid sequence shows close similarity with the SNF1-N terminal domain, whereas the regulatory domain is more divergent and is responsible for specific interactions with several putative partners identified by two hybrid experiments. The function of SnRK1 in the crosstalk between stress and metabolic responses will be discussed.

P19

Identifying Protein-Protein Interactions Among Starch Biosynthetic Enzymes

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Starch biosynthesis is a central metabolic process in plants that requires a number of enzymes working in concert to form a highly organized, crystalline structure. Genetic and enzyme activity analyses of mutant maize plants with altered starch content have indicated multiple proteins are involved in this process. Observed pleiotropic effects on enzyme activities imply various protein-protein interactions must occur during synthesis.

Four distinct families of enzymes are known to be involved in starch biosynthesis: starch synthases, branching enzymes, debranching enzymes, and D-enzymes. How these families of enzymes function and coordinate synthesis of crystalline starch is an area of intense research.

Extensive yeast two-hybrid analyses of maize proteins revealed evidence of interactions between members of the synthase and branching enzyme families; namely starch synthase I (SSI) and starch branching enzyme IIa (SBE IIa), and a portion of SSIII N-terminus with SSI and SBEIIa.
Affinity chromatography and peptide identification by tandem mass spectroscopy have confirmed these two-hybrid data and also shown evidence of a novel interaction between the SSIII homology domain and SSI.

P20

Induction and Metabolic Engineering of Sorghum Antifungal Compounds in Maize

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The significant contribution of phytoalexins to plant defense against pathogens has generated considerable interest in engineering of such compounds in the economically important crops such as cereals. Sorghum plants produce 3-deoxyanthocyanidin phytoalexins as a site-specific response to Colletotrichum fungus ingress. These phytoalexin compounds have structural similarities with flavan-4-ols, the precursors of the red phlobaphene pigments that accumulate in developing kernel pericarp of sorghum and maize. We have previously cloned a sorghum myb transcription factor yellow seed1 (y1), which is orthologous to the maize pericarp color1 (p1). These two genes are similar in their coding regions but are highly diverge in their regulatory sequences. They regulate the phlobaphene biosynthesis in the two species and have similar pattern of expression in the floral tissues. We showed that y1 has the exceptional expression in leaf as well as it is induced during fungal infection. Here, we describe the characterization of a null allele of y1 (y1-ww) and further study the role of y1 in phytoalexins biosynthesis. The molecular characterization of y1-ww indicated the presence of a partial internal deletion that genetically correlates with loss of phlobaphene pigmentation in kernel pericarp and leaf. Further, fungus inoculation studies will be presented to study the induced expression of the 3-deoxyanthocynidin phytoalexins in y1 null and wild type lines. The presence or absence of the phytoalexins in functional and null y1 alleles will be correlated with the resistance and susceptible responses, respectively. Maize transgenic lines carrying Y1 constructs will be used to test the heterologous expression of induced phytoalexins and their association with disease response.

P21

Isolation and characterization of maize sesquiterpene cyclase2 (stc2) gene involved in insect resistance

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In response to herbivorous insects, plants synthesize and release volatile chemical signals that will attract the natural enemies of the herbivore to defend themselves. Here we report the isolation and characterization of the maize sesquiterpene cyclase2 gene (stc2) that is ortholog of stc1, a gene induced in response to the attack of beet armyworm larvae. A full-length stc2 cDNA cloned by RT-PCR is predicted to contain an ORF encoding 633 amino acids. Analysis of genome sequences from different inbred lines showed a large extent of polymorphisms. In particular, a 2kb non-LTR retroelement was present in the 3rd intron of Ki3, A636, and I137TN stc2. Differentially-spliced stc2 transcripts were isolated from Ki3, which were possibly due to the retroelement insertion. stc2 was mapped to 6L, a region that contains a quantitative trait locus (QTL) for southwestern corn borer resistance (SWCB) in tropical maize, making stc2 a possible candidate gene for the resistance. The fusion proteins of both McC STC2 and STC1 with GFP are localized to the plastid, in agreement with the prediction of the ChroloP program. However, since several amino acids are absent in the transit peptide of Ki3 STC2, it will be interesting to further characterize its subcellular localization.

P22

Maize Beta-Glucosidase Aggregating Factor (BGAF) is a Polyspecific Jacalin-related Chimeric Lectin and its Lectin Domain is involved in -Glucosidase Aggregation

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In certain maize genotypes called "nulls," beta-glucosidase does not enter the gel and therefore cannot be detected on zymograms. Such genotypes were originally thought to be homozygous for a null allele at the glu1 gene. We have shown that a beta-glucosidase aggregating factor (BGAF) is responsible for the null phenotype. BGAF is a chimeric protein consisting of two distinct domains: the disease response (also known as "dirigent") domain and the jacalin-related lectin (JRL) domain. It was not known whether the JRL domain in BGAF is functional and if it does, what its sugar specificity is. Secondly, we were interested to know which of the two domains is involved in beta-glucosidase binding and aggregation. To this end, we purified BGAF from maize H95 "null" line to homogeneity. The purified protein gave a single band on SDS/PAGE, and the native protein was a dimer of two 29 kD monomers. Native free BGAF, BGAF-beta-glucosidase (Glu1 isoform) complex and recombinant BGAF agglutinated rabbit erythrocytes and their hemagglutination activity was inhibited by galactose, mannose, N-acetylneuraminic acid, and galactosamine. The presence of saturating concentrations of galactose had no effect on the binding of BGAF to Glu1 and that the BGAF-Glu1 complex could still bind lactosyl-agarose lectin affinity column, indicating that the sugar-binding site is distinct from the Glu1-binding site. To investigate which of the two domains is involved in Glu1 binding, we cloned the dirigent and JRL domain encoding regions of the bgaf cDNA separately and expressed them in E. coli. Aggregation assay showed that neither the dirigent nor the JRL domain produced beta-glucosidase aggregates that can be precipitated by centrifugation. However, in the gel-shift assay the JRL domain showed retardation of Glu1 mobility, suggesting that the binding regions for Glu1 in BGAF are in the JRL domain. Indeed, in the competitive assay in the presence of increasing concentrations of the dirigent or the JRL domain, only the JRL domain showed marked inhibition of beta-glucosidase aggregation by intact BGAF. These results together with the results from deletion mutagenesis and by exchanging the JRL domain of maize BGAF with that of BGAF-like protein from sorghum, which does not bind to Glu1, allowed us to conclude that the JRL domain of BGAF is responsible for both its lectin activity and beta-glucosidase aggregation.

P23

Maize CENPC - a DNA and RNA binding protein

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Centromeric DNAs are highly repetitive and non-conserved, making it difficult to understand how centromeres maintain their interactions with kinetochore proteins. Henikoff and colleagues have suggested that centromere repeats evolve selfishly to increase their binding affinity for inner kinetochore proteins. Currently the best candidate for the protein that mediates this interaction is Centromere Protein C (CENP-C). We have tested the binding capacity of maize CENP-C to DNA in a variety of assays. Gel shift assays show that maize CENP-C is a DNA binding protein, however, we found no evidence that the binding is sequence specific. We are now considering the possibility that chromatin-associated RNA may be the specific target for CENP-C binding, since prior data suggest that RNA is an integral component of the maize kinetochore. Encouragingly, recent data show that CENP-C is indeed a RNA binding protein. We are further testing for specific RNA-protein interactions. A detailed subdomain analysis of the protein suggests the major DNA/RNA binding region maps to an area that is undergoing negative selection.

P24

Maize flavonoid 3'-hydroxylase encoded by red aleurone1 (pr1) is required for cyanidin and luteoforol biosynthesis

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Flavonoid biosynthetic pathway produces a number of end products and depending on the genetics of the plant, these products include anthocyanins, flavonones, flavones and phlobaphenes. Maize genotypes carrying a functional pr1 have purple kernel due to the accumulation of cyanidin in aleurone cells. Mutation in the functional pr1 leads to accumulation of pelargonidin instead of cyanidin and thus kernel color in mutant plant is red as oppose to purple. For the synthesis of cyanidin, dihydrokaempferol (DHK) is required to be converted into dihydroquercitin (DHQ) by the action of flavonoid 3'-hydroxylase (F3'H), a cytochrome P450 dependent enzyme which adds a hydroxyl group at the 3' position of B-ring of flavonones. We have cloned the functional pr1 gene and genetic analysis of test-cross progeny plants showed that the cloned f3'h1 co-segregates with pr1 kernel phenotype. Genetic complementation experiments using CaMv 35S::f3'h1 gene construct confirmed that the putative protein product is capable of performing 3' hydroxylation reaction in vivo. Maize f3'h1 mRNA was detected in various tissues of plants segregating for Pr1 while pr1 plants did not show any detectable f3'h1 mRNA, indicating that pr1 plants has a mutation that affects transcription. In addition to its role in anthocyanin biosynthesis, our recent results indicate that functional pr1 may also affect phlobaphene biosynthesis. Phlobaphenes are formed through flavan-4-ols, under the regulatory control of pericarp color1 (p1) locus. Further, transcriptional and biochemical results will be presented to determine the dual role of pr1 in anthocyanin and phlobaphene biosynthesis.

P25

Making, mapping and characterizing tassel seed mutants in maize

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The maize tassel contains male flowers and the ear contains female flowers. This separation of sexes is due to selective abortion of the pistil in male flowers and growth arrest of stamens in the ear. The tasselseed1 or tasselseed2 mutations are due to a failure to abort the pistil in the tassel and a concomitant loss of stamen development in the tassel. Other mutations such as tasselseed4 or Tasselseed6 have additional defects in the maintenance of meristem identity, which are revealed by extra branching. We have used the tassel to screen for new mutants that affect the inflorescence. The occasional silk in the tassel is often a sign of other defects; for example, mutations in Zea mays agamous1 (zag1) and polytypic2 (pt2) have occasional silks in the tassel but are otherwise fertile. Both zag1 and pt2 mutants have significant defects in the ear. We are screening material generated by EMS in inbred and hybrid backgrounds. The use of different starting materials allows us to uncover new mutations that might be suppressed in one particular inbred. We present a progress report of walking to pt2 and the characterization and mapping of other dominant and recessive mutations that show a failure of pistil abortion.

P26

Map-based cloning of the nsf1 (nicosulfuron susceptible 1) gene of maize.

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Inheritance of susceptibility in maize (Zea maize L.) to the sulfonylurea herbicide nicosulfuron (DuPont Accent) is conditioned by a single recessive gene, designated nsf1 (Kang, 1993). It is known that resistant lines metabolize the herbicide faster than susceptible ones; the characteristics of the metabolism suggest the involvement of a cytochrome p450 mono-oxygenase (Burton et al., 1994). A map-based cloning approach was taken using a BC1 population. An initial small-scale experiment established the location of nsf1. A total of 2584 resistant BC1 plants were analyzed to leave a region containing a single candidate gene on a sequenced BAC. These results show that although present at a low frequency, this sensitive allele is widespread in North American open-pollinated varieties and synthetics. This gene has a variety of potential applications.

P27

Mapping of BGAF Binding Regions on the Maize Beta-Glucosidase Isozyme Glu1

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Beta-glucosidase (beta-D-glucoside glucohydrolase, E.C 3.2.1.2.1) catalyzes the hydrolysis of aryl and alkyl-beta-D-glucosides as well as beta-linked oligosaccharides. In maize, two beta-glucosidase isozymes (Glu1 and Glu2) have been identified, cloned and expressed in *Escherichia coli*. In certain maize genotypes "null," beta-glucosidase fails to enter the gel and cannot be detected on zymograms. We have shown that a specific beta-glucosidase aggregating factor (BGAF) is responsible for "null" phenotype, and it specifically interacts with beta-glucosidases and form large insoluble aggregates. Furthermore, we have mapped BGAF-binding regions on beta-glucosidase by domain swapping between maize beta-glucosidase isozymes Glu1 and Glu2, to which BGAF binds and sorghum beta-glucosidase (dhurrinase) isozyme Dhr1, to which BGAF does not bind. The previous results have shown that an N- terminal region (Glu^{50}-Val^{145}) and an extreme C-terminal region (Phe^{466}-Ala^{512}) together form the BGAF binding site. The goal of the present study was to define more precisely the BGAF-binding regions on the maize glucosidase isozyme Glu1 and to identify the specific amino acids involved in Glu1-BGAF interaction. To this end, we swapped the N-terminal (Ile^{72}-Thr^{82}) and C-terminal (Phe^{466}-Ala^{512}) regions of Glu1 with those of Dhr1. The results of binding assays suggest that the amino acids essential for BGAF binding are located in the N-terminal region (Ile^{72}-Thr^{82}) whereas the C-terminal region plays only a minor role in that it either increases the affinity of the N-terminal region for BGAF binding or enhances the stability of Glu1-BGAF complexes. Furthermore, to identify the specific amino acids, we mutated the unique amino acids in Glu1 (Ile^{72}/Val^{73}, Asn^{75}/Asp^{76}, Lys^{81}/Ala^{82}, and Thr^{82}/Glu^{83}) to those in Dhr1. Of these single amino acid substitutions, the replacement of Thr^{82} in Glu1 with the corresponding residue Glu^{83} in Dhr1 completely abolished binding to BGAF, suggesting that Thr^{82} plays a critical role in BGAF binding.

P28

Photosystem II genes display a potential mechanism of Lepidopteran resistance.

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Fall armyworm (FAW) and southwestern corn borer (SWCB) are Lepidopteran pests of maize that cause crop damage in the United States. Previous proteomic analysis of resistance and susceptible siblings revealed that several proteins involved in photosynthesis were differentially expressed in resistant compared to susceptible whorl-stage maize leaves. The *high chlorophyll fluorescence (hcf)* mutants have defects in photosystem I or II which lead to aberrant electron transport. Preference tests of several *hcf* mutants compared to wild-type confirmed that genes involved in photosynthesis affected insect damage. Antibiosis feeding trials were performed on leaf sections of mutant and wild-type plants to determine the effect of the *hcf* mutants on larval weight gain. Plant damage was determined using the AlphaEase software from digital images and larval weight was determined in milligrams on an individual insect basis. *hcf-N1029D* significantly decreased larval weight of both FAW and SWCB while *hcf*-88-3005-33* significantly increased larval weight gain of both Lepidoptera. *hcf11-N1250A* significantly increased FAW larval weight but had no effect on SWCB larval weight. Conversely, *hcf48-N1282C* significantly increased SWCB larval weight but had no effect on FAW larval weight. The data from this experiment together with the preference tests indicates that the photosystem II genes have a significant effect on larval feeding that is not related to differences in leaf color. These genes are localized to the stromal thylakoid which is also the location for synthesis of the fatty acid precursors to epicuticular waxes. Previous data indicates that epicuticular waxes can confer Lepidopteran insect resistance. To determine whether epicuticular waxes are altered in *hcf* mutants, we performed electron microscopy of the leaf surface waxes. Comparisons of mutant and wild-type leaves show marked differences in wax distribution on the leaves. Further tests are underway to characterize the composition and amount of leaf wax in mutant and wild-type plants.

P29

Pre-mRNA Splicing And rRNA Processing Are Altered in the Endosperm of mto38, a Maize Opaque Mutant.

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A maize mutant, mto38 (Mutator-tagged opaque 38), was identified from a genetic screen for kernels with opaque endosperm. Homozygous mto38-1 kernels have uniformly starchy endosperm and reduced levels of zein storage proteins. Cloning and sequence analysis revealed that the Mutator-tagged gene for mto38-1 encodes a spliceosomal protein. We compared the RNA transcript profile of mto38-1 endosperm with that of its wild-type siblings. Genes that are differentially expressed between the wild-type and the mutant endosperm, or DEGs (Differentially-Expressed Genes), include those encoding ribosomal proteins, ribosomal RNA (rRNA) processing factors, translation factors, zeins and their transcriptional regulators (e.g. opaque2). Remarkably, sequence alignments of some microarray probes to the genomic sequences of corresponding DEGs indicated that pre-mRNA splicing patterns are altered in the mutant. Splice variant-specific RT-PCR confirmed DEGs with altered splicing patterns in the mutant. These results are consistent with our hypothesis that mto38-1 phenotypes are caused by a defective gene encoding a spliceosomal protein and that the MTO38 gene may provide a layer of post-transcriptional regulation that is unique in maize endosperm.

P30

Rigidity of Carbohydrate Metabolism in Maize Kernels

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Relative fluxes through pathways of the central carbohydrate metabolism were determined. Developing kernels were grown in sterile culture and supplied with a mixture of [U-13C6]glucose and unlabeled glucose. The abundances of individual glucose isotopologs were analyzed by 13C isotope ratio mass spectrometry and high-resolution 13C NMR spectroscopy. In order to reveal the influence of different genotypes on the fluxes the starch mutants amylose extender (ae), waxy (wx), sugary1 (su1), brittle1 (bt1), brittle2 (bt2), shrunken2 (sh2), Sh2-Rev6, shrunken1 (sh1), shrunken1 sucrose synthase1 (sh1 sus1), miniature1 (mn1), as well as heterotic kernels were investigated. Similar glucose isotopolog patterns were identified for all samples, which points to a high degree of robustness of the central carbohydrate metabolism in maize kernels. Flux partitioning between glycolysis and PPP was identified as a very rigid metabolic branch point in every sample whereas direct carbon flux into starch is a slightly more flexible part of the metabolic network.

P31

The Maize TILLING Project: Progress Report for Year 3.

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TILLING (Targeting Induced Local Lesions In Genomes) is a high-throughput, reverse genetic method to identify point mutations in specific gene targets among a mutant population. Non-silent, sub-lethal and sub-sterile mutations will be valuable for functional genomics, studying protein function(s), protein-protein interactions and enzymatic activities. The Maize TILLING Project (MTP, http://genome.purdue.edu/maizetilling/) has developed EMS-mutagenized B73 and W22 populations for TILLING and forward genetic screens. To date, MTP has received 87 TILLING requests, delivered mutations for 44 of these and are either TILLING or sequencing identified mutations in 20 more targets. TILLING requests are considered completed when we obtain an allelic series in which there is a 95% confidence level that at least one of the mutations has a damaging effect on the protein. Users receive sequence information, predictions of mutation effects and stock numbers for seed. Orders not completed with our current population remain active for screening with additional mutant lines as they become available. Our current TILLING population contains ~2900 mutant lines with ~165,000 mutations in exons. In the next month we will add ~850 new W22 lines and, by the end of the spring, ~800 new B73 lines (~3100 total) and another ~1000 W22 lines (~2300 total).

The remaining 23 TILLING requests are in Primer Pre-screening. This Pre-screening has been valuable in increasing our success rates; only one primer set that passed the pre-screen has failed at the TILLING stage. More importantly, we can identify in advance primers and targets that are problematic (e.g., targets with high GC% or long stretches of identical bases, etc.), as well as the solutions to those problems. If a primer pair fails, we send the user a report describing the problem and sequence information we generated for the target. The user then selects (at no additional charge) another set of primers for the target and, in most cases, the second set of primers is successful.

P32

The RAMOSA Pathway in Maize Inflorescence Determinacy

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Three ramosa genes, ramosa1, ramosa2 and ramosa3 have been identified to regulate inflorescence branching architecture in maize. ra1 is expressed in emerging spikelet pair meristems. It encodes a zinc finger transcription factor and its expression predicts branch determinacy. ra2 encodes a LOB domain protein, expressed in the anlagen of the bract and meristem early in inflorescence development, and determines the fate of stem cells in the axillary meristem. ra3 encodes a trehalose-6-phosphate phosphatase. It is expressed in discrete domains subtending axillary inflorescence meristems and can also regulate inflorescence branching. Further experiments revealed that ra1 expression is lowered in ra3-R mutants and significantly reduced in ra2-R mutant; ra2 has the same expression pattern in tassels of ra1-R mutant and ra3-R mutant; there is also no significant change in level or localization of ra3 expression in ra1-R mutant or ra2 mutant. Based on these results, a ramosa pathway to regulate branch determinacy in the maize inflorescence was described. ra2 and ra3 act upstream of ra1, and ra3 may act parallel with ra2 in this pathway. We are further investigating the ramosa pathway at the molecular level. Techniques include generation and characterization of antibodies for RA1, and yeast two-hybrid (Y2H) analysis. Y2H will be used to investigate the relationships between these three RAMOSA proteins and to screen cDNA expression libraries for interacting proteins.

P33

The Role of the Maize Terpene Synthase Gene Family in Plant Defense

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Despite the remarkable abundance and diversity of terpenoid secondary metabolites in plants, there are still large gaps in our knowledge of their biological and evolutionary origin. However, the availability of genetic and genomic resources for the model plant species maize and Arabidopsis provides an exciting array of new tools for exploring the ecological and evolutionary significance of this enormous class of natural products. The key step of terpene biosynthesis is catalyzed by the enzyme class of terpene synthases which employ an electrophilic reaction mechanism to form multiple products from single prenyl diphosphate substrates. In maize, terpene synthases are encoded by a large family of genes that share a common evolutionary origin. To identify the function of terpene blends generated by the terpene synthases in the tritrophic interactions between maize, lepidopteran larvae and parasitoids, we overexpressed the corresponding genes in Arabidopsis and measured the attraction of the transgenic plants to the parasitoid Cotesia marginiventris using an olfactometer. A second tritrophic interaction was studied in the roots of maize which emit sesquiterpene hydrocarbons after insect damage. Feeding by larvae of the Western corn rootworm caused release of (-)-(E)-beta-caryophyllene which was shown to attract enemies of the corn rootworm.

P34

The maize DIP1 (DBF1-interactor protein1) containing an R3H domain is a potential regulator of DBF1 activity in stress responses

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The maize DRE-binding protein DBF1 is a member of the AP2/ERF transcription factors family and is involved in regulation of the abscisic acid-responsive gene rab17 through the drought-responsive element in an ABA dependent pathway. Here, we analyzed the functionality of DBF1 in abiotic stress responses and found that Arabidopsis plants overexpressing DBF1 are more tolerant to osmotic stress than control plants. In yeast two hybrid analysis DBF1 interacts with DIP1 (DBF1-Interactor Protein 1) a protein containing a conserved R3H single strand DNA-binding domain. Subcellular localization of DIP1 shows that the protein fusion DIP1-RFP is mainly localized in the cytoplasm. However, after co-transformation of DBF1-GFP and DIP1-RFP both proteins co-localize in the nucleus. Interestingly, when the N-terminal DBF1-GFP was co-expressed with the DIP1-RFP, both proteins co-localized predominantly in the cytoplasmic speckles observed for N-terminal DBF1-GFP fusion protein. These results clearly show in vivo interaction of DBF1 with DIP1 in the cell and that this interaction is necessary for the nuclear localization of DIP1 protein. Analysis of the regulatory effect of DBF1 and DIP1 interaction on the maize rab17 promoter activity indicated that co-transfection of DBF1 with DIP1 enhances promoter activity in normal conditions. We suggest that the regulated association of DBF1 and DIP1 may control the levels of target gene expression during stress conditions.

P35

The role of sorbitol and sorbitol dehydrogenase in maize kernel

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At 10 DAP (days after pollinization) sorbitol dehydrogenase (SDH) was found to be the most abundant transcript, as depicted by the number of reads in the MAIZEST database. SDH catalyzes the NADH-dependent reduction of fructose to sorbitol or the oxidation of sorbitol to fructose. In Rosaceae, this enzyme has an important role in the sorbitol translocation and fructose storage in fruits. Maize endosperm, however, does not store fructose or sorbitol. Sucrose arriving at the kernel base is metabolized into fructose, which can be converted to sorbitol by SDH. This is a highly active enzyme in maize endosperm, but not in embryos. Still, there is considerable sorbitol in maize embryos. We verified that embryos could grow having only sorbitol as a carbon source. We have found sorbitol transporter ESTs in endosperm and aldose-reductase, which can convert sorbitol into glucose, ESTs in the embryo. By injecting 13C sorbitol into the maize endosperm and performing mass spectrometry analysis we were able to demonstrate that sorbitol can be transported into the embryo, where it is metabolized into fructose or glucose. The role of sorbitol in developing maize kernels is potentially pivotal, but remains undefined. Our results seem to indicate that sorbitol contributes as a non-vascular transport sugar moving from endosperm to embryo. This may be especially important in a situation when the embryo competes, against the strong starch-synthesis machinery of the endosperm tissue, for sucrose that arrives to the kernel.

P36

Viral-induced gene silencing of cellulose synthase (CESA) and cellulose synthase-like (CSL) genes in barley.

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Growing plant cell walls are composed of independent, interacting networks of cellulose microfibrils, tethered by various cross-linking glycans, which are embedded in a matrix of pectins. The cell walls of the Poales order are distinct from non-commelinoid plants in that they contain mixed-linkage (1,3), (1,4)-beta-glucan (beta-glucan) and glucuronoarabinoxylan (GAX) as major cell wall crosslinking glycans. beta-Glucan plays essential roles in cell wall architecture, cell growth and development, and also the human diet. While a related form of GAX is found to lesser extents in non-commelinoid plants, beta-glucan is not. Owing to numerous mechanistic and topological similarities with cellulose biosynthesis, members of the CSL gene families are likely candidates for encoding the backbone glycan synthases of the non-cellulosic cell wall polymers.

To elucidate the functions of select members of the CESA/CSL superfamily in cereals, we have chosen viral-induced gene silencing (VIGS) in barley using the barley stripe mosaic virus. VIGS targets were designed based on sequence alignments of CESA and CSL genes in rice. The corresponding regions were then identified in barley. Viral particles tagged with our VIGS targets were generated in vitro and barley seedlings were inoculated. Infected plants showed significant decreases in the expression of respective target gene(s) and corresponding alterations in cell wall monosaccharide composition, cellulose amount, and beta-glucan profile. While VIGS experiments have identified candidate genes that encode the beta-glucan synthase, heterologous expression of these genes in non-commelinoid systems should provide definitive evidence of their function.

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P37

Ab Initio Protein-coding Gene Finding In Maize Genome

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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. A large-scale effort to sequence the maize genome will commence in 2006. To better annotate this monocot genome 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. The essential idea is that functional sequences, such as protein coding regions and splice sites, show different patterns of evolutionary conservation than sequences under little selective pressure, such as the central regions of introns. Here we describe an NSF funded project to improve *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. This training set will be constructed from currated alignments of full-length cDNAs to maize genomic sequences collected from both public and proprietary sources. Our model collection strategy, current progress and expectations will be presented.

P38

A Computational Approach for Characterizing Standardized Phenotypic Images for Maize

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Experimentation with mutant maize plants is an effective method for understanding the roles of specific genes as well as for visualizing the phenotypic effects of these mutations. For visually observed phenotypic effects, annotations are made by scientists to document the physical state of the mutated plant; however, the language used to describe the mutations can be vague, especially in terms of color, texture, and size (e.g. the leaf is pale green, the kernel is variegated, the plant is short). Color descriptions are further complicated by the fact that "light green" to one person may be described as "yellow green" by another. To combat this vagueness or uncertainty in mutant descriptions, image processing and computer vision algorithms can be developed to quantify these types of visual features, eliminating the subjective component of human perception in these kinds of descriptions.

We are developing a web-based phenotypic information management system, VPhenoDBS, which will use these features to allow biologists to perform complex queries (query by image example, query by text annotation/ontology, and query by physical and genetic map information) on maize images. The web-based system will be publicly accessible to the plant community, particularly for the maize community for the initial stage. We propose simple standards to capture phenotypic images for various body parts and development stages using commercially available digital cameras, color palettes, rulers, and homogeneous background settings under a consistent lighting condition. All images deposited to the VPhenoDBS using the simple standards, along with their corresponding text annotations, will be searchable and cross referenced to various maps with a unique visualization tool.

This project is supported by NSF grant #DBI-0447794.

P39

A novel bioinformatics method for identifying candidate active transposable elements

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Most transposable element-search programs can be divided into two general classes. The first class annotates DNA sequences with known transposable element libraries such as RepeatMasker, MaskerAid and/or CENSOR. The second search program class utilizes self-by-self comparisons to identify novel repeats that are usually transposable elements. Such programs include RECON and REPuter. Here we present a novel de novo method that searches for and identifies polymorphisms due to transposition events in otherwise identical or nearly-identical genomic sequence. The putative transposition polymorphisms are definable in either inter-subspecies comparisons (ESC) or intra-subspecies comparisons (ASC). A transposition event that is identified by ESC means the polymorphism has occurred recently and the element may still be active. Application of this program to the rice genomic sequence data led to the successful identification of 70 elements including complete copies of three active elements (mPing, TOS17 and Dart). We are in the process of using this program to identify maize elements in available and newly emerging genomic sequence and will report our progress to date.

P40

A simulation approach to measure crossover interference in linkage maps

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Interference during meiosis reduces the probability that two crossover events will occur close to one another. Improving our knowledge of this phenomenon and of how it varies along chromosomes and among different crosses and species would be useful to refine the mathematical models used to derive genetic distances from recombination rates. Interference can be easily measured from any group of three loci linked on a genetic map by calculating the coincidence coefficient. However, modeling the distribution of this coefficient, as it can be measured from usual genetic maps, is not straight-forward. We generated simulated backcross and F2 datasets with and without interference, and we observed the resulting distributions of the coincidence coefficient. Then we developed a generalized interference model and used it to measure the degree of interference in a barley linkage map.

P41

Analyzing, Annotating, and Visualizing the Maize Genome Sequence

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The NSF-funded Maize Genome Project, a collaboration between the Washington University Genome Sequencing Center, the Arizona Genomics Institute, Iowa State University, and Cold Spring Harbor Laboratory, aims to sequence the maize genespace to a finished quality using a BAC-based approach (cf. "Sequencing the maize genome"). Given the complex and highly repetitive topology of the genome, ongoing analysis and annotation is crucial to guiding the sequencing effort. A multifaceted informatics framework has been built at CSHL that provides streaming feedback regarding sequencing progress and presents the latest annotations to the maize community. The annotation pipeline, driven by Ensembl, performs automated analysis on nearly-finished BAC sequences. Analysis includes whole-genome alignment to rice, ab initio gene prediction, alignment to known sequence and marker data sets, and repeat analysis. Curated repeats and markers are used internally in finishing activities to annotate potentially difficult regions on BAC assemblies. Well-supported gene models facilitate mapping activities by redefining gene boundaries and order-and-orienting contigs.

The BAC sequences will be uploaded from GenBank and analysis results are made available to the public through an extensible genome sequence browser. Built on Ensembl, the browser visualizes sequence assembly and related data tracks. CMap provides views of comparative species maps, as well as maize genetic and physical maps. The interface presents reciprocal links to external data sources, notably Gramene and MaizeGDB, and encourages user feedback. Additional tracks will be incorporated as needed to serve the maize community.

P42

Applications of Plant Ontologies for describing and comparing phenotypes and gene expression data in plant databases.

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The Plant Ontology Consortium (POC) (www.plantontology.org) is a collaborative effort of several plant databases and experts in plant systematics, botany and genomics. A primary goal of the POC is to develop generic controlled vocabularies that reflect the biology of plant structures and developmental stages. The POC has adopted the ontology structure and rules as well as the software infrastructure previously developed by the Gene Ontology Consortium. However, Plant Ontology is conceptually different from GO, has its own principles, and is governed independently from GO. The initial releases of the Plant Structure Ontology and Plant Growth and Developmental Stages Ontology included integration of existing ontologies for Arabidopsis, maize and rice. Currently, we are expanding PO to encompass legumes, Solanaceae and other plant families. As a part of ongoing functional annotation efforts, participating databases (TAIR, Gramene and MaizeGDB) have been using Plant Ontologies to annotate gene expression data, describe mutant phenotypes and biological samples. Over 4,000 gene annotations have been contributed to the POC database. These annotations can be queried using our ontology browser, the Plant Ontology AmiGO. We will present a summary of the organizing principles and rules followed in developing Plant Ontologies. Standards and methods for using Plant Ontologies to annotate gene expression patterns and mutant phenotypes will be addressed with examples from member databases.

P43

Automatic and 3 dimensional phenotyping of complete corn plants.

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At PAG 2006 LemnaTec presents the *Scanalyzer 3-D*, a fully integrated automatic high-throughput screening system for complete plants like rice, corn, arabidopsis, poplars, barley or wheat in the greenhouse, combining information from all 3 dimensions. Due to the waterproof conveyor belt the system is able to 3-dimensionally screen up to 4.000 plants per day efficiently and precisely. With the *Scanalyzer 3-D* a wide range of visual evaluation parameters of plants can be sampled for a complete and reproducible and non-destructive analysis free of subjective influences (e.g. leaf area, leaf color, leaf length, internode length etc.) Fluorescence or temperature measurements are also possible and deliver a comprehensive growth and effect measurement. The produced data allows identification of statistically relevant phenotype effects by biotic or abiotic factors taking natural variability into consideration due to high numbers of plants to be analysed. Using transparent pots additional information on root growth can be quantified during the whole growth phase.

P44

Cell wall genomics at Purdue: A website resource for cell wall related genes in maize, rice, and Arabidopsis

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Cell wall composition differs greatly between commelinoid monocots (Type II cell walls), such as Oryza sativa (rice) and Zea mays (maize), and all dicots, such as Arabidopsis thaliana (Arabidopsis) and other non-gramineous monocots (Type I cell walls). Both cell wall types contain cellulose microfibrils but differ in the molecules that interlock the fibrils, glucuronoarabinoxylans for Type II and xyloglucans for Type I. Type II cell walls also have decreased abundance of pectins compared to Type I walls. The commelinoid monocots have a mixed-linked (1,3),(1,4)-beta-glucan that accumulates transiently during cell elongation. We estimate that over 2000 genes are required for synthesis and organization of the cell wall. This gene subset provides an opportunity to compare and contrast genes responsible for a fundamental plant cell process that can differ between species.

At our cell genomics website (http://cellwall.genomics.purdue.edu) we provide substantial information about cell wall related genes for Arabidopsis. We are expanding this information to include maize and rice using a bioinformatics approach to compare known genes of available Arabidopsis, maize, and rice sequences related to the cell wall. Our site provides a resource to compare cell wall related genes in these three very important plant species by providing annotated dendrograms with links to major databases, such as: TIGR, Gramene, MIPs, Orygenes, SALK, and TAIR. Our site aims to provide researchers with a tool for study of genomic differences in cell wall formation in plants that lead to very different cell wall composition and architectures.

Supported by the National Science Foundation Plant Genome Research Program

P45

Co-expression analysis of the metabolic network in Arabidopsis thaliana

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We investigated networks of co-expression for 1,330 genes encoding metabolic enzymes in Arabidopsis. We found that genes associated with the same metabolic pathway are, on average, more highly co-expressed than genes from different pathways. Positively co-expressed genes within the same pathway tend to cluster close together in the pathway structure, while negatively correlated genes typically occupy more distant positions. Genes with multiple paralogs are co-expressed with fewer genes, on average, than single-copy genes, suggesting that the network expands through gene duplication, followed by weakening of co-expression links involving duplicate nodes. Co-expression links per gene follow a power law distribution, with a small but significant number of genes having numerous co-expression partners but most having fewer than ten. The slope of link frequency distribution is steeper for negative versus positive co-expression, indicating a more highly connected, robust network structure for positively co-expressed metabolic genes. Using an algorithm based on intersection of co-expression (ICE), we identified and prioritized novel candidate pathway members, regulators, and cross-pathway transcriptional control points for over 140 metabolic pathways. These methods and results facilitate the prioritization of candidates for genetic analysis of metabolism in plants.

P46

Comparative Genome Analysis in Gramene

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Gramene (www.gramene.org) is a database for rice genome and genetics, and a resource for comparative grass analysis. The grass family, Gramineae, comprises a large and diverse set of agronomically important crops including rice and maize. Gramene currently uses the rice genome assembly (~390Mb) as a framework to associate many of unsequenced grass genomes represented by genomic sequences, ESTs, or genetic/physical maps. These sequences/markers are ordered and oriented based upon their synteny in rice, aiding researchers in the discovery of candidate genes and to develop genetic and physical marker resources in other crops.

Gramene currently provides a view of the complete rice and Arabidopsis genomes and the partial maize genome. Several million sequences from more than 60 sequence datasets (e.g., ESTs and BACs) have been mapped to the rice genome and part of them to the maize genome. Orthologous gene sets and syntenic regions between the two grass genomes are provided, as well as orthology relations to gene models from the model dicot Arabidopsis thaliana, thereby aiding the assignment of gene functions. Protein sequences are annotated through comparison with databases of known functional domains, e.g., Interpro. In addition to the sequenced genomes, Gramene hosts more than 160 genetic/physical maps from more than 20 cereal species in its comparative map view.

The project is supported by National Science Foundation grant No. 0321685 and the USDA Agricultural Research Service.

P47

Contribute your data to MaizeGDB!

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MaizeGDB is the community database for maize genetics and genomics and can be accessed online at http://www.maizegdb.org. The success of MaizeGDB project largely can be attributed to the involvement of the community of maize geneticists: members of the community have made their data available by contributing to MaizeGDB and have helped to guide the efforts of the MaizeGDB Team by giving lots of needed input and perspective. This poster demonstrates various methods for contributing data to MaizeGDB, emphasizing the use of the MaizeGDB Community Curation Tools.

P48

Gene Expression and Visualization Application (GENEVA): Development and Use in Shoot Apical Meristem Gene Expression Analysis

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Microarray analysis generates large sets of raw data to which automated and manual processing is applied. A project is ongoing to analyze the genes involved in shoot apical meristem function and leaf initiation in maize. The Gene Expression and Visualization Application (GENEVA) was designed to store, maintain, and facilitate the annotation process of the thousands of expressed sequence tags (ESTs). The application automates techniques previously performed manually by researchers including accessing multiple BLAST reports, Maize Assembled Gene Islands (MAGIs: http://magi.plantgenomics.iastate.edu/), six Open Reading Frames, and UniGene information. GENEVA is highly searchable and allows for analysis across multiple DNA microarray chips. The application presents statistical analysis of correlations in the data by categories for both up-regulated and down-regulated genes. GENEVA has significantly improved the speed and the quality of the EST annotation process.

P49

IRILmap: linkage map distance conversion software for intermated recombinant inbred lines

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Intermated Recombinant Inbred Lines (IRILs) in plants, or Advanced Recombinant Inbred Strains in animals, are constructed by carrying out generations of intermating between F2 individuals before starting recurrent inbreeding generations by selfing or sib-mating. IRILs are powerful for high-resolution genetic mapping because they have undergone more recombination than usual Recombinant Inbred Lines (RILs). However, there is no mapping software able to generate actual centiMorgan distances from segregation data obtained with IRILs. We developed the IRILmap software which converts genetic distances computed with any linkage mapping program designed for RILs (e.g. MapMaker), so that IRIL-derived segregation data can be used to get actual centiMorgan distances, directly comparable to F2, backcross, or RIL-derived maps. A Windows version of IRILmap with user-friendly interface, as well as a perl script for embedding in other applications are freely available from http://moulon.inra.fr/~bioinfo/mapping/irilmap1.html

P50

Integrated Software for SNP Discovery in Maize

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Maize (Zea mays L.) has been one of the most important crops used to feed different cultures, playing an important role in their economies. To promote maize research, one of the goals of the Maize Diversity Project is discovery of single nucleotide polymorphism (SNP) markers which have become increasingly valuable to biological research. The development of software tools to aid researchers in the SNP discovery process across several maize, teosinte, and Tripsacum lines has been our focus. An integrated set of tools consisting of a relational database and applications for data loading, editing and reporting has been developed. All stages of SNP discovery from tracking sequences, alignment generation, alignment editing, and reporting are covered. Central to this system is an intuitive, quality score based alignment editing tool designed to simplify manual editing of the highly polymorphic and complex Zea alignments. Additionally, the system has been extended to integrate data from other sources and to submit data to the publicly available Panzea database.

P51

MaizeGDB Standard Operating Procedures

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The Maize Genetics and Genomics Database (MaizeGDB) is the community resource for maize data and can be accessed online at http://www.maizegdb.org. To enable an understanding of the more technical aspects of the project, here we illustrate the breadth of information made available through MaizeGDB, convey the method by which information is curated and made accessible, relate how the database infrastructure was built and is currently maintained, and explain the machine architecture(s) and schema in detail. How each copy of the database is utilized is illustrated, and standard operating procedures employed at MaizeGDB are described.

P52

MaizeGDB: The Maize Community Genetics and Genomics Database

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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. Among the data sets included in MaizeGDB are sequences, including integration with various contig assemblies; references; detailed genetic, physical, and cytogenetic maps; primers; and a wealth of other datatypes. MaizeGDB includes integrated tools for map comparisons, sequence similarity searches, and comparisons with and links to other databases, such as Gramene and NCBI. MaizeGDB provides web-based community curation tools that enable researchers to edit and annotate their own data and to enter new data into MaizeGDB directly. MaizeGDB also provides informatics support for maize community initiatives such as the annual Maize Genetics Conference and community-wide workshops, and maintains data for maize community research projects. MaizeGDB is funded by USDA/ARS and can be accessed online at http://www.maizegdb.org.

P53

Microarray analysis of maize chromatin mutants

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Using oligonucleotide microarrays, we are conducting a comparative global transcription study of approximately 70 different mutants deficient in chromatin related proteins. Various procedures and types of equipment were tested for their utility in large scale microarray experiments. A streamlined protocol has been designed, including the use of a Tecan HS 4800 Pro hybridization station. This protocol was used on control samples, and resulting data has been used to test data processing methods. Hybridizations follow a 5- or 6-membered loop design, and loops are connected through a common reference to enable cross-experiment comparisons. Bioinformatics are approached using LIMMA, a software package for use within the R software environment, and with two in-house programs, MATT and SpotTracker, that were developed for more user-friendly data presentation and quality control, respectively.

P54

Plant Orthologous Groups: A resource for comparative genomics with a focus on predicted RNA binding proteins.

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To efficiently access the diverse molecular and genetic tools available in the major plant model systems, we have clustered all rice and Arabidosis genes into putative orthologous groups (POGs) using reciprocal best BLASTp hits. All gene models were examined using InterproScan and several intracellular targeting predictors. Maize sequences are associated with existing POGs via BLASTn homology to the rice member(s). Results from these analyses are stored in a web-accessible relational database (http://POGs.uoregon.edu).

Users may search by gene, domain, targeting prediction, and/or POG ID, or use BLAST to identify POGs of interest. The POG detail page includes a graphical display of domain and targeting predictions for each POG member, allowing users to quickly spot trends such as consistent targeting prediction across species or incongruities suggesting faulty gene-models. Also displayed is a tree of the POG members and their closest homologs, allowing users to evaluate the assignment of orthology versus parology and explore nearby POGs.

Users may view the sequences and alignment of the POG members or of closely related homologs. Our primary focus is on plant RNA binding proteins, so we will concentrate annotation efforts on POGs with known or suspected RNA binding activity. Such POGs will be hand-curated to (i) fine-tune POG assignments by incorporating information from phylogenetic trees, (ii) correct faulty gene models, and (iii) include experimental evidence. However, we feel the POG database will be widely useful to plant biologists who wish to identify proteins with specific predicted intracellular locations and domain architecture, or to view the features of orthologous rice/maize/Arabidopsis genes at a glance.

P55

Predicting Chromosomal Locations of Genetically Mapped Loci in Maize Using the Morgan2McClintock Translator

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The Morgan2McClintock Translator (http://www.lawrencelab.org/Morgan2McClintock) permits prediction of meiotic pachytene chromosome map positions from recombination-based linkage data using recombination nodule frequency distributions. Its outputs permit estimation of DNA content between mapped loci and help to create an integrated overview of the maize nuclear genome structure. The alpha version of the Morgan2McClintock Translator converts linkage map locations to predicted cytological positions in an automated fashion, but loci that are near one another (i.e., within approximately 3 cM of each other on a genetic map) cannot be resolved using the existing conversion equations due to the RN data collection procedure (RN frequencies on pachytene synaptonemal complexes were measured at a 0.2 micron length interval resolution). It should be possible to better resolve these positions if the conversion equations are refined. In addition, it should be possible to accomplish the map conversion in the opposite direction (i.e., using cytological map coordinates to predict genetic map positions). Theoretically, this approach is applicable to other organisms with comparable cytological crossover-distribution data such as tomato and mouse, and we plan to develop a set of similar tools for these organisms that should be useful in comparing genetic and chromosomal aspects of genomes in various species.

P56

Statistical analysis of diverse sequences identifies putative functional domains in maize RABs and SODs

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Gene function can be predicted from conserved sequences that encode known functional domains. In some cases, these predictions are good starting points for designing functional experiments because highly conserved sequences are coupled to robust studies. However, broad sequence searches may reveal mostly top-level domains and may not uncover subsets of functional domains. We are exploring a simple method of sequence comparison across widely divergent taxa to test whether subsets of domains can be uncovered in maize. The method uses a sliding window to identify regions of high conservation across diverse taxa and we hypothesize that these identified regions may be under functional constraint. DNA sequence signatures are generated from coding regions from selected taxa and nucleotide substitutions classified statistically. Taxa are removed selectively from the analysis to uncover sites in the signatures of new or of varying conservation level. To test this method, we are starting with two maize gene families, Rabs and Sods. Rabs encode GTP binding proteins in other systems and 54 ZmRabs in 8 sub-groups have been identified from current databases. Two closely related sub-groups, Rab1 and Rab2, are predicted to function in the same cellular compartment. HsRAB1 and RAB2 show reduced sequence diversity at the amino acid level, even outside the conserved GTP binding domains. Similarly, ZmRAB1 and ZmRAB2 have shared identity, but function in maize is unknown. We examined signatures from 55 Rab1 and Rab2 sequences from representatives across available phylogenetic groupings. Phylogenetically based removal of taxa uncovered differences in conserved sites (outside known GTP binding sites) when maize and other plants were compared with non-plant systems. We are testing the system further by comparing chloroplastic to cytosolic superoxide dismutase sequences, since these divergences may uncover unique plant-specific sites in the signatures. We will discuss how sequence signature comparisons could inform future functional studies.

P57

TASSEL 2.0: A Software Package for Association and Diversity Analyses in Plants and Animals

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TASSEL (Trait Analysis by aSSociation, Evolution, and Linkage) is a software package which performs a variety of genetic analyses. The analyses include association mapping, diversity estimation and calculating linkage disequilibrium. The association analysis allows users to incorporate complex field designs, environmental interactions, and epistatic interactions into analyses. The analyses can be performed by either a general linear model or a mixed linear model. The mixed linear model is specially designed to handle polygenic effects at multiple levels of relatedness which can be derived from pedigree or a set of random molecular markers. These new analyses should permit association analysis in a wide range plant and animal species. In addition to these association tools, TASSEL permits the analysis of diversity estimates include average pairwise divergence and segregating sites. Linkage disequilibrium is estimated by the standardized disequilibrium coefficient as well as r^2, and P-values.

One of the convenient features of TASSEL is allowing users to use GDPC (Genomic Diversity and Phenotype Connection) to select data sources, retrieve and filter data and export data to chosen formats. TASSEL also has a variety of data extraction utilities and visualization tools, such as alignment viewer, extraction of SNPs and indels from alignments, neighbor-joining cladogram, K-nearest neighbor missing data imputation, and miscellaneous data graphing. TASSEL is open source software written in Java. The application and source code can be downloaded from maizegenetics.net

P58

TE Nest: Automated chronological annotation and visualization of maize nested transposable elements

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Eighty five percent of the maize genome consists of transposable elements (TEs). A majority of TEs occur in clusters of nested repeats, where a transposable element inserts into the genome within the boundaries of an existing element. TE nesting breaks up the sequence of previously inserted repeats, creating short segments of different types. Mapping of nested TEs is therefore difficult as the resulting smaller fragments are not easily identified or classified. In maize, more than 70% of TEs are found nested within one another, necessitating an accurate nested TE identification tool for complete annotation of the maize genome. Current software does not address nested TEs, making it especially laborious to resolve and determine the evolution of even moderately clustered repeat regions.

TE Nest was developed to facilitate the annotation of our 1.5 Mb chromosome 3 centromeric rf1-spanning sequence, constructed from 19 contiguous BAC clones. TE Nest contains an up-to-date database of maize canonical TEs and their associated long terminal repeats (LTRs), if applicable. This database consists not only of true full length TEs found complete in the genome, but also artificial full length elements reconstructed from nesting fragments. With use of the TE database, TE Nest identifies and maps repeat incorporations into the original genome sequence while also providing chronology of insertion events in Mya based on LTR base pair substitution rate. A triangle insertion graph is produced to give an accurate visual representation of the TE integration history by showing timeline, location and classes of each TE identified, thus creating a framework from which evolutionary comparisons can be made among various regions of the maize genome.

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P59

The TIGR Rice Genome Annotation Database

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We have been funded by the National Science Foundation to annotate the rice genome and provide access to the annotated genome for the community. We have created a rice annotation database using Sybase called Osa1 that houses sequence and annotation information. We released version 4 of our rice genome annotation in early January that includes a number of updates and improvements. We have constructed pseudomolecules of the 12 rice chromosomes that represent ~95% of the total genome. We have created gene models using a combiniation of ab initio gene finders and experimental evidence. We have incorporated full length cDNA and EST evidence into gene models and improved our functional assignments. We have performed a comprehensive analysis of alternative splicing in rice and compared it with the data for Arabidopsis. We have generated a series of other annotations for the rice genome including gene ontology assignments, domains, motifs, alignments with flanking sequence tags, and a variety of expression data. We have also developed a series of alignments with other plant species including alignments with ESTs and with genetic markers from wheat, maize and sorghum. The annotation is available to the community through the TIGR Rice Genome Annotation website (http://rice.tigr.org) where the annotation can be viewed in the Rice Genome Browser or downloaded using the Data Extractor Tool. We are currently implementing a Community Annotation tool which allows community experts to refine the structural and functional annotation of our gene models.

P60

discorcdia1 is required for asymmetric cell division and encodes a PP2A phosphatase regulatory subunit

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Asymmetric cell divisions in the maize leaf epidermis give rise to the cells that form stomatal complexes as well as cork and silica cells. In plant cells, the future division plane is indicated by the position of the preprophase band (PPB) that forms prior to mitosis. During cytokinesis, a new cell wall is constructed by the phragmoplast, which is guided to the position of the former PPB by an unknown mechanism. *discordia1 (dcd1), dcd2,* and *dcd3* are maize mutants with disrupted asymmetric cell divisions in the leaf epidermis. Detailed phenotypic analysis of *dcd1* and *dcd2* revealed defects in phragmoplast guidance in asymmetrically dividing cells (Gallagher and Smith, 1999). We used co-segregation analysis and inverse PCR to clone a *Mu1* insertion allele of *dcd1* and found that *dcd1* encodes a conserved B regulatory subunit of PP2A, a serine/theronine phosphatase. Two additional *dcd1* alleles had lesions in the same gene, confirming the identity of *dcd1*. *dcd1* is homologous to the *Arabidopsis* gene *TONNEAU2 (TON2).* *Arabidopsis ton2* mutants are severe dwarfs with misshapen cells that lack PPBs and have disorganized cortical microtubule arrays (Camilleri et al., 2002). This phenotype differs strongly from that observed in the maize *dcd1* mutants. Since database searches of the maize genome uncovered a second *ton2* homologue, functional redundancy between the two maize *ton2* homologues could explain the difference between the *dcd1* and *ton2* phenotypes. Alternatively, DCD1 may have a more specialized role in maize cell division compared to TON2 in *Arabidopsis* cell division. Due to the similarity of the *dcd2* and *dcd3* phenotypes to the *dcd1* phenotype, we are currently sequencing the second maize *ton2* homolog in *dcd2* and *dcd3* mutants looking for genetic alternations. Our progress on this as well as preliminary molecular characterization of *dcd1* will be reported.

P61

Analysis the MAD1 gene of Plants

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The mitotic arrest deficiency 1 (MAD1) gene is known to be involved in checkpoint control during mitosis in yeast, and animals, however little is known about the function of MAD1 in plants. Analysis of alignments of the annotated MAD1 sequences from Saccharomyces cerevisiae to Arabidopsis thaliana or Zea mays show 21% identity and 25% identity, respectively. These results are similar to the reported 23% percent identity between the MAD1 sequence from Homo sapians and yeast (Jin et al., 1998). An additional multiple sequence alignment was done between H. sapian, S. cerevisiae, and A. thaliana to determine the conserved residues in all three MAD1 genes. To further understand the function of the MAD1 gene of plants a complementation experiment was preformed. In S. cerevisiae, mutations in MAD1 are deleterious in the presence of a spindle fiber inhibitor (Li and Murray 1991). To test for the conservation of gene function the MAD1 cDNA of A. thaliana was transformed into S. cerevisiae MAD1 knockout lines. The non-transformed lines did not survive when grown with a spindle fiber inhibitor, whereas the transformed S. cerevisiae lines were rescued. This experiment suggests the function of the A. thaliana cDNA is similar to the S. cerevisiae MAD1 function through its ability to rescue the S.cerevisiae mutant. Initial phenotypic characterization of A. thaliana MAD1 T-DNA knockout plants shows no apparent phenotype under standard growth conditions. To confirm MAD1 is not transcribed RT-PCR is being used. Preliminary RT-PCR data indicates the MAD1 gene is not transcribed in several T-DNA knockout lines; further analysis is necessary to confirm the initial data. Additional phenotypic analysis of the T-DNA knockout lines will use a spindle fiber inhibitor during plant growth with the expectation of gaining further insight into the role of MAD1 in plants.

P62

Characterization of pearl millet prolamins

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Originating in western Africa, 40 million hectares of pearl millet (Pennisetum glaucum) is harvested annually worldwide, providing the main source of proteins for over 500 million people. Because it is a drought-tolerant, ruddy crop with higher protein content and quality than other cereal crops, many hope to increasingly use it as a feed grain for both animals and humans alike. Yet despite the agricultural significance of pearl millet, little is known about its nutritionally important seed storage proteins. To facilitate such changes and open the door for future genetic improvements of the crop, it is necessary to first understand the essential seed storage proteins which contribute greatly to the processing and nutritional values of any cereal crop.

Although work on the prolamins of pearl millet have revealed partial amino acid sequences for several alcohol-soluble storage proteins (Marcellino et al. 2002), the genes encoding them have not yet been isolated. We constructed a cDNA library from developing seed tissue and screened it using maize zein gene probes. From the library we have identified several clones with sequences that share homology to alpha-zeins. We also compared the extracted prolamin fraction of pearl millet with other panacoid cereals using SDS-PAGE and immunoblotting. Finally, we imaged the ultrastructure and deposition of these proteins within developing endosperm using transmission electron microscopy.

P63

Differential Accumulation of ZmDerlin RNAs and Proteins during Prolonged ER Stress

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Maintenance of a functional protein secretory pathway is facilitated by removal of terminally misfolded proteins through an endoplasmic reticulum-associated degradation (ERAD) pathway. We have identified four maize genes (ZmDerlin1-1, 1-2, 2-1, 2-2) that encode proteins proposed to act with other ERAD components to form a retrotranslocation pore through which misfolded proteins can be transported from the ER to the cytosol for destruction by the proteasome. Both ZmDerlin1 and ZmDerlin2 proteins are present in ER of the endosperm and are also associated with protein bodies, the ER derived protein storage organelles of the seed. Quantitative RT-PCR analysis showed that ZmDerlin1-1, 1-2 and 2-1 were induced by ER stress while ZmDerlin2-2 expression appeared to be unaffected. During prolonged ER stress in the floury-2 endosperm mutant, we observed a reduction in the accumulation of ZmDerlin1 protein, disproportionate to that of the molecular chaperones BiP and calnexin. RT-PCR analysis indicated that this reduction was not reflected in the ZmDerlin RNA population associated with ribosomes. We observed a progressive decrease in both polysome and monosome fractions during seed maturation. This shift was much more pronounced in the floury-2 mutant than in the normal inbred. These data are suggestive that control over ZmDerlin1 protein accumulation occurs post-transcriptionally and that both ERAD and translational inhibition occur during ER stress in maize.

P64

Endophytic biological associations between bacteria and maize are genotype specific

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Endophytism is both a life habit and biological association with ecological and physiological relevance. It refers to some bacteria and fungi that actively colonize host plant tissues and establish with plant lifelong symptom-less associations. Endophytic bacteria have been isolated from leaves, stems, roots and fruits of different plants. Additionally, although there occurrence in seeds is still controversial, there are several reports of seed-born endophytic bacteria. We examined 195 kernels from four maize cultivars and isolated from them 15 bacterial strains, which were determined by molecular detection of eubacterial 16S rDNA. The bacterial strains belonged to six genera, Pantoea, Microbacterium, Bacillus, Paenibacillus, Frigoribacterium and Sphingomonas, and their occurrence was maize genotype dependent. The detected bacterial strains have already been reported in endophytic associations with different plants, but were mostly isolated from other parts of plant than seeds. Germinating kernels of the cultivar W22 were generally associated with a bacterium Pantoea sp. and fungus Fusarium verticillioides. Consistency of Pantoea association with this cultivar was further confirmed by its re-isolation from the kernels harvested in consecutive years. F. verticillioides is a saprophytic fungus that can infect maize plant from the outside, but this horizontal transmission can be reduced or eliminated by certain fungicides. However, F. verticillioides can also act as a maize endophyte, which produces toxins fumonisins and is transmitted vertically to the next generation of plants via clonal infection of seeds. This endopytic phase of infection is important, because it is not controlled by seed application of fungicides, and it remains reservoir from which infection and toxin biosynthesis takes place in each generation of plants. The results of our study demonstrate the exclusionary principles of F. verticillioides or Pantoea occurrence in W22 kernels and the inhibition of fungus growth triggered by Pantoea.

P65

Marker Assisted Breeding for Transformability in Maize

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Corn lines with improved culturability and transformability were produced using Marker Assisted Breeding (MAB) to introgress specific regions from the highly transformable hybrid, Hi-II, into the elite line, FBLL that responds very poorly in culture. FBLL is a female inbred parental stiff-stalk line that has been used to produce a series of some of DEKALB's historically best selling hybrids.

Five unlinked regions important for culturability and transformability were identified by segregation distortion analysis and introgressed into FBLL to produce the highly transformable FBLL-MAB (Marker Assisted Breeding) lines. Agrobacterium mediated transformation was used to screen the FBLL-MAB lines and select the most efficient lines for transformation using immature embryo explants. Two highly efficient transformation systems were developed using kanamycin and glyphosate as selective agents.

To evaluate agronomics, two testcross hybrids were produced for each of the three lead FBLL-MAB lines. A 25-location, 3-replication yield trial was used to evaluate grain yield, yield stability, and agronomic characteristics of the hybrids. Yields were found to be 2-5% lower and more stable (across a diverse set of environments) among hybrids produced with the FBLL-MAB lines as compared to the same hybrids produced with FBLL.

P66

Members of the Oligopeptide Transporter family function during germination in rice

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The seeds of cereal plants contain a large endosperm filled with nutrient stores that are accessed by the seedling following germination. In addition to starch, the endosperm contains storage proteins that serve as a source of amino acids for protein synthesis. Accessing these amino acids is a coordinated effort involving gibberellic acid induced production of cysteine proteases followed by the importation of the degradation products by membrane bound transporters. In rice, storage proteins are thought to be hydrolyzed primarily into small peptides because of low carboxypeptidase levels. We investigated if the Oligopeptide Transporter (OPT) family is involved in moving these peptides from the endosperm to the seedling. The OPT family contains two major phylogenetic divisions: the Yellow Stripe (YS) clade which transports iron phytosiderophores and the Peptide Transport (PT) clade which translocates peptides of three to five residues. PTs were chosen as candidates because their substrates are peptides of 3-5 residues, they function as importers not efflux pumps, and importation is driven by the symport of protons and the endosperm is an acidic environment. Using RT-PCR we have determined that four of the nine OPTs present in rice are expressed during germination. Furthermore, since the production and secretion of hydrolases from the aleurone is coordinated by GA3, it seems quite plausible that the transporters necessary for translocating hydrolysis products are also regulated by this hormone. We therefore have begun investigating if the transcription of these four transporters is regulated by GA3.

P67

The distribution of AM1 during the meiotic cell cycle suggests that it functions both as a cell cycle switch and as a regulator of meiotic prophase progression

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The switch from the somatic to the meiotic cell cycle in maize is regulated by ameiotic1. In the original allele, am1-1, and most other mutant alleles of am1, meiocytes undergo mitosis instead of meiosis or arrest in pre-division interphase. In contrast, in the am1-praI allele, the meiocytes enter meiosis and arrest at the leptotene-zygotene transition. We previously cloned the am1 gene and showed that it encodes a protein with unknown biochemical function. It contains three regions with similarity to a domain found in the Sac3/GANP protein family and two putative coiled-coil domains. The am1 transcript is ubiquitously expressed in all plant tissues. To study protein localization, we used a polyclonal antibody generated against a peptide located in the central region of AM1. The antibody shows diffuse staining in the nuclei of pre-meiotic interphase and leptotene cells. At the leptotene-zygotene transition, the antibody shows punctate staining on chromosomes and then in the late zygotene stage it becomes diffuse within the nuclei again. In am1-1 meiocytes, the antibody shows diffuse staining in nuclei during interphase and prophase of mitosis, but no staining was observed after the prophase. In am-praI meiocytes, the protein is diffuse in nuclei, and no foci were observed on chromosomes even after meiosis arrest. Based on the analysis of the am1-praI phenotype and the chromosomal localization of AM1 at the leptotene-zygotene transition in wild type meiocytes, we propose that an additional function of AM1 is to control of the progression of early prophase. The AM1 antibody also shows diffuse staining in tapetal cells and somatic cells of root tips. This observation, as well as the inability to identify null alleles, suggests that AM1 may also play a role in somatic cell division cycle. Interestingly, the AM1 staining is not readily apparent during metaphase either in mitotic and meiotic cells. To investigate the mechanism of AM1 function during the switch from the mitotic to the meiotic cell cycle, at the leptotene-zygotene transition and its possible function in somatic cells, we are using our antibody in co-immunoprecipitation experiments to identify interacting proteins.

P68

A Comparison of Centromere Mapping Techniques

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We have mapped maize centromeres using telosomes and isochromosomes produced by spontaneous chromosome breaks, radiation-induced chromosome breaks recovered in T-B translocation lines or in oat-maize radiation hybrid lines, single-locus FISH, and half-tetrad analysis. These data have been compiled together with results from other work including Ed Coe's retrospective analysis of genetic data, Bor-Yaw Lin's work with T-B translocation lines and r-X1 induced terminal deficiencies, and Laurie Anderson's recombination nodule map. The data were insufficient to make useful comparisons for chromosomes 5, 7, and 8. Centromere locations on chromosomes 2, 4, 6, 9, and 10 were consistent with all, or all but one, of the studies. Results clustered in a distinct region spanning less than 5% of the genetic map. In contrast, centromere positions on chromosomes 1 and 3 were scattered over 10% or more of the genetic map. The inconsistencies found for centromere map positions on 1 and 3 were not obviously linked to a particular mapping technique, nor were they tied to materials used. Indeed, the same material used in different studies gave different centromere locations on chromosome 1.

P69

Alleles of AFD1 uncouple axial element elongation and bouquet formation from RAD51 distribution and homologous pairing

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REC8 is a master regulator of chromatin structure and function during meiosis Here we dissected the functions of ABSENCE OF FIRST DIVISION (AFD1), a maize REC8 homolog, using a unique afd1 allelic series (afd1-1, ?afd1-2, afd1-3, afd1-4). A novel series of alleles of afd1, the maize rec8 homolog, allows us to develop for the first time a unified model for chromosome behavior during meiotic prophase I in maize. The first observable defect in afd1 mutants was the inability to make a leptotene chromosome. AFD1 was required for elongation of axial elements but was not essential for their initial recruitment, thus showing that AFD1 acts downstream of ASY1/AtHOP1. Rescuing 50% of axial element elongation in the weakest afd1 allele restored bouquet formation demonstrating that extent of telomere clustering depends on axial element elongation. However, rescuing bouquet formation was not sufficient for both proper RAD51 distribution and homologous pairing, providing the basis for a model in which AFD1/ZmREC8 controls homologous pairing via its requirement for full axial element elongation and subsequent RAD51 proper distribution, independent of bouquet formation.

P70

Compound B-A Translocations: Creating B-A-A Translocations and Subdividing the Maize Chromosomes

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Maize B-A translocations result from reciprocal exchanges between a supernumerary B chromosome and an arm of an essential A chromosome. Because of the frequent non-disjunction of the B centromere at the second pollen mitosis B-A translocations have been used to locate genes to chromosome arms and study the dosage effects of specific A segments. Compound B-A translocations are created by bringing together a simple B-A translocation with an A-A translocation, wherein one of the chromosome arms of the A-A translocation is the same arm as that borne on the B-A translocation. Recombination in the region of shared homology of these A chromosome segments creates a B-A-A translocation.

The breakpoints of most of the A-A translocations have been cytologically defined by earlier investigators. The 18 simple B-A translocations can be recombined with regions of most of the nearly 900 A-A translocations, thus B-A-A translocations can be used to delineate and analyze hundreds of chromosome segments on a rather fine cytological scale throughout the maize genome. Previous investigators have produced 16 B-A-A translocations and one B-A-A-A translocation which collectively define 35 A chromosome breakpoints. We have enlarged this group by creating 43 new B-A-A translocations. We present a summary of the total of 60 B-A-A translocations showing their distribution among the chromosome arms and the 121 cytologically defined chromosome segments delimited by them. We also illustrate the method of construction of these B-A-A stocks, as well as the simple B-As, the A-As, and the recessive kernel trait testers used to create, identify, and maintain them. We show how these stocks can contribute to the integration of the DNA physical and genetic maps with the maize cytological map.

P71

Constructing a Cytogenetic Map of Maize Core Bin Markers in Oat Addition Lines Using Sorghum BACs as FISH Probes

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We are developing a pachytene cytogenetic FISH map of the maize genome using sorghum BACs corresponding to the 90 maize Core Bin Marker (CBM) loci. These loci were chosen because they are uniformly distributed and they delineate the genetic bins derived from the UMC98 (Genetic 2005) maize linkage maps. We are using the single-locus cytogenetic FISH mapping system previously described by Koumbaris & Bass (2003, Plant J. 35:647). Maize marker-selected sorghum BACs are used as FISH probes on maize pachytene chromosome spreads from alien addition lines of oat. Progress on maize 9 is described by Amarillo et al. (accompanying poster). The project presented here is specifically focused on mapping chromosomes 1, 3, 4, 5, 6, and 8. The procedure for selecting a sorghum BAC is illustrated here using the RFLP probe umc161 (CBM 1.11, GenBank Acc. AY771212). In addition, we have been obtaining full-length insert sequences for all of the CBM probes as summarized online http://www.plantgdb.org/prj/RFLP_FLIS/. The project is described at cytomaize.org with mapping, image, and RFLP sequence data being released through NCBI, MaizeGDB.org, and plantgdb.org. This project will provide insights into the structure of the maize genome, create new technologies and reagents for chromosome research, assist in genome assembly, and integrating the physical, genetic, and cytological maps of maize with those of sorghum.

P72

Construction of a High-Density Cytogenetic Map of Maize Chromosome 9 Using Sorghum BACs as FISH Probes

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The overall goal of this project is to produce a cytogenetic map of the maize genome using RFLP marker-selected sorghum BACs as FISH probes (www.cytomaize.org). Here we describe the recent findings from our detailed pachytene FISH mapping studies in which we have examined a large number of loci spanning chromosome 9. Maize marker-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). Following collection and deconvolution of the 4-color 3-D images, the chromosome fiber paths were computationally straightened and the FISH signals were mapped using the relative arm position coordinate system that now we refer to as the centiMcClintock map unit (cMC). We have also mapped centromere-linked markers, allowing us to more precisely define the centromere location within linkage map. This project provides unique data that integrates information from a variety of gene maps of maize and sorghum. These results will provide valuable new information for current research on structural genomics, positional cloning, and comparative genomics among the grasses. The cytogenetic FISH map data and chromosome images available online at http://www.maizegdb.org/cgi-bin/displaymaprecord.cgi?id=892372 through the MaizeGDB.

P73

Cytogenetic Analysis of Transposon-Induced Chromosomal Rearrangements in Maize.

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At the maize p1 locus, alleles containing multiple copies of Ac termini are unstable and give rise to various rearrangements, including deletions, duplications, and inversions. These rearrangements can be easily identified because of 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. To test this, we identified candidate translocations 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, University of Minnesota) to identify the chromosome involved in the translocation. In this way, we isolated 6 new putative translocation stocks. Candidate translocation stocks were studied by cytogenetic analysis at meiotic diakinesis of sporocytes. In each case, the predicted associations of four chromosomes (rings or chains) were observed, confirming the presence of translocations. These cytogenetic data, together with the molecular and phenotypic results, provide conclusive evidence that reciprocal translocations can be generated by alternative transposition reactions.

As part of the educational activities of this project, a new course in maize cytogenetics (GDCB 519X; "Chromosomal Genomics") is being offered at Iowa State University during the second half of fall semester, 2006 and 2007. The course includes twice-weekly 3-hour combined lecture and lab sessions. Anyone interested in attending the course please contact Dave Weber or Tom Peterson for further information. 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, J. Zhang, and D. Weber.

P74

Development of PCR based FISH probes for identification of maize mitotic chromosomes.

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High polymorphism of somatic chromosome landmarks among corn varieties complicates karyotyping of mitotic cells using Fluorescent In Situ Hybridization (FISH). FISH probes that label unique genetic loci would simplify karyotype analysis in maize. Even though a few existed, a unique FISH marker on each of the ten chromosomes does not, and obtaining those probes is difficult due to the highly repetitive nature of the maize genome. By analyzing DNA sequences available on public databases, unique sequences were identified that were devoid of retroelements. PCR primers were designed to amplify these regions from genomic DNA or cDNAs. Sequence data were obtained from three sources: (i) gene sequences, (ii) sequenced BACs and (iii) large cDNAs. The PCR products were fluorescently labeled and used as FISH probes on somatic chromosomes. The probes showed clear signals on mitotic chromosomes 1S, 5L, 7, 8L and 9S. These probes combined with already existing ones have enabled us to obtain a single locus FISH marker on all of the ten maize homologs, allowing unambiguous identification of each chromosomes. Many additional FISH probes can be generated as progress is made in maize genome sequencing. Using the collection of probes and the techniques described, the cytogenetic location of unanchored BACs can be determined, new cDNAs and transgenes can be positioned on the cytogenetic map and chromosomal aberrations could be identified on corn somatic chromosomes.

P75

FISH as a means for detecting gene targets in mitotic Zea mays chromosomes

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Large cDNA collections have been developed and sequenced for maize. Due to the labor involved, most of these cDNAs remain unmapped. The use of fluorescent in situ hybridization (FISH) provides a method to determine the cytological location of specific sequences in maize. Here we demonstrate the use of cDNAs as a method for locating genes on mitotic chromosomes. In combination with other FISH probes that allow the individual chromosomes to be identified in mitotic chromosome spreads, this approach represents a rapid way to determine the approximate genetic position of maize genes.

P76

Organelle DNA Insertions into Maize Chromosomes

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We have examined mitochondrial DNA (mtDNA) insertions into the nuclear genomes of ten inbred lines of maize 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 of the following lines: B73, B37, A188, A632, KYS, Mo17, Oh43, W22, W23 and Black Mexican Sweet corn. Detectable sites of insertions varied dramatically among the lines. Initial studies of plastid sequences using FISH onto B73 chromosomes show additional sites of organelle DNA insertions. These results show that organelle DNA insertions are significant contributors to genome diversity within maize.

P77

Partitioning of the maize epigenome by the number of methyl groups on histone H3 lysines 9 and 27

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This report addresses the issue of whether there is a distinction between classical, cytologically-defined heterochromatin and heterochromatin defined biochemically by Histone H3 methylation. We focus on histone H3K9 and H3K27, each of which has three possible methylation states. 3D light microscopy is combined with high-resolution pachytene chromosome mapping and 16 commercially available anti-histone antibodies. The data reveal that the intensity of H3K27me2 staining closely follows the intensity of DAPI (DNA) staining, as expected for markers of condensed chromatin. H3K27me3 is limited to seven apparently non-heterochromatic but highly focused domains on chromosomes 1, 2, 6 and 10 (KYS inbred). Surprisingly, we find that H3K9 methylation is not strictly a heterochromatin mark in maize, though it can be detected there. H3K9me2 in particular shows a strong association with gene-rich portions of the genome. In centromeres, where the primary histone H3 is a variant (CENH3), H3K9me2 and H3K9me3 are interspersed in blocks. We also confirm by two assays that histone H4K20 di and trimethylation is rare or absent. Our data demonstrate that in maize only H3K27me2 marks classical heterochromatin. Trimethylation at K9 and K27 is rare and highly specialized, whereas H3K9me2 shows a cytological association with genes, consistent with its known roles in gene regulation.

P78

Reactivation of nondisjunction for an inactive B centromere and minichromosomes derived from the B chromosome

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Twenty-two minichromosomes different in size and structure were induced by the BFB cycle in the progeny of a hemizygous B-9-Dp9 chromosome together with two 9-B chromosomes. Six of these contained two B centromere sequence sets as confirmed by FISH using the B repeat, knob, CentC and CRM probes, but one of the two sets in each case is inactive. Pollen FISH results indicated that all the minichromosomes lost nondisjunction during the second pollen mitosis, which is typical of the normal B chromosome. However, when a normal B chromosome is present, all but minichromosome #12 can recover nondisjunction. A plant carrying an A-B translocation was also found among the progeny of a plant undergoing the BFB cycle. Multicolor FISH results indicate that this translocation chromosome was derived from chromosome 9 and the B chromosome centromeric region. It is referred to as 9-Bic-1 (inactive centromere-1) because its B centromere is inactive. 9-Bic-1 also lost nondisjunction as confirmed by pollen FISH and test crosses. When 9-Bic-1 was combined with B chromosomes, 9-Bic-1 recovered nondisjunction, which often caused chromosome breakage because centromere 9 disjoins. In outcrosses of this genotype as a male, various genotypes were recovered in the progeny including two 9-Bic-1 chromosomes, chromosome 9 fragments and 19 chromosomes, which is the expected result for nondisjunction of chromosome 9. In two cases, the B centromere of 9-Bic-1 was broken and translocated to the short arm of chromosome 7 and another unknown chromosome.

P79

The Effect of Interploidy Crosses on the Structure of Endoreduplicated Chromosomes

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The maize endosperm is an excellent model system to study developmental and cellular biology. Our lab is studying endosperm development by examining the consequences of interploidy crosses. This often results in abnormal development of both the embryo and the endosperm, but the nature of abnormal development remains elusive on the molecular level. Previous examination has revealed that one consequence of interploidy crosses is the disruption of endoreduplication. Endoreduplication occurs in later stages of development, and is characterized by a cessation of the normal cell cycle and a start of one that finishes DNA synthesis but does not complete mitosis. This results in nuclei that increase in size and ploidy. Endoreduplication can be detected as early as 10 days after pollination (DAP), and has the highest mean of DNA content per nuclei at 16 DAP. After 16 DAP, cell death and DNA degradation begins. Interploidy crosses disrupt developmental timing of endoreduplication. This disruption is different depending on the direction of the cross. In a diploid (2x) by tetraploid (4x) cross, the endosperm enters the endoreduplication phase later than normal and endoreduplicates to a lesser degree. When a 4x plant is crossed by a 2x, the endoreduplication phase begins and ends earlier than normal. Because of this change in developmental timing on endoreduplication, our lab examined whether interploidy crosses would also disrupt the structure of endoreduplicated chromosomes. It has been recently shown that during endoreduplication the entire set of chromosomes are copied many times, but they remain associated throughout the entire length of the chromosomes, and also stay closely attached at the centromere and the knob loci. Centromeric histone H3 deposits only at the centromere and not at the knob, indicating that the mechanism that keeps the chromatin attached at the knob loci is not centromeric in nature. Interploidy crosses destabilize the chromosomes at the centromeric and knob regions, resulting in dissociation at these sites. The effect of destabilizing the cohesion of chromosomes is slightly different depending on the direction of the interploidy cross.

P80

nrm2, a mutant defective in cytokinesis during meiosis

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nrm2 is a novel maize mutant identified in a screen for functional non-reduced gametes. The screen exploited the ploidy barrier of the endosperm, where viable maize kernel development strictly requires a 2m:1p ratio of maternal to paternal genomes. If an unreduced central cell is fertilised by a normal sperm, the kernel aborts due to an imbalance of this genome dosage ratio in the endosperm (4m:1p). Seed abortion is also observed if a diploid female is crossed with a tetraploid male resulting in a 2m:2p ratio, and any other deviation from the 2m:1p ratio. However, if an embryo sac is unreduced and then fertilised with pollen from a tetraploid plant, a 4m:2p ratio in the endosperm results, ensuring normal endosperm development. For the screen, families derived from individuals with high *Mu* activity were used as female parents and scored for segregating plants that produce plump kernels when pollinated with a 4n pollen-donor. *nrm2* (non-reduction mutant) was one of three mutants isolated that produces plump kernels when crossed with pollen from a 4n plant. Meiosis of *nrm2* in both male and female flowers was analysed using confocal laser scanning microscopy. Failure of cytokinesis was observed after both meiotic divisions in male meiosis, though more frequently after meiosis 2. Abnormalities of chromosome behaviour were observed during meiosis I, and failure of karyokinesis was observed during meiosis 2 in female flowers. A failure of karyokinesis during meiosis 2 would explain why non-reduced embryo sacs are produced in *nrm2*, and therefore also why viable kernels are produced from crosses to pollen from a 4n plant.

P81

A Distant Upstream Enhancer at the Maize Domestication Gene, tb1, has Pleiotropic Effects on Plant and Inflorescent Architecture

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While quantitative trait locus (QTL) mapping has been successful at describing the genetic architecture of complex traits1-4, the molecular basis of quantitative variation is less well understood, especially in plants such as maize that have large genome sizes. Regulatory changes at the teosinte branched1 (tb1) gene have been proposed to underlie QTLs of large effect for morphological differences that distinguish maize (Zea mays ssp. mays) from its wild ancestors, the teosintes (Z. mays ssp. parviglumis and mexicana)1,5-7. We have used a fine mapping approach to show that intergenic sequences ~58-69 kb 5' to the tb1 cDNA confer pleiotropic effects on Z. mays morphology. Moreover, using an allele-specific expression assay, we show that sequences > 41 kb upstream to tb1 act in cis to alter tb1 transcription. Our findings show that large stretches of the non-coding DNA that comprise the majority of many plant genomes can be a source of variation affecting gene expression and quantitative phenotypes.

P82

A Genetic Basis for Variation of Complexity in Simple and Compound Leaves

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Although large advances in developmental biology in model species such as Drosophila melongaster and Arabidopsis thaliana have provided insight into the mechanisms by which developmental patterns are designated, how these mechanisms are modified to produce the diversity of organismal forms observed in nature is largely unexplored. To understand the process by which leaf shape varies and to identify genetic elements that contribute directly to alteration in leaf morphology we have initiated Quantitative Trait Loci mapping experiments. Solanum species derived mapping populations encompassing both BC1 and F2 designs were scored for leaf complexity and QTL controlling these traits were identified. Comparison of QTL utilizing marker colinearity allowed for the identification of putative loci that influence complexity in both simple and compound leafed species suggesting common genetic control for both structures. In addition, colocalizing QTL positively and negatively influence complexity suggesting multiple mutational events at the same locus. The ultimate goal of this analysis is to determine the genetic mechanism by which variation in leaf shape is achieved.

P83

A Screening for Enhancers/Suppressors of the Ramosa1 Mutant Uncovered Two Novel Mutations Enhancing Branching in Maize

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In the ramosa1 mutant (ra1) both maize inflorescences, tassel and ear, are highly branched, as a result of a switch from determinate to indeterminate fate of most second-order meristems. In an effort to uncover additional loci involved in the ramosa1 branching pathway, and to identify putative interactors of RA1, we initiated a search for modifiers of a weak allele of ra1 (ra1-RS) by mutagenesis with ethylmethane-sulphonate. More than 1300 M2 families have been screened for enhancement of ear branching, and for either suppression or enhancement of tassel branching. Among several families segregating recessive mutations affecting inflorescence branching we have focused on three manifesting an enhanced branching phenotype. One of these represents an intragenic enhancer, a new ra1 allele due to a single amino acid substitution in the EAR repressor domain of the Ra1 protein. The second (ramosa1 enhancer-1, ren1) enhances the degree of tassel branching with only mild effects on ear branching, while the third (ramosa1 enhancer-2, ren2) has increased branching in both tassel and ear. The ren1 mutant shows an increase in primary and secondary branches in the tassel and a widespread indeterminacy in the spikelets, resulting in the formation of additional florets. We genetically show that this mutation is manifested only when the ra1-RS allele is also present. The ren2 mutant shows a dramatic and moderate increase in branching of the ear and tassel, respectively. The tassel resembles a ramosa2 mutant with upright primary branches. This characteristic is maintained independent of the ramosa1 mutation, although no clear ear phenotype is observed in the absence of the weak ra1-RS allele. The ren1 and ren2 mutants have been mapped on chr.1L and chr.10L, respectively, by mean of SSR markers in regions where no other similar mutations have been mapped. These data, together with complementation tests, show that we identified two novel loci involved in the branching of maize inflorescences.

P84

A potential role for the rough endosperm 3 (rgh3) mutant in endosperm-embryo interactions during seed development

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The rough endosperm 3 (rgh3) mutant was identified by phenotypic screening of the UniformMu transposon tagging population (McCarty et al, 2005). Mutant rgh3 kernels have aleurone defects similar to the previously characterized sal1 mutant (Shen et al., 2003), which is required to limit the number of aleurone cell layers in the endosperm. B-A translocation experiments mapped the rgh3 mutation to the long arm of chromosome 5. Further analysis of the B-A mosaic kernels suggests that the Rgh3 gene is non-tissue autonomous. Non-concordant kernels with the rgh3 endosperm phenotype fail to develop normal embryos. To our knowledge, only one other defective kernel mutation, dek26, shows a similar non-autonomous phenotype (Chang and Neuffer, 1994). Complementation tests with dek mutants mapped to 5L (prg1, dek9, dek26, dek27, and dek33) indicate that rgh3 is a novel mutant. These data suggest that the Rgh3 locus is involved in endosperm-embryo interactions during kernel development. Over proliferation of the aleurone cells and poor development of the basal endosperm transfer cell layer in rgh3 mutants suggest that Rgh3 is involved in cell fate differentiation. We have identified a 7 kb EcoRI fragment containing a Mu1 transposable element that is closely linked to the rgh3 phenotype. The cloning and characterization of Rgh3 will help to elucidate the molecular mechanisms of endosperm-embryo interactions.

P85

Analysis of the maize mutant compact plant2 (ct2)

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We are interested in maize inflorescence architecture particularly in meristem size regulation. In compact plant2 (ct2) mutants main spike and lateral branches of the tassel are shorter and thicker than normal, resulting in a club shaped tassel with a higher spikelet density. In addition, in the majority of cases ct2 ears are strongly fasciated, indicating that the ct2 mutation affects meristem size regulation.

ct2 mutants are also significantly shorter than their wild type siblings, on average around one half, as they develop compressed nodes, resulting in a sturdier, more robust plant. However, the total number of nodes seems not to be affected. ct2 leaves are significantly shorter and wider than normal.

Currently we are investigating the inflorescence development of ct2 mutants in greater detail by using SEM and in situ hybridizations. In addition we are analyzing the genetic interaction between ct2 and other fasciated mutants like fea2, fea3 and td1.

ct2 has been mapped to the distal end of the short arm of Chromosome 1 (27cM; Genetic 5 map). By using a combination of SSR and CAPS markers and 150 mutant individuals we were able to narrow down the chromosomal location of ct2 between the flanking markers umc1568 (141.8cM) and lim504 (151.6cM) on the IBM2 map. Fine mapping of the ct2 locus using ~1000 mutants is in progress and results will be presented.

P86

B class gene expression in a basal grass and non-grass outgroups confirms that the lodicule is homologous with the second whorl of monocot flowers

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B class MADS-box genes in the core eudicots include orthologs of APETALA3 (AP3) and PISTILLATA (PI), and are necessary for stamen and petal identity. Mutations in these genes show homeotic conversion of stamens to carpels and petals to sepals. However, the role of B class genes in establishing petal identity outside the core eudicots has been controversial. Some expression studies in basal eudicots indicate that while B class gene expression is conserved in stamen development, expression is patchy or weak in petals. Additionally, petals are thought to have arisen independently multiple times in the angiosperms. Unfortunately there is little information on B class function outside the core eudicots. The only exception is for the maize and rice AP3 orthologs Silky1 (Si1) and SUPERWOMAN1 (SPW1). Both si1 and spw1 mutants show homeotic conversion of stamens to carpels, and lodicules to palea/lemma-like organs. These mutant phenotypes suggest that the lodicule of the grasses is homologous with petals, and B class activity is conserved in the common ancestor of monocots and eudicots. However, it is also possible that in the lineages giving rise to the grasses B class genes specified stamen identity only, but during grass evolution were independently recruited to specify a lodicule identity. In order to understand the relationship of lodicules to the sterile organs of more traditional monocot flowers we have isolated and observed the expression of B class genes from a basal grass species Streptochaeta that diverged before the evolution of lodicules, as well as non-grass outgroupts Joinvillea (Joinvilleaceae) and Chondropetalum (Restionaceae). In every case B class genes are expressed in stamens and the whorls of sterile organs just outside the stamens. Our results confirm the genetic data that lodicules are modified second whorl organs. Furthermore these results suggest that B class genes control a differentiated second whorl as opposed to a "petal" identity as the second whorl in these species is distinct from the first whorl, but does not have many of the characteristics often interpreted as petaloid.

P87

Bladekiller_1 is Required for Shoot Meristem Maintenance and Development of the Maize Leaf Blade

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A unique maize mutant, currently designated bladekiller_1, shows a meristem maintenance and truncated leaf phenotype. The mutant was found in the F2 progeny of EMS-mutanganized material and segregates as a recessive, single locus mutation. The phenotype of the mature mutant 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 seem to be unaffected. Plants with the bladekiller1 phenotype typically fail to develop functional reproductive organs, although small tassels and ears may form in less severe mutants. SEM analysis has revealed that bladekiller shoot meristems (SMs) are often smaller in size than their wild-type siblings, suggesting a deficiency in meristem maintenance. Interestingly, abnormal levels of cell death at the shoot apical meristem and within leaf primordia have been observed. Furthermore, expression analysis using qRT-PCR has revealed that several genes implicated in SM development, including TD1, FAS1 and a maize homologue of CLAVATA3, are down-regulated in mutant axillary meristems. Further characterizations of bladekiller1 will include additional phenotypic analysis using SEMs, and global expression analysis of bladekiller1 shoot meristems using SAM-enriched microarrays. A long-term goal of this project is to utilize a map-based approach to clone the mutated locus. These experiments are designed to elucidate the function of the BLADEKILLER gene product during shoot meristem maintenance, and to understand the link between the leaf and meristem size phenotypes.

P88

Cell-type specific transcriptome profiling of shoot-borne root initiation in maize (Zea mays L.)

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An elaborate root stock architecture with an extensive shoot-borne root system secures optimal water and nutrient uptake as well as anchorage of the maize plant in the soil. The monogenic recessive mutant rtcs (rootless concerning crown and seminal roots; Hetz et al., 1996) is completely devoid of all postembryonically formed shoot-borne roots and the embryonic seminal roots. Crown roots are initiated from the innermost cortical cell-layer of the coleoptilar node (first shoot node) about 5 days after germination. Comparative proteome and transcriptome analyses of whole coleoptilar nodes were performed to identify putative genes involved in crown root initiation downstream of rtcs. Moreover, cell-type specific microarray experiments of the innermost cortex cell-layer, which was isolated via Laser Capture Microdissection (LCM), will be discussed.

P89

Clonal mosaic analysis indicates that Tie-dyed1 functions in the innermost layer of leaves

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tie-dyed1 (tdy1) is a recessive mutation that conditions a yellow/green sectoring pattern in the leaves. In certain genetic backgrounds, red anthocyanin accumulation occurs in the epidermal layers of yellow sectors. Previous work showed that the yellow sectors hyperaccumulate starch and soluble sugars relative to wild type tissue, and display reduced levels of photosynthetic pigments. In contrast, tdy1 green tissue is not discernibly different from wild type. Because of the nonclonal nature of sectoring, cell-cell signaling is hypothesized to play a role in tdy1 sector formation. In order to evaluate the nature of Tdy1 signaling, a clonal mosaic analysis experiment was performed using the white14 (w14) marker cis-linked proximally to tdy1. Gamma-ray induced chromosomal loss in germinating seeds generated white, aneuploid leaf sectors mutant for tdy1 and w14, with neighboring green tissue wild type for both traits. Within the white sectors, the equivalent of tdy1 yellow sectors were observed, as evidenced by anthocyanin expressing regions containing high levels of starch. In the transverse dimension, the epidermis was not found to play a role in determining sector identity. Regardless of its genotype, the epidermis phenotypically reflected the identity of the internal layers. However, in the lateral dimension, tdy1 sectors never reached the green/white border, indicating that wild type tissue produces a compensatory effect over a few veins. In cases in which either the upper mesophyll layer (L2) or lower mesophyll layer (L4) were genotypically wild type, but the remaining inner layers white, all layers were phenotypically tdy1. These data demonstrate that a mobile signal produced in the tdy1 mutant tissue is able to transform the phenotype of wild type cells to mutant. Conversely, no cases were found in which the innermost leaf layer (L3), which contains the veins, bundle sheath and intervening mesophyll cells, was genotypically wild type and transformed into a tdy1 fate in the presence of a mutant L2 and/or L4. Based on these observations, we propose that the L3 layer is the site of Tdy1 function.

P90

Comparative genetic analyses of WOX4 function in the development of shoot lateral organs in Arabidopsis and tomato

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Plant shoot lateral organs and vascular tissues arise from founder cells, stem cells that are recruited from meristematic tissues. Previous studies have shown that the members of the WUSCHEL related homeobox (WOX) gene family function to organize various stem cell populations throughout plant development. A wox gene (termed LeWOX4) was cloned from tomato that encodes a putative transcription factor of 242 amino acids. Conservation of gene structure and phylogenetic analysis of the homeodomain region suggests that it is closest related to the Arabidopsis gene WOX4. RT-PCR and in situ hybridizations reveal that WOX4 is expressed in the stem cells of the vascular cambium in both Arabidopsis and tomato, although these species differ in the arrangement of vascular bundles. Specifically, Arabidopsis exhibits collateral vascular bundles (xylem adaxial to phloem), whereas tomato contains bicollateral bundles in which phloem is sandwiched between two patches of xylem, Reverse genetic approaches (RNAi and constituitive overexpression) are being used in comparative analyses of the function of the previously uncharacterized WOX4 homologs during the development of the morphologically distinct shoot lateral organs of Arabidopsis and tomato. In addition, the response of Atwox4 transcription to the hormone auxin, and the genetic interactions between AtWOX4 and the vascular patterning gene IFL1/REV are investigated.

P91

Divergent selection for vegetative phase change and its affects on resistance to common rust (Puccinia sorghi) and European corn borer (Ostrinia nubilalis)

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The vegetative tissues of maize (Zea mays L.) undergo a transition from a juvenile phase to an adult phase, and each phase has its own distinct, individual characteristics. An obvious difference between the phases is the presence of epicuticular wax on the juvenile leaves, which gives them a dull-bluish hue. Adult tissue lacks epicuticular wax and the leaves have a glossy-green appearance. Seven cycles of divergent recurrent selection for the last leaf with juvenile wax (LLJW) were performed in the maize population Minnesota 11. The goals of the study were to evaluate the effect of divergent selection for phase change on resistance to Puccinia sorghi and Ostrinia nubilalis as well as the impact of selection on agronomic characteristics. Data was collected on a total of twenty traits from each entry. The experiment was conducted in a randomized complete block design with three replications in two locations. The direct effect of selection, LLJW, was strongly affected by selection (late transition R2=0.95**). The average LLJW increased from 9.2 in cycle 0 to 14.8 in cycle 7 late. The transition zone between completely juvenile and completely adult leaves was also strongly affected by selection. The cycle 0 transition zone was 2.45 leaves while the transition zone of cycle 7 early was 0.60 and cycle 7 late was 6.41 leaves with an R2=0.96** for the late transition cycles. In contrast, the average ear weights showed no significant differences; the average ear weight of cycle 7 early was 104.4 g. while the average ear weight of cycle 7 late was 104.0 g. LLJW was correlated with leaf area damaged by common rust (r = 0.84**) and dead plants due to corn borers (r = -0.74**). While decreased rust damaged was associated with early transition, decreased corn borer was associated with late transition.

P92

Effects of Hsp101 activity on adventitious root formation at the coleoptilar node in maize seedlings.

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In maize, adventitious roots originate from stem nodes located belowground and little is known about the factors that regulate their formation. When maize seedlings were heat-shocked for 1 h, between 45 to 49 C, primary and seminal roots were more sensitive than coleoptiles and the first adventitious roots emerged from the coleoptilar node. Removal of the primary root at optimal temperature was not sufficient to promote adventitious root formation in the coleoptilar node. However, heat shock in the presence of the primary root induced more adventitious roots than in its absence. Heat-shock temperature for maximal induction of adventitious roots was higher at 36 h than at 60 or 84 h after imbibition. Similarly, seedlings were more heat-shock resistant at 36 than at 60 or 84 h. These results correlated positively with embryo-accumulated heat shock proteins (hsp) that disappeared earlier from the primary root than from the coleoptile during seedling growth. We asked if hsp have a role in the heat shock-dependent induction of adventitious roots at the coleoptilar node. The response of two different maize lines with homozygous Mu insertions in the Hsp101 gene (hsp101-m4::Mu1 and hsp101-m5::Mu1) was compared with that of their corresponding wild type siblings. Preliminary results indicated that Hsp101 has a dual role in this process. It repressed adventitious root formation in the coleoptilar node both under non-shock conditions and upon heat shock up to 45 C at 36 h after imbibition. After a heat shock between 46 to 49 C, Hsp101 protected the coleoptile and triggered the emergence of adventitious roots. Thus, Hsp101 seems to influence a developmental switch that controls the appearance of adventitious roots in the growing coleoptile.

P93

Evidence of possible cross-talk between sugar and hormone signaling in developing endosperm of maize

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Important cellular and metabolic processes such as cell division, cell elongation, endoreduplication and sink strength are known to be controlled by hormones. However, very little or no knowledge exists on genes related to hormone biosynthesis and metabolism in developing seeds in any plant. In Arabidopsis, sugar signaling mutants have unraveled a complex network that link sugar response to specific hormones. Maize has the richest reservoir of well analyzed carbohydrate endosperm mutants. Some of these are an ideal system to study sugar hormone connections. In this regard, the miniature1 (mn1) seed mutant is of special interest as it shows pleiotropic changes both at cellular and metabolic levels due to an early loss of the Mn1-encoded cell wall invertase that controls the flux of sucrose metabolism in developing endosperm (Planta: 223: 159-167, 2006). Indeed our recent results indicate that the mn1 endosperm show greatly increased accumulation of sucrose and decreased levels of glucose and fructose in the basal endosperm that functions as sucrose unloading and sucrose turn-over zone in developing seed. More remarkably, both basal and the upper storage region of endosperm also exhibited greatly reduced levels of an auxin, indole-3-acetic acid (IAA), in mn1 relative to the WT throughout the 8 - 30 DAP developing endosperm. The maize G-protein alpha subunit homolog ZmGPA1 was also reduced in the mn1 mutant. Overall, these results show that the mn1 has a reduced hexose:sucrose ratio that correlates with significant decreases in IAA and ZmGPA1; we suggest that sugar-hormone signaling may function through a G-protein mediated pathway to regulate cell division, cell size and sink strength in developing kernels.

P94

Exploring the Role of ramosa1 in the Derivation of Domestic Maize

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Modern maize was domesticated from Zea mays parviglumis, a teosinte, 5000-10,000 years ago in Mexico. Genes thought to have been selected upon during the domestication of crops such as maize, are commonly known as domestication loci. The ramosa1 (ra1) gene encodes a transcription factor that is part of a pathway that regulates meristems to control branching architecture of inflorescences, thus playing a major role in the ordering of kernels on the ear. Patterns of nucleotide diversity indicate positive selection on ra1 at some point in the evolution of domestic maize, suggesting that ra1 is a domestication locus. Through an initial survey of nucleotide diversity at ra1, we found a greater genetic diversity in the teosintes than in maize, as expected. However, ra1 nucleotide diversity is also low in Z. m. parviglumis as compared to an average gene. One interpretation of these results is that selection at ra1 occurred both during the evolution of Z. m. parviglumis and during domestication. Current work involves widening the scope of the diversity survey to include more maize landraces as well as to determine what part(s) of ra1 was targeted for selection. We are also examining the molecular evolution of ra1 in other grasses.

P95

Expression Studies in Maize Homologs of Arabidopsis Floral Regulators.

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The transition from vegetative growth to reproductive growth is a major developmental phase for Angiosperms. Although regulation of this transition in maize is not well characterized, a lot of work has been done in the model system Arabidopsis. Evidence from additional species, including rice, suggests that this process is quite conserved. To further understand this critical aspect of maize development, we have identified putative maize floral regulators based on sequence similarities to Arabidopsis floral regulators such as the floral repressor *SVP* and the floral activator/inflorescence identity gene *FUL*. We are establishing expression profiles for these maize genes. We used real-time RT-PCR to quantify the level of transcripts for each gene in different tissue types and at different development stages. Our data reveal that their expression patterns are often comparable to their Arabidopsis counterparts. We are now working on the functional characterization of these putative maize floral regulators.

In a parallel project, we monitored the expression level of these putative maize floral regulators in a similar manner in the maize mutant, *mop1-1*. The wild-type allele of *mop1* (for *mediator of paramutation1*) is required for an epigenetic phenomenon, paramutation (Dorweiler et al., 2000). The homozygous *mop1-1* mutant plants display pleiotropic developmental defects, including delayed flowering and feminized tassels (tasselseed). Although the molecular mechanism of the late-flowering and abnormal development of the *mop1-1* mutant is not yet fully understood, our data suggest that some of these putative maize floral regulators are differentially expressed in this late-flowering mutant. Our preliminary data also suggests that additional genes, such as *indeterminate spikelet* (*ids*) and maize genes similar to Arabidopsis *SPINDLY* (*SPY*) and *GIBBERELLIC ACID INSENSITIVE* (*GAI*), appear to be differentially expressed in *mop1-1* mutants, and thus may be responsible for the observed tasselseed phenotypes. Future work will attempt to dissect whether these assorted genes may represent potential targets of *mop1*.

P96

Functional role of ATRX and MOT-1 in female gametophyte development

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The formation of the female gametophyte requires the establishment and maintenance of a precise pattern of cell fate. To control cell fate and gene expression in a flexible way, plant cells have developed robust epigenetic mechanisms that play pivotal functions in plant development. In Arabidopsis thaliana, several genes encoding chromatin remodelling factors have been shown to be involved in reproductive development; however, their potential role during female gametogenesis remains unknown. We are investigating the gametophytic role of MOT1, as well as the Arabidopsis homologues of the human ATRX genes (available at www.chromdb.org). We have taken advantage of the RNA interference (RNAi) strategy to inactivate each of these genes using a promoter specifically acting in the female gametophyte. pFM1 is a 844 bp regulatory sequence that was obtained from an enhancer detector line showing reporter gene expression in the functional megaspore stage on, until full differentiation of the female gametophyte. We modified the dsRNA vectors generated by the group of R. Jorgensen (U. of Arizona) by replacing the CaMV35S promoter with pFM1, and transformed wild-type individuals with palyndromic RNAi constructions corresponding to each of our candidate genes. After plant transformation between 25 and 30 T1 plants resistant to the herbicide BASTA were analyzed. We found between 6 and 14% showing a female sterile phenotype in which 50% of ovules abort before fertilization. In all cases ovule abortion appeared to be related to the developmental arrest of the female gametophyte at the functional megaspore stage. These results suggests that female gametophyte development is likely to be highly sensitive to modifications of chromatin organization regulatory mechanisms.

P97

Genetic Analysis of Somatic Embryogenesis in Maize Inbred A188

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Maize transformation is an important tool for both genetic research and crop improvement. Current cereal transformation protocols require the use of tissue culture for selection and regeneration of transgenic plants. Somatic embryogenic tissue cultures are preferred to organogenic cultures for maize transformation; however, many maize genotypes are recalcitrant to this type of tissue culture. Maize inbred, A188, has been shown to produce highly embryogenic callus in culture, leading to its frequent use in maize transformation experiments and investigations into the genetic control of embryogenic response in tissue culture. Two independent mapping studies using A188 as a parent to the mapping populations reported a total of seven QTLs affecting tissue culture and transformability in A188. To further dissect the genetic basis of embryogenic response in maize, we have developed a mapping population of 101 recombinant inbred (RI) lines from the cross of A188 to the popular (nonembryogenic) maize inbred, B73. The A188xB73 lines (BC3S5) are estimated to contain approximately 3% of the A188 genome and 97% B73. The RI lines were grown in the field in 2005 and 25 immature embryos (1.2-1.5mm) were isolated from two ears of each of the 101 lines. We identified 6 lines that produced a higher than expected number of somatic embryos when we cultured them for two weeks on a regeneration medium containing auxin, cytokinin and abscisic acid, that differed from the media in the previous studies. When we screened the RI population for the QTL markers we did not match the previously established A188 markers to our responsive lines. We hypothesize that we have found at least one new locus that controls the production of somatic embryogenesis in A188 and we are working to further validate our findings and identify genes responsible for somatic embryogenesis in general.

P98

Genetic analysis of Bif1 demonstrates a role in axillary meristem initiation

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The "barren inflorescence" (bif) class of mutants in maize is characterized by reduced branching in the male inflorescence, reduced production of spikelets and floral defects. These mutations are thought to be due to defects in auxin signaling that cause the failure of axillary meristem initiation and maintenance.

We have focused on the characterization of one of the members of the bif group, Barren inflorescence 1, (Bif1), a semi-dominant mutant. In order to further understand the function of BIF1 and its role in the formation of the inflorescence, double mutants were constructed between Bif1 and other maize mutants with axillary meristem defects. These included ramosa1, a mutant that produces extra branches and spikelets, teosinte branched1, a mutant with vegetative axillary meristem defects, barrenstalk1 and barren inflorescence2, members of the bif group.

The results showed that BIF1 has an important role in the initiation and maintenance of inflorescence axillary meristems. We also determined that BIF1 does not function in vegetative axillary meristems.

P99

Gibberellic acid stimulates expression of microRNA172 to promote vegetative adulthood in maize

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Shoot development in plants proceeds through phase changes, where meristems and organs transition from one set of identities to another. In maize, gibberellic acid (GA) has been shown to promote both the juvenile to adult and the vegetative to reproductive phase transitions. However, the molecular connections between GAs and the downstream effectors of these changes are largely unknown. We have focused on how GA affects glossy15, an APETALA2-like gene that is both necessary and sufficient to promote the juvenile phase. We recently demonstrated that microRNA172 post-transcriptionally antagonizes glossy15 to promote the transition to the adult phase. Here we show that reductions in GA biosynthesis due to the dwarf1 (d1) mutation delay the adult phase, flowering time, and miR172 expression relative to wild-type plants. Further, we demonstrate that exogenous application of GA to both wild-type and d1 mutant shoots accelerates vegetative phase change and increases miR172 accumulation. Because an oligonucleotide complementary to the fully processed and highly conserved miR172 was used as a Northern hybridization probe, our initial assay was not specific for any of the five miR172 genes we've predicted in maize. Gene-specific assays for the transcript intermediates of two miR172 genes show differential expression across a 42-day timecourse of WT shoot development. Based on these expression studies, one of the miR172s remains a candidate for regulating vegetative phase change and is being tested for differential expression in response to GA. Other candidates for GA regulation by this mechanism include 6 other maize genes with two AP2 domains and a microRNA172 target site, including two other meristem identity regulators (Vegetative to generative transition1 and indeterminate spikelet1). Functional redundancy among this class of proteins inferred from our GL15 overexpression studies suggests more generally that GAs promote phase changes in maize by increasing miR172 activity, which subsequently downregulates the 2AP2-miR172 genes.

P100

Global Expression Analysis of Early Events in Maize Leaf Initiation

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Leaf morphogenesis begins with recruitment of founder cells at the flank of the SAM (P0). Treatment of maize apices with NPA, an inhibitor of polar auxin transport (PAT), blocks leaf initiation by arresting vesicular trafficking and PAT. Towards the elucidation of important transcriptional events occurring at the SAM during leaf initiation, laser microdissection-microarray (LMM) is being used for comparative expression analyses of PAT-arrested and unarrested meristems. However, NPA inhibits vesicle transport in general and is not a specific inhibitor of PAT-related transport. Therefore, these NPA microarray data will be pooled with results obtained from LMM comparisons of incipient leaf primordia (P0) and undifferentiated meristematic tissue. Identification of transcripts differentially expressed in both experiments will minimize transcriptional noise unrelated to leaf initiation and identify genes required for the earliest events in leaf development.

P101

Global Gene Expression Patterns in the Maize Shoot Apical Meristem

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As part of a project aimed at understanding the cellular networks involved in shoot apical meristem function and in the formation of leaf primordia, we are analyzing the results of several microarray experiments. BLASTn and BLASTx analyses were evaluated in an attempt to provide an annotation, based on nucleic acid sequence identity and/or amino acid sequence identity/similarity, for each of several thousand differentially regulated (p-value of less than 0.0001) cDNA probes on the microarrays. In addition, EST probe sequences were used as queries of the Maize Assembled Gene Islands (MAGI) database (http://magi.plantgenomics.iastate.edu/) to identify longer ESTs or genomic sequences for which BLASTn and BLASTx could be performed and evaluated. Each differentially regulated cDNA was annotated with a gene name and placed into a functional category. To date our group has annotated over 3,500 differentially regulated EST probes. Evaluation of these data is ongoing and will be presented.

P102

Interaction of barren inflorescence 2 (bif2) and barren stalk 1 (ba1) in maize inflorescence development

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It has been shown that barren inflorescence 2 (bif2) and barren stalk 1 (ba1) play very important roles in maize inflorescence development. The chemical N-naphthylphthalamic acid (NPA) inhibits polar auxin transport and causes abnormal phenotypes similar to the barren inflorescence mutants. RNA in situ hybridization analysis shows that bif2 is still expressed in the NPA treated tassel meristem while ba1 is not. Therefore, we can propose a hypothesis that bif2 acts upstream of auxin transport and ba1 acts downstream to control axillary meristem initiation in maize tassel development.

To test this hypothesis, we determined the bif2 expression pattern in the ba1 genetic background and ba1 expression in the bif2 genetic background through RNA in situ hybridization analysis. We also tested their genetic interaction by constructing the bif2;ba1 double mutant. Preliminary double mutant analysis implies that the interaction is more complicated than we expected.

P103

Interactions between ABPHYL1 and auxin polar transport in the shoot apical meristem

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Shoot apical meristem activity determines the aerial morphology of plants, including leaf phyllotaxy. Auxin has recently been implicated in phyllotaxy regulation. However, identification of the ABPHYL1 gene in maize also suggests a cytokinin involvement in this regulation. ABPHYL1mutants have altered leaf arrangements, and the gene is homologous to type A two-component response regulators and is cytokinin-inducible. A yellow fluorescent protein fusion to ABPHYL1 shows nuclear localization, and rice calli over-expressing the rice ABPHYL1 homolog failed to regenerate shoots. These results suggest that ABPHYL1 functions in nuclei as a repressor of cytokinin signaling.

To investigate interactions between cytokinin and auxin signaling in the shoot apical meristem, ABPHYL1 expression was examined in plants grown on auxin polar transport inhibitors. This treatment strongly reduced ABPHYL1 expression. In addition, expression of the maize PINFORMED1 auxin polar transport facilitator homolog, was reduced in abphyl1 mutants. Taken together, these results suggest that polar auxin transport activity and ABPHYL1 are interdependent for their expression and presumably their functions in phyllotaxy regulation. To investigate ABPHYL1 gene targets, gene expression profiles in mutant embryos are being compared using the long oligo maize microarrays, and these results will be presented.

P104

KNOX evolution in monocots

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ClassI Knox genes are members of the Homeobox Transcription factor family. Mutations in ClassI Knox genes can result in loss of meristematic identity, Increases in leaf compoundness and ectopic cell proliferation. Virtually all studies of KNOX genes in monocots have been in the Grasses. The grasses are often utilized as a model monocot. Monocots however are an ancient and highly diverse group of plants and grasses are relatively young and highly derived within the monocots.

There are a number of important differences between the KNOX composition between the Grass species and Dicot species. The Grasses have representatives of only two of the four seed plant KNOX genes. The Grass AtKNAT1 like gene underwent an ancient duplication prior to the origin of extant grasses resulting in two paralogs of the gene in all grasses, the Knotted1 like and the RS1/Gn1 like. We have used degenerate PCR cloning and phylogenetic analysis to show that Monocots and Dicots shared a common complement of Knox genes and that the Grasses are a special case with gene duplications and losses specific to the lineage.

To determine where the STM loss and KNAT1 like duplication events occurred, KNOX sequences have been cloned from many orders of Monocots and we placed these onto the evolutionary tree of Monocots. The closest relative to the grasses found to possess an STM ortholog is Musa in the Zingiberales.

Knat1 like clones from Asparagus and Washingtonia robusta do not form a clade with Knotted1 and do not show Knotted1 specific amino acids suggesting the duplication occurred after the divergence of Arecales from the Zingiberales and Poales.

This shows that each step in the KNOX rearrangements leading to the grasses is represented by living taxa, and thus the implications for each change can be inferred using extant species.

P105

Localization of three Actin Genes and their Proteins during Maize Seed Development

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Plants do not crawl or migrate from the place they were sown but it is well known that they exhibit a multitude of fascinating and relevant intracellular processes like cytoplasmic streaming of certain cells. The cell shape and the division plane determination as in the asymmetric cell divisions, the formation of the cytokinetic phragmoplast as well as in the cytokinesis, karyocinesis and the organelles movement among many other processes. At the core of these movements is a dynamic network of polymeric molecules, associated with motor molecules called the cytoskeleton. This complex and dynamic network consists of mainly three components: microtubules, intermediate filaments and actin filaments. The actin framework is perhaps the most dynamic of the three. In cells it exists in two pools: G-actin (monomeric) and actin-filament (F-actin). In cells these two forms are in constant interaction and they are controlled by a multitude of accessory proteins, by post-translational modifications and the physiological state of the cells. The families of plant actin genes have been classified according to their differential expression, in two main groups: reproductive genes expressed in pollen and ovules and the vegetative genes expressed in roots, stem, seeds and leaves. In Maize eight actin genes partially sequenced has been reported. We have analyzed by Northern blot the expression of three maize actin genes: MAct1, Maz81 and Maz87 at different stages of embryo and endosperm development, using as specific probes the 3' UTR region of each gene. As well as RNAs from immature anthers and ovaries. The results of this analysis suggest that the actin gene MAct1 and Maz87 are vegetatives, while the Maz81 is reproductive. Preliminary results of the proteins from immature anther and 20 DAP endosperm by two dimensional analysis so far has not revel clear resolution in the number of the actin isoforms.

P106

Maize Brittle Stalk2 encodes a COBRA-like protein expressed in early organ development and is necessary for development of tissue mechanical strength at maturity

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The maize brittle stalk2 (bk2) is a developmentally programmed mutant in which all parts of the plant turn brittle, even the pre-existing tissues, at about the 5-leaf stage, around four weeks. Consequently, bk2 plants snap easily, making it difficult to grow them unassisted. The outer region of the stalk had fewer vascular bundles, and the sclerids underlying the epidermis had thinner secondary cell walls. Total cell wall mass and relative cellulose content was decreased in leaves and stalks after the appearance of the brittle phenotype. The Bk2 gene was cloned with a combination of transposon tagging and a candidate gene approach. Bk2 was found to encode a COBRA-like protein with similarity to both rice Brittle culm1 (BC1) and Arabidopsis COB4. COBRA-like proteins have been found to be glycosylphosphatidylinositol (GPI)-anchored, which may lead to cell wall patterning that predisposes all tissues to become brittle. Semi-quantitative RT-PCR revealed that Bk2 expression is present at higher levels during early growth stages (week 1 and 2) than at later growth stages (weeks 3, 4, and 8). The decrease in cell wall synthesis and anatomical changes may predispose the plant to the brittle phenotype, but it is not the direct cause. The bk2 mutant had no significant changes in total lignin content, but when phloroglucinol-stained sections were fixed with formalin-acetic acid (FAA), loss of staining was evident as well as loss of autofluorescence. The appearance of brittleness and loss of mechanical strength occurs during later organ development coincident with known changes in lignin architecture. Supported by the Purdue University Agricultural Experiment Station and the National Science Foundation Plant Genome Program.

P107

Microarray analysis of vegetative phase change in maize

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Vegetative phase change is the process during which plants gain competency to flower. In maize, the nonflowering juvenile plants also differ from the flowering-competent adults with respect to a number of vegetative traits, such as leaf anatomy and composition of the cell wall. Phase is reset to juvenility in each sexual generation. Phase can also be reversed experimentally by isolating the shoot tip from a plant in the adult phase and growing it in culture. We have exploited this technique to identify phase-specific genes using DNA microarrays. We compared mRNA populations from plastochron 5-6 primordia of leaf 4 (juvenile), leaf 9 (adult) and leaves 3-4 from plants grown from meristems isolated from adult plants that had initiated 12 leaves (rejuvenated). Using 6 biological replicates, we identified as phase-specific any gene that was up- or down-regulated in both juvenile and rejuvenated samples compared to adult. Using a threshold of at least a 2-fold difference in expression at p> 0.005, we found 272 juvenile-specific genes and 26 adult-specific genes.

P108

Molecular study of camouflage1, a gene involved in the chlorophyll biosynthesis pathway

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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. Transmission electron microscopic investigations of cf1 yellow-green leaf tissues reveal that bundle sheath cells undergo cell death and are the primary cell type affected. Reverse transcription-PCR (RT-PCR) analyses showed reduced mRNA levels of a bundle sheath cell-specific gene, rbcS, in yellow-green sectors but not the green sectors of the mutant. The cf1 gene was cloned via Mutator (Mu) transposon tagging and encodes a gene involved in chlorophyll biosynthesis. We found in the cf1-m1 mutant allele, a Mu8 transposon cosegregated with the mutant. Analyzing two additional cf1 alleles derived from Mu active populations revealed independent Mu1 insertions in the 5' end of the gene, which demonstrated we cloned the correct gene. Surprisingly, RT-PCR analyses showed reduced but detectable cf1 mRNA expression in cf1-m1 mutant leaves. This result suggests the cf1-m1 allele is not a null allele. We found by 5' RACE that the transcriptional start site of cf1 is 110 base pairs upstream of the start codon. The Mu8 insertion site in cf1-m1 is 1 bp downstream of the transcription initiation site. 5' RACE experiments also revealed that cf1 transcription begins about 600 bp downstream from the start codon in the cf1-m1 mutant. Further investigations of how this altered transcription pattern affect the function of camouflage are underway. A model discussing the enzymatic function and phenotype will be presented.

P109

Neural network analyses of infrared spectra for classifying cell wall architectures

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The cell wall is an integrated amalgam of structurally complex polymers. The "Type II" walls of maize and other cereals are composed of cellulose microfibrils, glucuronoarabinoxylans and mixed-linkage (1,3),(1,4)-beta-D-glucans, together with smaller amounts of glucomannans, xyloglucans, pectins, and a network of polyphenolic substances. We estimate that a "cell wall gene network" comprising well over 2000 genes is required to construct a cell wall. The precise biochemical function has been established for very few genes, but one of the more useful ways to establish function is to examine mutants with defects in wall architecture. The range of cell-wall mutants maize and other species with Type II walls is limited. However, maize cell walls display considerable and well-characterized variation in polymer synthesis, assembly, & hydrolysis during coleoptile growth. Dynamic changes in cell walls of etiolated maize coleoptiles, sampled at one-half-day intervals of growth, were analyzed by chemical & enzymatic assays, and Fourier transform infrared spectroscopy. During coleoptile development, changes in cell wall composition include a transient appearance of the (1,3),(1,4)-beta-D-glucans, a gradual loss of arabinose from glucuronoarabinoxylans, and an increase in the relative proportion of cellulose. Although infrared spectra of the distinctive walls from embryonic, elongating, and senescent coleoptiles could be discriminated from each other by exploratory Principal Component Analysis, Artificial Neural Network (ANN) algorithms (both genetic & Kohonen) could correctly classify the ages of most individuals to their one-half-day interval of growth. The ANNs were proven robust in classifying walls of Wisconsin 22 to their developmental stage using hybrid maize as the training set. Our results establish a paradigm for classification of a comprehensive range of cell wall architectures altered by mutation as a major step towards defining function of the component genes of the cell wall gene network. Supported by NSF PGRP.

P110

New mutants affecting maize floret and spikelet development

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Screens for maize mutants affecting inflorescence development have been typically performed in the field. Though productive, such screens tend to uncover phenotypes that are more pronounced, owing to the time constraints imposed on careful screening for subtle defects. The flowers of grasses (florets) are found in a unique structure called the spikelet. In maize, tassel spikelets are composed of two glumes entirely enclosing an upper and lower floret making it difficult to identify subtle spikelet and floret phenotypes in a field-based mutant screen. Both the spikelet & floret have derived morphologies unique to the grasses, and thus developmental mutants affecting these structures are likely to uncover genes not identified in other model systems such as Arabidopsis. To assess the kinds of spikelet and floret mutant phenotypes to be found in maize we have undertaken a careful screen of 1145 M2 families from an EMS-mutagenized A619 background (http://www.maizegdb.org/ems-phenotype.php). For each M2 family, the basal branch from every individual tassel was collected, placed into labeled coin packets, & dried in a seed dryer. At leisure, spikelets from each branch were rehydrated to screen for mutant phenotypes with the aid of a dissecting microscope. We identified 15 putative mutants affecting floret and/or spikelet development that were missed during field-based screening of these same families. Here we present a preliminary phenotypic description for two of these mutants with novel floral phenotypes, and no apparent resemblance to single floral mutant phenotypes of Arabidopsis. F2 mapping populations have been established for four of the mutants & bulked-segregant mapping is underway. Our results suggest that even with the high redundancy of the maize genome, novel floral mutant phenotypes are to be found, and more extensive screens (in maize or another grass) are likely to be very productive.

P111

Parasitic castration by a stinking smut increases sexual reproductive allocation of its host buffalograss, in part by down-regulating a homologue of Tasselseed2 (Ts2)

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Here we present a parasitic smut fungus-perennial grass host system that exemplifies the ability of parasitic castration to dramatically increase a host's sexual reproductive allocation. The parasite, Tilletia buchloeana Kellerman and Swingle, does this in part by down-regulating a sex-determining gene homologous to Tasselseed2 (Ts2), resulting in the formation of pistils (female sex organs) in otherwise male plants of its dioecious host buffalograss [Buchlo dactyloides (Nutt.) Engelmann]. This fungal-induced sex change of male buffalograss mimics maize ts2 mutants. Because the fungus dramatically increases pistil production in both male and female plants of buffalograss and sporulates only in pistil ovaries, we refer to the stinking smut fungus as "pistil smut". Pistil smut infection substantially reduces overall plant dry weight and is thus a virulent parasite, however, in a remarkably coordinated fashion, it greatly increases overall sexual reproductive allocation in both male and female plants. In female plants, the fungus induces an 11.8-fold increase in ovary production. In male plants, the fungus induces a 1.9-fold increase in flower number and over 95% of these flowers contain well developed ovaries. Such intricate regulation by the fungus seems to be directed at the determinacy of vegetative meristems (transition from non-flowering to flowering) and spikelet meristems (inducing an extra flower per spikelet in both sexes). Overall, as a result of infection, we observed a 16-fold increase in potential seed yield (Harvest Index) for female plants and a 42-fold increase per male-female pair combined. If such increase in seed yield were capable of being realized without the fungus, it would certainly seem to have application for improving perennial grain crops whose low seed yields are problematic for commercialization. Future research of this interesting system will clearly enhance our understanding of plant resource allocation, host-parasite coevolution, unisexual flower evolution, and seed production improvement of perennial grain crops.

P112

Photoperiod Regulation and Expression Profile of *CONSTANS-like* Genes in Maize

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We are interested in the transition to flowering in maize. The focus of our research has been maize genes similar to *CONSTANS*, a known floral activator in Arabidopsis. In Arabidopsis, this *CONSTANS* / *CONSTANS-like* gene family consists of a total of 17 members. We are using real-time RT-PCR to study the expression of several maize genes showing similarity to this family. Our initial studies were focused particularly on one member of this gene family in maize, which is most similar to *COL4* in Arabidopsis. This gene was initially identified as showing reduced expression in late flowering *mediator of paramutation1* (*mop1-1*) mutants. Consistent with the expression of Arabidopsis *CONSTANS*, this maize *constans-like* gene appears to be expressed at very low levels. Despite its low levels of expression, the *constans-like* message is detectable in seedlings within 5 days of planting. We are beginning functional characterization of this *constans-like* gene, as well as expanding our focus to additional family members.

Given the circadian regulation of *CONSTANS* in Arabidopsis, we have tested for circadian variation in maize *constans-like* genes. Seedlings grown in a growth chamber under short-day conditions (8 hours daylight) were harvested at 3-hour intervals over 48 hours. Expression levels of *constans-like* genes were then assayed by real time RT-PCR. Expression of at least two family members fluctuates throughout the day indicating their expression is likely to be influenced by the circadian clock. Furthermore, we have compared this circadian expression pattern in *mop1-1* mutants relative to their wild-type siblings. Interestingly, our data suggests that there are fluctuations in the presence of particular circadian peaks, or shifts in the relative timing of those peaks in mutant versus wild-type plants. Future work will focus on characterizing the functional significance of this altered expression.

P113

Regulation of C-Allocation in Developing Maize Florets: Implications for Seed Set and Grain Yield

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The pre- and early post-pollination phases of maize reproductive development are critical for optimal seed set. A reduced anthesis-silking interval (ASI) has been continuously favored in breeding efforts to improve yield. However, asynchrony between pollen shed & silk emergence is enhanced under drought stress where normal growth of female reproductive tissues is delayed. The cost is reduction in grain yield, yet processes governing growth & development of female florets prior to pollination have received comparatively little attention at the molecular/metabolic level. Therefore, in the present work we investigated potential signals regulating pre-pollination adjustment of sink strength among tissues within maize female florets. Rates of fresh & dry weight increase showed that the major sinks, rather than ovaries themselves, were silks and fleshy floral structures surrounding the ovaries (glumes, lemmae, & paleae). Abundant transcript levels of a vacuolar invertase, Ivr2, preceded rapid expansion of these tissues. Generation of two hexoses via invertase -mediated cleavage of each sucrose molecule could contribute to turgor-based expansion and promote sugar signals. Ivr2, which is rapidly repressed under drought stress, is both sugar- and ABA-responsive. Sensitive Q-PCR and GC/MS techniques were used to quantify transcript levels of sugar-regulated genes and endogenous hormones in individual female floret tissues up to 12 days prior to pollination. A transient ABA peak at 10 days prior to silking was enhanced under drought stress and coincided with elevated maternally-localized transcripts of VP1, an activator/repressor of ABA-based signals. Detailed analyses of vp1 mutants showed that developmental patterns of Ivr2 expression in floral structures were altered compared with those of wild-type. This suggests that signals generated in a network involving ABA/sugar crosstalk may influence progression of female reproductive growth and adjust timing of silk emergence.

P114

Striga KNOX1 (KNOTTED1-like homeobox) RNAi as a resistance mechanism in maize and sorghum

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RNA interference is becoming a powerful tool for mRNA-targeted degradation & subsequent silencing of the corresponding gene. We will use RNAi as a way of controlling the maize parasitic weed Striga. Striga KNOX1 (KNOTTED1-like homeobox) genes required for meristem maintenance & proper leaf patterning of organs will be a target for RNAi. Since our strategy is to RNAi Striga KNOX genes in the maize host, we did multiple sequence alignments & phylogenetic analyses of the known KNOX genes (as well as KNOX genes cloned in our lab). These alignments were used to design degenerate primers to clone Striga othologs of STM and KNAT1. A 4kb fragment each was cloned for both STM and KNAT1 like genes from Striga. Our analyses indicate that grasses have no STM like gene. Careful comparisons were done between the maize KN1/RS1 and Striga KNAT1 sequence to find regions of no sequence conservation. A 400-bp fragment each from STM and KNAT1 unique to Striga were used for RNAi construct building and cloned into the maize vector pTF101. In addition, we are using the same strategy to bioengineer Striga resistance in sorghum. Due to problems associated with transgene silencing & especially silencing in multicopy insertion events, it is desirable to generate transgenic plants containing only a single copy of the transgene. Previous research has shown that use of transposable elements as a transporter of transgenes leads to the generation of independent single-copy transgenic plants from crossing two transformats, one with Ds & one with Ac. Once Ac is crossed away, the tranformants have been found to have stable transgenes in later generations. Striga KNOX gene RNAi cassette (as described above) was inserted between nonautonomous Ds-element inverted repeats. This Ds construct was used to transform sorghum embryogenic green tissue cultures by particle gun bombardment. We have since selected three lines for regeneration.

P115

The *Wpk1/Vp8* gene regulates aleurone differentiation in a region-specific pattern.

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We have identified a new class of mutants from the UniformMu W22 population that have abnormal aleurone development in a region of the endosperm surrounding the embryo. One of the mutants, designated as *widow's peak 1* (*wpk1*), arrests embryo development and has defective aleurone development in the germinal region of the endosperm. We analyzed three independent Mu tagged *wpk1* alleles by MuTAIL microlibrary sequencing. *In silico* analysis of the MuTAIL sequences identified allelic Mu insertions in a candidate gene from two of the alleles. We subsequently confirmed that the third allele also contained a Mu insertion in the same gene. The *Wpk1* gene encodes a membrane localized peptidase which shows similarity to human prostate specific antigen. Further genetic analysis revealed that the *wpk1* is allelic to *viviparous8* (*vp8*) mutant. Unlike the *wpk1* mutants, *vp8-R* in its original background develops a viviparous yet viable embryo. Upon introgression into the *W22* background, *vp8-R* expression was transformed to a defective embryo phenotype indistinguishable from the three UniformMu *wpk1* alleles. Further genetic tests suggest that the Stock Center *vp8-R* appear to contain a single dominant suppressor of the *wpk1/vp8* mutant.

P116

The maize flowering time regulator, INDETERMINATE1, interacts with novel proteins

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The transition to flowering is a key aspect of plant development, and thus its manipulation has important implications in agriculture and horticulture. Despite this, little is known about the pathway(s) controlling floral transition in maize. Mutations at the *indeterminate1* (*id1*) locus cause maize plants to flower late, implying that *ID1* positively regulates flowering. ID1 encodes a putative transcription factor that is the founding member of a plant specific family of zinc finger proteins found in both monocots and dicots. This family is characterized by a conserved domain called the ID domain (IDD), which contains 4 zinc fingers and a putative nuclear localization sequence. We have shown that the two middle zinc fingers of ID1, Z2 and Z3, bind to a DNA consensus sequence *in vitro*, providing evidence that ID1 functions as a transcription factor. However, the outer zinc fingers, Z1 and Z4 do not appear to have DNA binding activity. Another possible role for zinc fingers is the mediation of protein-protein interactions. We are testing whether ID1 function may be carried through interaction with other proteins, as is common with other transcription factors and regulatory proteins. We have performed a yeast two-hybrid screen with an immature leaf library to search for novel maize proteins that interact with ID1. A number of putative interactors have been isolated and are being confirmed by co-immunoprecipitation experiments and bimolecular fluorescence complementation (BiFC).

P117

The protein kinase ZmPTI1 from maize co-localizes with callose deposition in developing and germinating pollen

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In the process of deciphering the function of genes involved in maize fertilization, we cloned a functional serine/threonine protein kinase (ZmPTI1) which is highly similar to the tomato Pto Interactor 1 kinase (LePTI1). The tomato gene acts in a specific Pseudomonas syringae pv. tomato defense signaling cascade that is linked to hypersensitive response (HR) in leaves.
In contrast, ZmPTI1a was found to be expressed strongly and specifically in pollen. Crossing studies with heterozygous ZmPti1a-PTGS (post transcriptional gene silencing) plants revealed a significantly decreased transmission of the transgene by PTGS-pollen suggesting that a lack of ZmPti1a expression results in reduced pollen fertility.
Expression of GFP fusion constructs demonstrated ZmPTI1a to be sequestered at the plasma membrane due to N-terminal acylation. Plants stably expressing ZmPTI1a-GFP revealed a co-localization of the fusion protein with callose (1,3-glucan) at different stages of pollen development and germination. Thus, it seems conceivable that ZmPTI1a is involved in crucial stages linked to callose deposition during pollen maturation.
In addition, three further ZmPti1 homologues were cloned. However, these kinases showed neither plasma membrane targeting nor pollen specific expression. Initial experiments gave evidence that one of these genes is likely involved in pathogen defense similar to LePTI1 from tomato. Pathogen induced HR is often associated with cell wall reinforcement through callose deposition. Hence, one working hypothesis suggests that PTI1-like kinases may act as a general feature of signaling processes linked to callose, but during different developmental programs and in different tissue types.

P118

The tasselseed4 gene encodes a negative regulator of floral homeotic gene expression

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Unlike other recessive tasselseeds, tasselseed4 (ts4) mutants display a change in meristem fate in addition to converting the normally male flowers in the tassel to female. We cloned the ts4 gene by chromosome walking and found that it encodes a novel negative regulator of a well characterized family of floral specific transcription factors. Four alleles of ts4 have been isolated, three of which were found to be caused by insertions of the newly discovered Helitron transposable element family within the promoter of the gene. The expression pattern of ts4 suggests that cells at the base and adaxial side of the meristem are involved in non-cell autonomous signaling pathways that control meristem fate. Furthermore, through double mutant analysis we identified a target gene of ts4. This allowed us to construct a single pathway that controls both sexual identity and meristem fate within maize.

P119

Tie-dyed1 encodes a novel gene controlling carbohydrate accumulation in leaves

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A recessive maize mutant, tie-dyed1 (tdy1), develops non-clonal yellow & green sectors in leaf blades. The sectors develop soon after leaf emergence & do not change in size or shape once formed. Previous research showed that yellow sectors accumulate higher levels of sugars & starch and have less photosynthetic pigments than green tissues. Green tdy1 tissues have carbohydrate & pigments levels similar to wild type (wt) leaves. To investigate the dynamics of starch accumulation & degradation, we compared starch levels in wt & mutant leaves collected at the end of the day & night. Interestingly, the starch staining results showed that yellow sectors in leaves collected at the end of the night still retained some starch, while wt contained none. This indicates that the large amount of starch accumulated during the day in tdy1 yellow sectors can be metabolized at night, though not completely. To characterize the molecular function of Tdy1, we performed a directed Mutator (Mu) transposon tag. Four new alleles were recovered from screening ~50,000 plants. A co-segregation analysis identified a Mu1 element tightly linked to tdy1. From examining 110 chromosomes, we found the tdy1 mutant allele & the Mu1 element always cosegregated indicating they were located within 1 cM of each other. A subgenomic DNA library was screened to isolate the Mu1 containing fragment. Sequencing the fragment revealed the transposon inserted into the 5' UTR of a novel gene. By PCR using a gene specific primer & an outward facing Mu terminal inverted repeat primer, we discovered two independent alleles both contain Mu insertions seven base pairs downstream of the first allele. These results further confirmed that we cloned the Tdy1 gene. Current research is focused on characterizing the expression of TDY1 to understand how it functions to regulate carbohydrate metabolism and export in maize leaves.

P120

Transgenic plants over-expressing the HD-ZIP transcription factor ZmOCL1 show delayed flowering

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The OCL1 to OCL5 genes (Outer Cell Layer) encoding putative transcription factors are expressed in the L1 cell layer of the embryo & several other maize (Zea mays) tissues. OCL1 was isolated by a differential display between microspores & androgenic embryos, genes OCL2 to OCL5 by cross-hybridisation with OCL1. The protein sequence revealed the presence of a homeo domain (DNA binding) at the N-terminus, followed successively by a leucine zipper (protein/protein interaction), a START domain (steroid binding) & a conserved domain with unknown function at the C-terminus. Thus OCL proteins belong to the HD-ZIP class IV family of transcription factors, which includes the Arabidopsis FWA, AtML1 PDF2 and GL2 proteins. The expression of the five OCL genes is more or less restricted to the L1 cell layer of the embryo, the endosperm, the apical meristem, leaf primordia & young flowers. Although the expression territories of the 5 genes show big overlaps, each gene has a distinct spatial expression pattern. Transgenic plants harbouring a pCsVMV-OCL1 fusion have been generated & their phenotype has been analysed. Compared to wildtype, plants over-expressing OCL1 showed a severe delay in flowering. Whereas wildtype plants produced 14 leaves before flowering, their transgenic siblings developed 16 to 22 leaves, depending on the transformation event. The severity of the symptoms was positively correlated with the transgene expression level. No effect on the timing of the juvenile to adult transition was observed. Additional phenotypes of the transgenic plants included narrower, shorter and paler leaves, and a tassel that did not fully open with the tassel branches staying upright. The transgenic plants were fertile, and self-pollination resulted in normal looking, viable kernels. However, no homozygous plants could be obtained for the transformation event with strongest transgene expression. A NIRS analysis evaluating over 80 biochemical and biophysical traits did not detect any statistically relevant differences between wildtype & transgenic kernels.

P121

Two-for-one sale: Discounting the evolutionary cost of Tasselseed2 (Ts2) as a female-sterility gene by resisting pistil smut sporulation in buffalograss

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Plants and parasites interact at a rate that is governed over the long-term by their resistance-virulence coevolution. The parasitic pistil smut fungus (Tilletia buchloeana Kellerman and Swingle) has the particular habit of sporulating only in the ovaries of its dioecious host buffalograss (Buchlo dactyloides (Nutt.) Engelmann). Even though male plants do not normally possess ovaries, infection by pistil smut induces pistils in staminate flowers by down-regulating a female-sterility gene homologous to Tasselseed2 (Ts2); a condition known as induced hermaphrodism. We assume that pistil smut was, once upon a time, a disease causing agent of the hermaphroditic ancestor of dioecious buffalograss and similarly sporulated only in ovaries much like the bunts and stinking smuts of wheat. It seems reasonable that if pistil smut parasitizes only the female sex then any host mutation that would allocate resources to the male sex would have a selective advantage. We postulate that such a selective advantage enabled Ts2 to evolve as a post-infection basal resistance gene against pistil smut sporulation by eliminating pistils, thus reducing parasitic virulence in the hermaphroditic ancestor of buffalograss. The selective advantage afforded Ts2 as a disease resistance gene would discount its establishment as a female-sterility gene within a hermaphroditic population resulting in an androdioecious breeding system (resistant males and susceptible hermaphrodites). Given the epistatic control of Silkless1 (Sk1) over Ts2 in maize, we reason that a Sk1 homologue would have had to establish within a Ts2 genetic background, giving rise to andromonoecious buffalograss capable of producing seed. Incorporation of male-sterility gibberellin biosynthesis genes along with disruptive selection pressure to avoid inbreeding depression completed the evolution of dioecy in buffalograss. The ensuing coevolution of an over-riding virulence mechanism by pistil smut, down-regulating Ts2 in dioecious buffalograss, thus reverts male host plants back to a more primitive morphological state, i.e. a "retrophenotype".

P122

Vegetative phenotype of *thick tassel dwarf1* reveals opposing roles of TD1 in vegetative and inflorescence meristems

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Mutants for the *thick tassel dwarf1* gene produce fasciated ears and tassels suggesting that the role of TD1 is too restrict growth in the inflorescence meristems. Paradoxically, these mutants produce fewer and narrower leaves and are shorter in stature, suggesting that the role of TD1 in the vegetative meristem is to promote growth. In Arabidopsis, genes of the CLAVATA pathway and SHOOT MERISTEMLESS (STM) genes act antagonistically to regulate shoot meristem development. Clavata mutants form fasciated shoot and floral organs while stm mutants fail to maintain embryonic meristems. In an effort to explore the role of the maize orthologues of these genes, double mutants at *thick tassel dwarf1-glf* and *knotted1-e1* (a loss of function allele of *Knotted1*) loci were analyzed. During floral development, *kn1-e1* was able to suppress the homozygous *td1-glf* phenotype. Therefore, the relationship seen in Arabidopsis where clv1 is able to suppress stm in a dominant fashion is not seen for floral phenotypes of the putative maize orthologues of these genes, with these particular alleles. Interestingly, *td1-glf* was dominant to *kn1-e1* in vegetative development. *td1-glf* mutants and double mutants each made one fewer leaf than *kn1-e1* mutants or normal plants. The width of the leaf at the ear was narrower in *td1-glf* mutants and double mutants than in *kn1-e1* mutants and normal plants. Careful observation of td1 mutants show that they transition from vegetative to floral development earlier than heterozygous plants. Other novel aspects of the vegetative phenotype of *td1-glf* are presented. Possible explanations for the dichotomous roles of TD1 are discussed.

P123

ramosa2 encodes a LOB domain protein that determines the fate of stem cells in branch meristems of maize

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Genetic control of grass inflorescence architecture is critical given that cereal seeds provide most of the world's food. Seeds are borne on axillary branches, which arise from groups of stem cells in axils of leaves and whose branching patterns dictate most of the variation in plant form. Normal maize ears are unbranched and tassels have long branches only at their base. The ramosa2 (ra2) mutant of maize has increased branching with short branches replaced by long, indeterminate ones. ramosa2 (ra2) was cloned by chromosome walking and shown to encode a LOB domain transcription factor. ra2 is transiently expressed in a group of cells that predicts the position of axillary meristem formation in inflorescences. Expression in different mutant backgrounds places ra2 upstream of other genes that regulate branch formation. The early expression of ra2 suggests that it functions in the patterning of stem cells in axillary meristems. Alignment of ra2-like sequences reveals a "grass-specific" domain in the C-terminus that is not found in Arabidopsis. The ra2-dm allele suggests this domain is required for transcriptional activation of ra1. The ra2 expression pattern is conserved in rice, barley, sorghum and maize, suggesting that ra2 is critical for shaping the initial steps of grass inflorescence architecture.

P124

Allelic Variation for R-Gene Expression in the Rp1 Resistance Gene Cluster.

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The Rp1 locus of maize is a complex resistance gene (R-gene) cluster that confers race-specific resistance to Puccinia sorghi, the causal agent of common leaf rust. An impressive amount of work has been done on Rp1 regarding the evolution of genes maintained at this locus. However, very little is known about possible allelic variation in gene expression at this locus. We have used RT-PCR with gene-specific and non-gene-specific primers to analyze Rp1 R-gene expression in F1 reciprocal hybrids and their parental isogenic haplotypes. Reciprocal F1 hybrids were generated with three different Rp1 (Rp1-B, Rp1-D, Rp1-M) haplotypes in two maize inbred backgrounds (B73 and H95). We have cloned and sequenced the coding region (~4 kb) of Rp1 genes in the three parental haplotypes that had not been previously characterized (HRp1-B, HRp1-M and H95). The Rp1-B, -M and H95 haplotypes maintain at least 21, 11 and 13 Rp1 R-genes, respectively. We have cloned and sequenced Rp1 cDNA sequences from the parental and reciprocal haplotypes and compared these sequences to the genomic clones. We identified 15, 7, 4 and 3 transcribed Rp1 members in HRp1-B, -M, H95 and B73 parents, respectively. Imprinting (parent-of-origin effects) were not observed for any Rp1 R-gene combinations. For most Rp1 R-genes, expression appeared to be additive in the hybrids, but 7 R-genes were more abundant (> two-fold) in both reciprocal hybrids, suggesting overdominance. Expression was not observed for any of the numerous truncated R pseudogenes in these regions, despite the use of gene-specific RT-PCR primers to attempt to find any low level of expression.

P125

Analysis of maize centromere transcripts

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Centromeres have rigidly-defined functions but exhibit a variety of forms. Their plasticities in DNA sequence composition, size, and location, extend not only among species but also within them. As such, there is no genetic definition common to centromeres; in fact they are one of the fastest evolving genomic regions. A potential resolution occurs if centromeres are specified by yet unknown epigenetic factors. Previous work from this laboratory has demonstrated that non-coding transcripts corresponding to the maize centromere core occur and are in physical association with the kinetochore-forming chromatin itself. Here we further investigate the nature of centromere transcripts and discuss their implications in centromere specification and function.

P126

DNA Methylation Analysis of the 5' Proximal Sequence of the Imprinted Gene Mez1

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Imprinting, the differential expression of a gene based on its parent-of-origin, is an epigenetic phenomenon that exists in both plants and animals. The maize gene Mez1 is maternally imprinted in maize endosperm tissue, resulting in exclusive expression of the maternal allele. In both plants and animals there is evidence that DNA methylation is required for the establishment and/or maintenance of imprinted expression patterns. A combination of techniques has been used to study DNA methylation of the Mez1 gene in tissues with and without imprinted expression. Bisulfite sequencing on reciprocal hybrid (B73 / Mo17 and Mo17xB73) endosperm and leaf DNA was used to determine the methylation status of every cytosine within ~2kb of the Mez1 transcription start site. The B73 and Mo17 alleles contain several single nucleotide (SNP) and insertion/deletion (IDP) polymorphisms. By determining the genotype of the bisulfite sequencing products it is possible to determine parent-of-origin affects on DNA methylation. The DNA methylation patterns observed using bisulfite sequencing are validated by performing real-time PCR quantification of DNA that has been subjected to methylation-sensitive and methylation-dependant restriction enzymes.

P127

Decoding The Knotted Phenotype: An Insight in the Transcriptional Regulation of knotted1 in Maize

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Our goal is to investigate the regulatory mechanism that allows the proper transcription of knotted1 (kn1) in maize. The kn1 gene is expressed in all meristems and the developing vasculature but it is absent in differentiated cells such as leaves. A number of loss-of-function and dominant kn1 alleles were previously described by our laboratory. Loss of function mutations include Mu insertions in intron one as well as exons one and three of kn1. Other transposon insertions in intragenic and 5' regions cause the kn1 gene to be ectopically expressed in leaves. The presence of this ectopic transcript correlates with the dominant knotted phenotype in which leaves are dramatically changed by the presence of pockets of undifferentiated tissue, or knots, and the disruption of the proximal-distal axis of the leaves. Phenotypic analysis of Kn1 alleles suggests the onset of the knotted phenotype is different for intragenic and 5' Mu insertions although no obvious sequence differences can be found. We propose that intragenic and 5' regions of kn1 are essential to maintain kn1 in a transcriptionally silenced state in differentiated tissues and that the phenotypic differences between alleles point to different regulatory mechanisms being disrupted by the above Mu insertions. Mu insertions in the ~5 kb intron three of kn1 are found in a 300 bp "Mu hot spot". Most of these insertions are surrounded by a number of Conserved Noncoding Sequences (CNS) that appear to be well conserved in homologous knox genes osh1 in rice, bknox3 in barley and kn1 in maize. To investigate the function of these CNSs in the regulation of kn1, we utilized the high forward mutation rate of the Mu system to find deletions of kn1 sequences near the Mu insertions by using pool PCR. We found one ~100bp deletion but the neighboring CNSs remained intact. In addition, the sequences in the third intron and 5' noncoding region are currently being investigated using expression constructs in rice and maize.

P128

Dissecting cis-acting Sequences Required for Maize p1 Paramutation

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Paramutation at the maize p1 gene is an interaction between epigenetic states of the same gene (epialleles) in which a low expressed and lightly pigmented P1-rr' (patterned pericarp and pink cob) state heritably silences a darkly pigmented and highly expressed P1-rr (red pericarp and cob) state. We used a transgenic approach to dissect the cis-acting requirements for P1-rr' paramutation. In a previous study this strategy allowed localization of sequences required for p1 paramutation to the 1.2 kb enhancer fragment of the P1-rr regulatory region (Sidorenko and Peterson, 2001, Plant Cell:13, 319-335). Our new data obtained from testing nine deletion constructs indicate that the center of epigenetic activity is located within a 405 bp sub-fragment of the 1.2 kb enhancer fragment. Even though the 405 bp deletion is able to efficiently induce P1-rr' silencing, it does not confer high heritability of the silenced state; heritability is drastically reduced when the inducing transgene segregates away (29%). This is in striking contrast with the full, 100%, heritability of P1-rr' silencing observed for transgenes carrying the full length 1.2 kb fragment. Therefore, the 405 bp sub fragment is sufficient to induce silencing, however the sequences outside this sub-fragment are important to set up a chromatin state at P1-rr' that can be maintained in subsequent generations. This is the first evidence that establishment and maintenance of paramutation are conferred by different cis-acting sequences.

P129

Effect of reduced chromatin gene expression on the epigenetically regulated maize gene, Pl-Blotched

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Eukaryotes can regulate gene expression using epigenetic mechanisms that involve changes in chromatin packing. Open, loosely packed chromatin is acccessibile and actively transcribed, whereas closed, tightly packed chromatin is less accessible and associated with gene silencing. Many studies have furnished information about how covalent modifications of the DNA and histone components of chromatin can alter its strucutre. However, much remains to be learned about regulation of those changes. To address this problem, we are analyzing the expression of an epigenetically-regulated gene, Pl-Blotched, that controls the synthesis of purple anthocyanin pigments in maize. The maize anthocyanin pathway offers an excellent system for studying gene control mechanisms, because the genetics is well understood and pigment levels are an accurate reporter for activity of the underlying genes. Pl-Blotched has a variegated phenotype that is associated with a unique pattern of DNA methylation and more closed chromatin structure than usual. To understand how these epigenetic features are controlled, we have introduced Pl-Blotched into lines with reduced expression of genes involved in DNA methylation, histone acetylation and chromatin remodelling. By measuring and comparing pigment levels in plants with and without the chromatin-gene mutations, we can gain insights about which chromatin genes might be involved in controlling the epigenetic state of Pl-Blotched. Our results thus far implicate multiple classes of chromatin genes--including several involved in histone acetylation--as regulators of Pl-Blotched expression.

P130

Gene targeting construct induces strong silencing in *PHYA* and *PHYB* locus.

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In an attempt to carry out homologous recombination mediated gene targeting in the *PHYA* locus of Arabidopsis, we discovered a potentially novel type of gene silencing. The native *PHYA* gene is strongly and very frequently (~75%: 9 out of 12 transgenic lines) trans-inactivated by a homologous transgene construct containing a disrupted full-length fragment of the *PHYA* gene not capable of transcribing dsRNA. Further, silencing occurred only in those lines containing multicopy T-DNA integration loci, and in certain lines is maintained in the absence of T-DNA locus. This implies that certain type of T-DNA repeat formation induces transcriptional gene silencing (TGS) of *PHYA*, however, extensive PCR and Southern analysis of six silenced and two non-silenced lines revealed no correlation between T-DNA repeat configuration and phenotype. Therefore, the identity of silencing T-DNA configuration is not obvious. A search for methylated sites within both the transgene and native *PHYA* loci reveals differential methylation of the coding region in silenced lines, but not of the promoter or 5"UTR. However, within lines homozygous for the transgene, a PCR-based assay revealed methylation of all *Hpa*II sites. Our analyses have not identified the presence of siRNA corresponding to the *PHYA* gene, but we have discovered a *PHYA* RNA gradient within the transgenic line (68LF3) from which was obtained two stable, non-transgenic, epigenetic mutants. To screen the transgene's effect in inducing silencing, several deletion constructs and an alternate construct were transformed into WT plants. The alternate construct, containing *PHYB* in place of *PHYA*, induced *PHYB* silencing in 59% of T0 transformants. Likewise, a construct lacking the *PHYA* promoter caused silencing in 59% of T0 transformants. We are currently performing experiments to determine the significance of this data, possibly leading to more efficient gene silencing constructs.

P131

Genetic and epigenetic features of maize gametes

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A notable feature of the plant life cycle is the alternation of haploid and diploid generations. In the mature diploid phase of the plant life cycle, specific cells undergo meiosis to generate, after two or three mitotic divisions, separate male and female multicellular haploid gametophytes, each containing two gametes. We have undertaken a molecular approach to characterize the genetic and epigenetic status of a group of genes in maize gametes. Our findings suggest that an apparent asymmetry in the epigenetic machinery exists between gametes, which may explain the divergent developmental programmes of both products of double fertilization.

P132

Genetic interactions between modifiers of *pl1* paramutation

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Meiotically heritable epigenetic changes in gene regulation occur at specific alleles or haplotypes of most maize flavonoid regulatory loci through a process known as paramutation. Despite the relative unknown nature of this mechanism, this process appears to be of general relevance to the orchestrated chromosome changes underlying development and evolution. Mutational analysis has been a useful tool for genetically identifying components of the paramutation machinery operating at the *purple plant1 (pl1)* locus (Genetics 157: 369-78). Ten recessive, *trans*-acting factors have been identified which are *required to maintain repression (rmr)* of gene expression associated with the repressed paramutant expression state (denoted *Pl'*) of the *Pl1-Rhoades* allele. A novel ethylmethane sulfonate-derived mutation, designated *ems98287*, also derepresses the *Pl'* state, but it exhibits unusual genetic characteristics that distinguish it from other *rmr* mutations. Genetic tests show that *ems98287* fails to complement *rmr1-1* and mutations identifying the *rmr6* locus while complementation of other *rmr* and *mediator of paramutation1* mutations occurs completely. As runon transcription experiments show that *Rmr1* affects post-transcriptional levels of *pl1* RNA while *Rmr6* is required for transcriptional repression of *Pl'* (Genetics 171: 725-740), *ems98287* defines a functional link between seemingly distinct mechanisms of gene regulation. Additionally, double mutants of *ems98287* and *rmr6-1* have severe developmental problems not observed to the same extent in either single mutant including stunted growth, narrow, twisted leaves, and failure to produce mature tassels. *ems98287* and mutations at *rmr1* and *rmr6* allow relatively high rates of reversion from *Pl'* to the highly expressed *Pl-Rh* state of the *Pl1-Rhoades* allele suggesting they all disrupt the maintenance of meiotic heritability, a hallmark of paramutation. Identification of the lesion responsible for *ems98287* promises to aid in understanding the biochemistry responsible for paramutation at *pl1* and how these mechanisms control genome function during plant development.

P133

Global expression profiling of zmet2-m1 mutants using Affymetrix and spotted microarrays

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Zmet2 is a chromomethylase that catalyzes CpNpG methylation. The zmet2-m1 allele contains a Mu insertion in an exon near the 3' end of the gene. The insertion results in an abnormal transcript that contains a frameshift mutation disrupting the 30 C-terminal amino acids. Analysis of DNA methylation found a co-dominant reduction of CpNpG methylation with no change in CpG methylation (Papa et al., 2001). No obvious morphological phenotypes were observed in BC6 plants from a B73 or Mo17 background. Microarrays were used to search for genes with altered expression in the zmet2-m1 mutant. Relatively few genes display altered expression levels in the mutant compared to wild type plants. Using the B73 BC materials and the maize Affymetrix GeneChip we identified 19 genes that were up-regulated in the mutant and 28 down-regulated genes, including zmet2 itself. This data will be compared to the results of hybridizations using the same RNA samples with a 70-mer oligonucleotide microarray. In addition, the results will be compared to hybridizations of Mo17 BC RNA samples with the Affymetrix GeneChip. Given the relatively low number of differentially expressed genes it is hypothesized that the targets of zmet2 are primarily transposable elements and changes in gene expression are secondary affects. It might be predicted that B73 BC and Mo17 BC would display differences in expression due to differences in the location and nature of repetitive elements surrounding genes.

P134

Mutator Transposon Insertion in One of the Six Tandem-Repeated Copies of a pericarp color1 allele causes Ectopic Gain of Pericarp Function

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Multicopy genes, repeated transgenes, and repetitive non-coding sequences are usual targets for silencing caused by DNA methylation and chromatin condensation, however little is known about why they are especially susceptible. To investigate tandem-repeat associated silencing, we utilized Trait Utility System for Corn (TUSC) to screen for *Mutator* (*Mu*) transposon insertions in a six copy allele (*P1-wr*) of the maize *pericarp color1* (*p1*) gene. We recovered 13 unique insertion alleles. *p1* encodes for a transcription factor that regulates the expression of brick-red phlobaphene pigments. The *P1-wr* allele conditions white pericarp and red cob glumes phenotype; however, 1 out of the13 *Mu* insertion alleles resulted in a red pericarp and red cob phenotype. The gain of function allele designated as *P1-wr-mum6*, results from the presence of a *Mu1* insertion in the 5'UTR in one of the *P1-wr* copies. In several reported cases of *Mu* suppression, *Mu* insertions in the 5'UTR region have provided alternative transcription start sites when the *MuDR* transposase is in an inactive state. However, the gain of function phenotype associated with *P1-wr-mum6* does not depend on *Mu* activity. Moreover, we did not detect alternate *P1-wr* transcripts using primer extension and 5' RACE-PCR. Interestingly, RT-PCR analysis suggests that that the insertion of *Mu1* into a single copy's 5'UTR increases the mRNA expression of other uninterrupted copy(s). Furthermore, the gain of function is associated with DNA hypomethylation at the *P1-wr* distal enhancer region. A model will be presented to explain the ectopic function associated with linked copies that are not carrying the *Mu1* insertion. In this model, we consider the possibility that the *Mu1* insertion disrupted a critical region of a single copy that is import for copy to copy associations that rely on heterochromatization and associated DNA methylation.

P135

Pericarp and cob specific expression patterns of pericarp color1 are attributed to DNA methylation modifications of two distinct regions

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Epigenetic mechanisms that establish and maintain tissue and allele specific expression are poorly understood. The white pericarp red cob glume allele of pericrap color1 (p1) known as P1-wr shows a stable inheritance of its phenotype over generations. This stability has been shown to be associated with hypemethylated multi-copy gene structure of this allele. However, this stability of expression is perturbed in the presence of a second site modifier known as Unstable factor for orange1 (Ufo1) leading to enhanced pericarp and cob color. These phenotypic modifications are unstable such that the gain of function reverts to wild type state in the subsequent generations. This gain/loss of expression has been correlated with changes in DNA methylation in both coding as well non-coding region of the P1-wr. To further understand the molecular mechanism underlying tissue specific expression of p1 alleles and their interactions with Ufo1, we have characterized a natural epiallele of P1-wr that shows white pericarp, white cob glume phenotype. This allele, designated as P1-wr*[RI41], is more methylated than its progenitor P1-wr allele. Interestingly, in the presence of Ufo1, P1-wr*[RI41] plants produced a range of gain of pigmentation only in cob tissue while pericarp remained colorless. Molecular and biochemical analyses demonstrated that extent of cob pigmentation had a linear correlation with decreased DNA methylation, increased p1 transcription, and increased accumulation of phlobaphenes in cob glumes. Our results indicated that the gain of cob color can be associated with hypomethylation of DNA sequence in the intron2 region. Interestingly, the comparison of genomic bisulfite sequencing results between P1-wr*[RI41] Ufo1 and P1-wr Ufo1 revealed another sequence in the promoter that may play a critical role in pericarp expression.

P136

Suppression of p1 expression by transposon-induced gene rearrangements: a novel type of gene silencing induced by the Ac/Ds system

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The maize p1 gene encodes a Myb homologous transcriptional activator conferring red pigmentation in the floral tissues such as kernel pericarp and cob. The P1-rr11 allele (specifying red pericarp and red cob) contains a fractured Ac (fAc, 2039 bp of 3' Ac) insertion in intron 2 and an Ac insertion 8835 bp upstream of the p1 transcription start site. The 3' end of the fAc element and the 5' end of the Ac element in P1-rr11 are in opposite orientation, and they can participate in alternative transposition reactions that generate various types of rearrangements such as deletions, inversions, translocations, and a novel type of local rearrangement (Zhang and Peterson, 2004. Transposition of Reversed Ac Element Ends Generates Chromosome Rearrangements in Maize. Genetics 167: 1929-1937). An example of this latter type is the p1-vv30 allele (specifying variegated pericarp and variegated cob), in which the DNA sequences from -23 to 4341 and -8835 to -24 have exchanged positions. The p1-vv30 allele was crossed with the standard P1-rr4B2 allele. Surprisingly, 90% of the F1 progeny ears had nearly colorless pericarp and cob, indicating that the p1-vv30 allele strongly suppresses P1-rr expression (designated as P1-rr'-s, for strong suppression); while 10% of the F1 ears have reduced pigmentation in both pericarp and cob (designated as P1-rr'-w, for weak suppression). The suppressed phenotype is heritable in the F2 and F3 progenies of P1-rr'-s. Whereas, the F2 progenies of P1-rr'-w exhibit variable levels of pigment suppression. Comparisons among related alleles indicate that the suppression by the p1-vv30 allele requires 1) a particular configuration of the p1 genomic sequences, and 2) the presence of an Ac element. Tests are in progress to determine whether the P1-rr'-s state is paramutagenic. These results indicate that alternative transposition-induced rearrangements can induce a novel type of gene silencing. 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, J. Zhang, and D. Weber.

P137

Suppressor of plant blotching1 (Spb1): an epigenetic modifier of Pl-Blotched

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Pl-Blotched is a stable epiallele of the anthocyanin regulatory gene purple plant1 (pl1). Pl-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 Pl-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 Pl-Blotched sequence. These observations led us to propose that Spb1 is an epigenetic modifier of Pl-Blotched, which acts to increase Pl-Blotched expression by altering the chromatin structure of Pl-Blotched. To evaluate this hypothesis, the effect of Spb1 on Pl-Blotched chromatin structure has been investigated through DNaseI sensitivity assays and chromatin immunoprecipitation (ChIP) assays. For ChIP assays, various antibodies against modified histones (e.g., methylation and acetylation of H3 and H4) have been used and the analyses have been carried out by quantitative real-time PCR (qRT-PCR). The results of these assays will provide detailed information about the organization of Pl-Blotched chromatin in plants with and without Spb1.

P138

The Maintenance of Silencing Associated with p1 Paramutation is Disrupted by a Dominant Mutation Unstable factor for orange1

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Paramutation is an epigenetic interaction in which one specific allele heritably changes the expression of another specific allele in a heterozygote. In contrast to many other epigenetic phenomena, with paramutation the newly established silenced state is not only heritable but also capable of silencing yet another active allele. Paramutation is associated with changes in DNA methylation and chromatin structure but the mechanism of paramutation remains unknown. Here we report results of our efforts toward identification trans-acting factors that affect paramutation at the maize p1 gene, a myb-like transcriptional activator of the flavonoid biosynthetic pathway. The active state of the paramutable P1-rr allele results in uniform red pigmentation of pericarp and cob, while paramutated state, named P1-rr, has patterned pericarp and pink cob. Our results show that the Ufo1 (Unstable factor for orange 1) mutation, disrupts silencing associated with p1 paramutation leading to recovery of the dark red pigmentation of pericarp and cob tissues. This up regulation is transient, because the silenced P1-rr state is restored upon Ufo1 segregation. This P1-rr behavior is similar to that of P1-wr, the p1 allele previously described to be affected by Ufo1. The P1-wr (white pericarp and red cob) becomes darkly pigmented in both pericarp and cob tissues in the presence of Ufo1, and demonstrates reduction of DNA methylation and increase of transcription rate of p1 (Chopra et al., 2003 Genetics 163:1135-1146). Similar to P1-wr, the analysis in P1-rr showed reduction of DNA methylation. However, reduction was only partial after two consecutive generations in the presence of Ufo1. It is possible that several generations of exposure to Ufo1 are required for a complete reduction of P1-rr hypermethylation. To test for such a possibility we are probing for changes in DNA methylation levels upon prolonged (four consecutive generations) P1-rr exposure to Ufo1. The results of our experiments employing Southern blot analysis and bisulfite genomic sequencing will be reported.

P139

The role of RNA in mediating *trans*-interactions affecting *pl1* paramutation

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Meiotically heritable changes in gene regulation occur at specific alleles or haplotypes of maize anthocyanin regulatory factors through a process known as paramutation. These changes occur in response to ill-defined allelic interactions hypothesized to require either physical homologue contact or the action of diffusible substances. Our analyses of diverse alleles at the *purple plant1 (pl1)* locus suggest that *pl1* RNA may mediate these *trans*-interactions. Transcriptionally repressed states of the *Pl1-Rhoades* allele (denoted *Pl*) invariantly facilitate heritable change of the highly expressed reference state (denoted *Pl-Rh*). Thus, only *Pl* states are transmitted from *Pl* / *Pl-Rh* heterozygotes. Based on our survey of nine *pl1* alleles from divergent sources, only *Pl1-Rhoades* exhibits these dynamic behaviors. Although *Pl* may revert to *Pl-Rh* when heterozygous with certain *pl1* alleles or when hemizygous, we found other *pl1* alleles stabilize the *Pl* state in *pl1* / *Pl* heterozygotes. Analyses of loss-of-function *Pl1-Rhoades* derivatives indicate PL1 protein is not required for the dynamic behaviors of *pl1*, yet this finding does not exclude *pl1* RNA as a diffusible mediator of *pl1* allelic interactions. In support of this hypothesis, *pl1* alleles that facilitate reversion of *Pl* to *Pl-Rh* confer weak or undetectable pigmentation, suggesting they are expressed at very low levels or not at all. Additionally, two derivative alleles of *Pl1-Rhoades* that simultaneously lose both the abilities to acquire a heritable *Pl* state and to stabilize *Pl* in *trans* confer vastly weaker pigment than *Pl1-Rhoades*. Preliminary evidence shows these derivative *pl1* alleles fail to produce detectable *pl1* RNAs. We are currently using RT-PCR on our entire allelic series to test our hypothesis of *pl1* RNA acting as a diffusible intermediate of *pl1* allelic interactions.

P140

Transcriptionally silent transgenes are activated by three mutations defective in paramutation

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Three maize mutants defective in paramutation were able to induce transcriptional activation of two different silent transgenes, suggesting shared mechanisms between paramutation and the transcriptional silencing of transgenes. The one mutant tested, mop1-1, had no effect on post-transcriptional gene silencing. With two of the mutants, mop1-1 and rmr2-1, the transgene could remain active in a subset of progeny even after multiple generations in the presence of the wild type protein. Interestingly, the immunity to silencing increased as generations progressed. These observations are consistent with an epigenetic state being formed in plants carrying mop1-1 and rmr2-1 mutations that becomes more resistant to silencing over multiple generations. With the third mutant, rmr1-1, the transgene was efficiently resilenced in the presence of the wild type protein. DNA methylation was increased at CNG sites in the silent transgenes relative to the active transgenes at all of the sites monitored within the promoter. However, asymmetrical methylation persisted at one site in rmr1-1 mutants with reactivated transgenes.

P141

Variability in the accumulation of phenolic and flavonoid compounds in maize carrying Unstable factor for orange1 and pericarp color1

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Unstable factor for orange1 (Ufo1) is a dominant modifier of pericarp color1 in maize. The p1 gene encodes a MYB type of transcription factor that regulates accumulation of phlobaphene pigments in tissues including pericarp and cob glumes. The P1-wr allele conditions white pericarp and red glume phenotype, while in the presence of Ufo1, P1-wr plants have red pericarp and dark red glume color. These P1-wr Ufo1 plants also exhibit enhanced pigment accumulation in vegetative parts of the plant including leaf sheath, husk, midrib and occasionally leaf blade (Chopra et al. 2003. Genetics 163:1135-1146). One pleiotropic effect of Ufo1 is stunted growth and bent stalks. A population obtained from a backcross {(P1-wr Ufo1)BC4F1 x P1-wr/P1-wr} was grown in the greenhouse. Ufo1 expresser and non-expresser plants were identified based on the presence or absence of pigment in the leaf sheath. We investigated if lignin accumulation is affected in tissues that are either hyper-accumulating phlobaphenes or that have bent stalk. Different tissues harvested at varying developmental stages were hand sectioned and stained with phloroglucinol. Stained tissue sections were observed under Nikon SMZ 1000 microscope and pictured using a Nikon digital camera attached with the microscope. Results will be presented to compare the expression of lignin in Ufo1 expresser and non-expresser sibling plants. This study will allow us to understand if over expression of phlobaphenes affects the channeling of common precursors that are required for a large class of phenolic and flavonoid compounds.

P142

Ancient conservation of cereal microRNAs

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The ability to engineer gene expression during plant development could be used to improve crop yields, increase resistance to disease, and enhance environmental adaptability. It has been suggested that microRNAs, or miRNAs - a class of noncoding genes - control developmental processes such as meristem cell identity, organ polarity, and developmental timing, by reducing transcript accumulation or inhibiting translation of their respective coding gene targets. Our understanding of the spatial- and temporal-specific expression is critical for elucidating miRNA functionality. Various miRNAs are highly conserved across multiple plant species demonstrating their ancient origins. The fact that they remain intact through the evolution and of plants implies a critical regulatory role. Within this analysis, we focused on identifying highly conserved miRNAs within syntenic spans of Zea mays and Oryza sativa japonica representing functionally important cereal miRNAs. Through a pattern matching approach, we were able to identify 40 maize miRNA genes, phylogenetically conserved with known Arabidopsis miRNAs from the miRNA Registry, within the public maize gene enriched genomic sequences. We successfully mapped 23 of the 40 maize miRNAs, 19 of which resided with syntenic spans between rice and maize. Ten successfully mapped maize miRNAs were found to have a rice miRNA counterpart within the syntenic span. The expression of these miRNA families across the cereals was validated via small RNA blots. Overall, we were able to elucidate the evolutionary relationships of cereal miRNAs, an important step for increasing our understanding of the small RNA regulatory network and their respective roles in regulating gene expression within agronomically important crops. The project is supported by National Science Foundation grant No. 0321685 and USDA ARS.

P143

DNA Sequence Diversity of the Fasciata1 Paralogs Among Inbred Lines and Open-pollinated Landraces.

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The Arabidopsis FASCIATA1 protein is a subunit of a chromatin assembly factor involved in the function of both shoot and root apical meristems. Previously, we cloned and sequenced a portion of the Zea mays Fas1 gene and demonstrated that it is expressed in shoot and root apical meristem-enriched tissues, as well as other tissues that are mitotically active. We now demonstrate that there are two Fas1 paralogs (ZmFas1a and ZmFas1b) in the maize genome. RT-PCR was performed on mRNA isolated from 14-day shoot apical meristem-enriched tissue and the amplified product was cloned and sequenced. This study indicated that both ZmFas1a and ZmFas1b are expressed in this tissue and confirmed the intron/exon boundaries that were predicted from our database analyses. To further characterize these two paralogs, DNA sequence diversity was accessed among North American inbred lines, and open-pollinated landraces from New Mexico and Mexico. Interestingly, among the maize lines studied ZmFas1a exhibited far more DNA sequence diversity than ZmFas1b.

P144

DNA Sequence Diversity of the Zeaxanthin epoxidase Paralogs Among Inbred Lines and Open-pollinated Landraces.

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In plants the enzyme zeaxanthin epoxidase catalyzes the conversion of zeaxanthin to antheraxanthin and violaxanthin which are precursors to abscisic acid. The Arabidopsis zeaxanthin epoxidase protein sequence was used as a query in tBLASTn searches to identify maize ESTs with obvious sequence similarity. Oligonucleotide primers were then designed that would amplify a ~500 bp sequence of the ZmZep gene containing both exonic and intronic sequence. These primers amplified two paralogs of the gene (ZmZep1a and ZmZep1b) which exhibit 82% DNA sequence identity. RT-PCR was performed on mRNA isolated from 7-day shoot and 14-day leaf tissue of maize, and the amplified product was cloned and sequenced. This study indicated that ZmZep1b is expressed in these tissues and confirmed the intron/exon boundaries that were predicted from database analyses. Our experimental design did not rule out the possibility that ZmZep1a is also expressed in these tissues. Maize genomic sequences from both paralogs were obtained from seven North American inbred lines, and five open-pollinated landraces from New Mexico and Mexico, respectively. In addition, genomic sequences were evaluated from various teosintes. DNA sequence diversity (silent pi) among the maize lines was assessed for these loci. Both paralogs showed similar overall DNA sequence diversity, however, ZmZep1a exhibited its highest diversity among the inbred lines, while ZmZep1b exhibited its highest diversity among the open-pollinated Mexican landraces.

P145

Defining the Multiple Related Copies Within the Maize Genome

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Maize contains a complex genome with multiple copies of related genomic regions throughout its chromosomes. These multiple related regions have developed from a duplication event of a grass genome(s) that already contained internal duplications. The model genome for grass, rice, also contains these ancestral duplications. Duplications within a genome diverge over time through gene loss, rearrangement, and differentiation that can mask the similarity between duplicate copies and related genome segments. We have developed a Perl script called "Crush & Compare" that recreates an ancestral gene order for the grass genomes from the rice genome sequence and its identified genomic duplications. Our scripts allow the direct comparison of the sequenced rice genome with genetic maps. DiagHunter, developed by Steve Cannon, defines syntenic regions using a mathematical matrix. This type of synteny classification allows for small interruptions and rearrangements in the colinearity of homologous sequences. Using the ancestral rice gene order, called rice blocks, as a reference the complex structure of the maize genome and the multiple related copies within the genome are identified. Extrapolating from rice genome calculations and their identified syntenic regions with maize estimates that the majority of rice duplications are again duplicated in maize. This comparative process also allowed for comparisons between the rice blocks and sorghum chromosomes allowing for an estimate of the genomic duplication within sorghum. Statistical analysis has been done on all identified syntenic regions to calculate their probability of occurrence by random in a dataset of this size.

P146

Differentiating centromeres and pericentromeres using repeat junction markers, ChIP-PCR, and recombinant inbred mapping

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Mapping maize centromeric and pericentromeric sequences is complicated by the fact that there are very few single copy sequences. The junctions between two repeat regions, however, are generally unique and can be used as molecular markers. As a test of this method we were able to develop seven markers from two BACs: 16H10, a presumed centromeric BAC, and 06E22, a presumed pericentromeric BAC. Roughly 70 different primers were required to identify the seven single-copy markers. Recombinant inbred mapping was used to place the BACs on chromosome 8 (16H10) and chromosome 9 (06E22). Anti-CENH3 antibodies were combined with Chromatin Immunoprecipitation (ChIP) and real-time PCR to determine if 16H10 was derived from the functional centromere. One 16H10 marker showed significant ChIP with CENH3. These data not only confirm the interpretation that 16H10 represents DNA from a functional centromere but also accurately map the centromere of chromosome 8. BAC 06E22 is presumably pericentromeric because it includes only a short array of the centromere-specific satellite repeat CentC and showed no significant ChIP with CENH3.

P147

Mutations Affecting Meiotic Recombination Rate in Maize

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The mechanisms and regulation of meiotic recombination are still not well-understood. Although great progress has been made in other model systems, the underlying genes of recombination and the control of these steps remain elusive in plants, including maize. We have used EMS mutagenesis on two differentially marked maize populations to identify mutations that significantly alter recombination ($p < .05$). Using two different crossing schemes and testcross assays that eliminate meiotic defects resulting in sterility, we monitored crossovers in the *C1-Wx1* interval of chromosome 9S and the *A1-Et1* interval of chromosome 3L. Thus far, the two screens have produced 56 candidate mutations segregating in M2 and M3 families (30 from the 9S screen and 26 from the 3L screen), including 39 putative mutations that significantly increase recombination, seven that significantly decrease recombination, and nine that segregate for an increased frequency of double crossovers (rarely observed in maize, where crossover interference is extremely high). Four families form an interesting fourth class in which both a significant increase and a significant decrease in recombination frequency over the same interval segregate in the same family.

P148

Nucleotide diversity and recombination distribution in the *AN1-CER1* interval of *Arabidopsis*

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Recombination is one of the most important evolutionary forces in shaping genetic diversity. In plants, cytogenetic analyses and genetic mapping projects have investigated meiotic recombination at megabase and whole-genome scales. High-resolution recombination studies across multigene intervals have rarely been performed in plants. To study recombination distribution at the molecular level, we targeted the 235 kb *AN1-CER1* interval on chromosome 1 from the model plant *Arabidopsis thaliana* . Accessions Columbia, Landsberg *erecta* (Ler), Cape Verdi Islands (Cvi) and Sapporo were employed to study nucleotide sequence variation. About 40 kb of genomic DNA was sequenced from the 3' and 5' ends of 37 genes. Seventy-seven nucleotide changes were found in 20 genes, most of which were SNPs between Cvi and other accessions. About 200 F2 recombinants were isolated from a cross of an *an1-cer1* double-mutant in the Ler background with Cvi. These recombinants are currently being confirmed and genotyped. The results of this work will shed light on the specificities of meiotic recombination in plants.

P149

Oat-Maize Addition and Radiation Hybrid lines for the physical and genetic mapping of the maize genome.

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Oat-Maize Addition (OMA) lines are available with maize chromosomes 1-10, and ones also with a maize B chromosome added individually to the oat genome by wide crossing. Previously, maize chromosomes 1-10 from Seneca 60 had been recovered individually in OMA lines. Self-fertile lines transmitting the added maize chromosome were obtained for maize chromosomes 1-9, but only for the short arm of chromosome 10. DNA is now available for OMAs of all 10 maize chromosomes in either a B73 or Mo17 background. Disomic additions have been recovered for chromosomes 1, 4, and 5 of B73 and chromosomes 2, 5, and 10 of Mo17. Gamma irradiation of the OMA lines has yielded several hundred Radiation Hybrid (RH) lines with only a fragment of the maize chromosome either after partial deletion of the maize chromosome or from translocation of the maize chromosome to an oat chromosome. RH mapping panels are now available for low- and medium-resolution physical mapping, as well as for contig assembly and map-based cloning. The total number of RH lines recovered follows: chromosome 1 (68), 2 (81), 3 (39), 4 (71), 5 (41), 6 (121), 7 (13), 8 (0), 9 (202), 10 (2). Preferential recovery of RH lines with breaks in the same intervals has been observed in some RH lines. Here, we report on the current status and availabilty of OMA lines and RH mapping panels. This material is based upon work supported by the National Science Foundation under Award No. 0110134.

P150

Structural annotation of the maize genome.

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Maize (Zea mays) genome sequencing projects will soon provide the community with large amount of contiguous genomic sequences. A next step will be to predict the gene content of these sequences. To do so, several software are available and among them the EuGene package. Like most of other similar software, Eugene needs to be trained with gene sets which are structurally well characterized. It is advised that a set should include at least a hundred genes to roughly represent a genome. The first goal of this project is to build such a gene set for maize. Genomic and full length cDNA sequences are retrieved from public databases and aligned with SIM4 or GeneSeqer. Information about gene function and expression is gathered from literature when possible. Structural and functional annotations of each gene are manually curated. The resulting, annotation will be released in the GeneFarm database and the annotated genomic DNA/cDNA cognates will be used to train the EuGene software. A major limitation of this approach is the availability of cognate genomic, cDNA and protein sequence for maize. However, the first gene set contains 117 cognate sequences and is under evaluation. This work is funded by Genoplante.

P151

The New IBM Neighbors: Genetic and Physical Probed Sites

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Our goal is to combine all physically and genetically mapped probes and genes onto a single frame. The strategy relies on a frame that has been refined & added to by BAC contig information available at Arizona (www.genome.arizona.edu). The frame includes over 2000 firm BAC contig anchors onto chromosomes. 1088 of these anchors are positioned by IBM2, a map based on an inter-mated, 302 line mapping panel that has a potential genetic resolution of 0.04 cM. The coordinate system is a hybrid between the 'IBM2 centiMorgan' and contig coordinate (CB). This new version of neighbors includes several new genetic maps that were integrated into MaizeGDB since last March: (1) the IDP maps of Pat Schnable (maize-mapping.plantgenomics.iastate.edu); (2) the Genoplante cDNA maps (Falque *et al*. 2005 Genetics 170: 1957-1966); (3) Genetic 2005 maps (Ed Coe); (4) SNP mappings, incorporated into the community IBM-94 maps (Mike McMullen *et al.)*. Additionally, over 45,000 loci identified only by wet-lab, BAC probings can be assigned a coordinate on neighbors. In the case of over 25,000 of these loci, at least 2 overlapping BACs are hit by a given probe; the remainder, some 20,000, have only one BAC hit in a given contig. Loci identified by at least 2 BAC hits have been integrated into MaizeGDB &, via the associated BACs, provide a direct link to the contig representation at Arizona. Almost all of these BAC probes are cDNA-based & provide a rough indication of the distribution of coding sequences along the maize chromosomes.

P152

The Oryza Map Alignment Project: A new resource for comparative genome studies within Oryza

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With the completion of a finished genome sequence we must now functionally characterize the rice genome by a variety of methods including comparative genomic analysis between cereal species & within the genus Oryza. Oryza contains 2 cultivated & 22 wild species representing 10 distinct genome types. The wild species, in particular, contain a largely untapped reservoir of agriculturally important genes that must be harnessed if we are to maintain a safe & secure food supply for the 21st century. OMAP was established two years ago to generate a comprehensive set of genomics resources to investigate genome evolution and enhance positional cloning efforts in the genus Oryza (Wing et al. 2005). To date we have generated 12 high quality BAC libraries that encompass the 10 genome types of Oryza (Ammiraju 2006); ~1000 Mb of BAC end sequence from these libraries; and SNaPshot fingerprint databases for all 12 libraries. All of these resources are publicly available via the AGI BAC/EST Resource Center, GenBank or at www.OMAP.org. The fingerprints & end sequences have been combined to develop 12 phase I physical maps. Six of these physical maps, O. nivara [AA], O. rufipogon [AA], O. glaberrima [AA], O. punctata [BB], O. officinalis [CC] and O. brachyantha [FF], have been heavily manually edited (HME) and aligned to the reference rice genome sequence. These alignments have revealed a large array of genome rearrangements relative to the Nipponbare genome and have allowed us to begin draw a more complete picture of Oryza genome evolution (The Rice Chromosome 3 Sequencing Consortium 2005). In this poster we will present the current status of OMAP and discuss recent analysis of the HME maps, comparative sequence analysis of select loci across Oryza, and a global analysis of structural variation among the AA genome species.

P153

A GeneTrek description of the maize genome

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Whole genome sequencing is a powerful tool, but its high cost necessitates the choice of cost-effective sequencing methods. To determine the best approach, it is useful to characterize the general composition and structure of the target genome. Analysis of random shotgun or BAC end sequences can provide an estimate of gene number and repeat content. However, this technique does not indicate gene or repeat distribution. Here, we present an alternative approach, GeneTrek, based on the sequencing and annotation of a small number of randomly-selected large insert clones. We demonstrate this method by applying it to 74 >80 kb BACs from B73 maize, representing a total of 12.2 Mb of sequence data. Our analysis reveals that >68% of the analyzed sequence is composed of LTR-retrotransposons. A total of 188 confirmed genes and 131 hypothetical genes were found, suggesting 37,000-63,000 genes in the maize genome. We observed an average gene density of 1 confirmed gene per 65 kb, with large variations between regions (from 0 to 7.9 genes per 100 kb), indicating that genes are unevenly distributed in the maize genome. The number of genes per gene island observed was from 1 to 8, with a mean of 1.8. Among the 74 BACs, 21 do not contain any confirmed gene. The detailed characterization of 6 of these BACs reveals that they are mainly composed of nested LTR-retrotransposons (87.1% of the total sequence). Few other repeats, such as DNA transposons (e.g., MITEs) or tandem repeats, were found on these gene-free BACs. Using markers derived from the nested patterns of LTR-retrotransposons, 3 of the gene-free BACs were mapped in B73 x Mo17 populations. The inserts in these BACs were derived from chromosomes 1, 6 and 7. They do not map at centromeres or knobs, revealing that other gene-free regions exist in the maize genome.

P154

A Physical Map of the Whole Maize Genome via Single Molecule Analysis

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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 (importantly, fragments are ordered; average fragment size of ~18 kb) 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 will 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 the assembly of these 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 good: approximately 389.32 Gb of raw genomic DNA single molecule maps have been acquired (155x genome coverage), and there are now 1391 map contigs, spanning 68% of the maize genome. Our current efforts are focused on the completion of this map, and the enablement of effective linkages to 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.

P155

A Two Component *Activator/Dissociation* Platform for Reverse and Forward Genetic Analysis in Maize

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We are utilizing the transposable elements *Activator* (*Ac*) and *Dissociation* (*Ds*) in a genome-wide mutagenesis program. Using classical genetic techniques and non-transgenic materials we are generating a collection of 10,000 families that each harbor a unique *Ds* insertion distributed throughout the genome. DNA sequences flanking the *Ds* elements are cloned and sequenced providing a precise physical location for each insertion in the maize genome. Thus, we will generate a sequence-indexed collection of up to 10,000 *Ds* knockout lines. Importantly, each *Ds* insertion is stable in the absence of *Ac*, but can be remobilized using a stabilized transposase source that we have recently characterized (see poster by Conrad et al.). As *Ds* tends to move to closely linked, gene-rich regions of the genome, each insertion will also serve as a platform for additional rounds of mutagenesis targeting linked genes. This project will be integrated with ongoing maize genome sequencing projects and the data disseminated as it is generated through two project websites at Iowa State University and the Boyce Thompson Institute. All genetic materials generated will be distributed by the Brutnell lab for a small fee (approx. $40/line) that will cover the costs of propagating and shipping maize seed.

P156

Analysis of Genes Induced by Drought Stress in Mexican Maize Landraces

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Two standard and three subtractive cDNA libraries were produced from three Mexican maize landraces, Cajete Criollo, Michoacn 21 and 85-2 grown under drought stress conditions. Cajete criollo and Michoacn 21 are considered to be drought tolerant genotypes whereas 85-2 is susceptible. Leaf water potential, photosynthesis, transpiration rate, soil water potential and relative humidity were monitored throughout the experiment. Separate subtractive libraries were produced from mRNA from leaves and roots of plants under 8 and 14 days of drought stress. The two standard libraries were also obtained from mRNAs from a mixture of leaf and root tissue from slightly and severely stressed plants. A preliminary microarray of 813 cDNAs has been hybridized with total RNA extracted from leaves and roots of maize plants under stressed and non stressed conditions. Northern blot analysis is currently being carried out on a selection of cDNAs in order to confirm their expression patterns under drought stress. To generate an RNAi construct the partial coding region of those selected genes will be inserted into the vector pMCG161 (provided by the Arabidopsis Biological Resource Center). Transgenic plants of the maize lines B73, Cajete criollo and Michoacn 21 will be produced by particle bombardment or Agrobacterium-mediated transformation using the RNAi construct.

P157

Advances in identification of Mu-induced knockout mutations for cell wall biosynthetic genes using reverse genetic screens of the UniformMu maize population.

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The UniformMu maize population is being screened via a PCR-based reverse genetics approach to identify Mu-induced knockout mutations of specific cell wall biosynthetic genes. The UniformMu population is ideal for this research, due to its low ancestral mutation load, high rate of new mutations, traceable pedigrees, a genetic mechanism for stabilizing Mu-induced mutations, and highly uniform, wildtype "controls" for comparative analysis of mutant phenotypes. Here we report advances in identifying knockout mutations and present our most recently identified putative inserts into target genes. In order to confirm the identity of PCR products, a reamplification step, using a nested Mu primer (TIR8), is now employed along with the gene-specific primer to amplify original PCR products for direct sequencing. This reamplification/sequencing procedure, together with Southern blotting, identifies knockouts more readily and with greater confidence. The importance of strictly gene-specific primers has enhanced our focus on the primer design phase of the process. This has been expanded to include a Short Oligo Genome Scanner Program to help avoid primers containing repetitive or highly represented sequences. Putative knockouts obtained most recently include: Beta-expansin 2 (implicated in cell wall modifications during tissue expansion in roots and initiating tillers of Festuca pratensis), Profilin 5 (an actin binding protein with possible roles in cytoskeletal organization and intracellular signaling), SCAMP2 (a secretory carrier associated membrane protein, possibly involved in export of cell wall components), and two cellulose synthase-like proteins (implicated in cell wall polymer synthesis and that exhibit diverse effects when nonfunctional in other species).

P158

All possible modes of gene action are observed in a global comparison of gene expression in a maize F1 hybrid and its inbred parents

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Heterosis is the phenomenon whereby the progeny of particular inbred lines have enhanced agronomic performance relative to both parents. Although several hypotheses have been proposed to explain this fundamental biological phenomenon, the molecular mechanisms responsible for heterosis have not been determined. The maize inbred lines B73 and Mo17 produce a heterotic F1 hybrid. Global patterns of gene expression were compared in seedlings of these three genotypes using a cDNA microarray. Over one thousand genes were identified as being significantly differentially expressed among genotypes. All possible modes of gene action were observed, including additivity, high- and low-parent dominance, underdominance, and overdominance. The largest proportion of these genes (78%) exhibited expression patterns that are not statistically distinguishable from additivity. Even so, 22% of the differentially regulated genes exhibited non-additive modes of gene expression. These findings are consistent with the hypothesis that multiple molecular mechanisms, including overdominance, contribute to heterosis.

P159

Analysis of Maize Full-length cDNA and Gene Expression Patterns of Maize Seedlings under Osmotic and Cold Stresses

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We report here the construction of a full-length enriched cDNA library of maize seedlings by oligo-capping method. Randomly selected 15075 clones from the library were subjected to one-pass sequencing from their 5'-ends. Finally, we gained 2073 completed cDNA sequences. Of 2073 full-length cDNA sequences, 1654 aligned to the maize GSS loci at >95% identity over their entire length, which accounted for more than 79.8% of our FL-cDNA genes. The remaining 419 clones not matched to any sequence are probably in the genomic region lacking in the current draft sequence. That is to say, 419 clones are new genes in maize. The results also indicated that 1921 full-length cDNAs sequences could be mapped in rice genome sequences. And 1596 sequences were mapped on both maize and rice genomes. At the same time, we also constructed osmotic and low temperature-stress subtractive cDNA libraries by the suppression subtractive hybridization (SSH). In all, these ESTs with significant protein homology were sorted into 14 functional categories, i.e., metabolism, cell growth, protein synthesis, protein folding, modification and destination, cellular transport and cell division. In these libraries, some reported osmotic or cold-stress genes could be found, such as ZMrab17/ZMDBF1/ZMGRP/ZMSUCS1 and so on. Macroarray has also been used to monitor the changes of maize gene expression profiles under various stresses and treatment of plant growth regulators. Some water-stress or cold stress-induced ESTs have been found. The functional analysis of several genes is under progress.

P160

Analysis of allelic variation in gene expression in B73 and Mo17 and implications for hybrid expression patterns

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A combination of Affymetrix microarray and allele-specific expression analyses were applied to investigate the differential regulation of maize alleles, and the regulatory alterations that occur following F1 hybridization. Seedling, immature ear and embryo tissues were profiled in the inbred lines B73, Mo17, and reciprocal hybrids B73xMo17 and Mo17xB73. Using microarray analyses, we identified many genes that are differentially expressed between inbreds B73 and Mo17. For a subset of the differentially expressed genes expression is only detected in one of the two inbreds. Further analysis of these genes shows that they are present in the genomes of both inbreds, but differ in their regulation. We proceeded to profile the expression patterns in the reciprocal hybrids. There was no evidence for parental affects in the tissues that we studied as no genes were differentially expressed in B73xMo17 relative to Mo17xB73. The genes that were not differentially expressed in B73 relative to Mo17 were also not altered in their hybrid expression state. The vast majority of the B73 versus Mo17 differentially expressed genes were expressed at mid-parent, or additive, levels in the hybrid. Almost no genes displayed expression levels in the hybrid that were outside the range of the inbred parents. We further studied a set of 27 genes that were differentially expressed in the two inbred lines by using allele-specific expression analysis; we investigated the respective transcriptional contribution of the inbred alleles in the hybrid. This analysis provided evidence that the majority of differential expression in B73 and Mo17 is due to cis-regulatory variation, not differences in trans-acting regulatory factors. This may explain the predominance of additive expression and relative lack of epistatic effects observed in the microarray data as genes subject to cis-regulatory variation are expected to be expressed at mid-parent, or additive, levels in the hybrids.

P161

Analysis of the IDD transcription factor family in higher plants

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The timing of the floral transition in plants is controlled by environmental and developmental signals that ensure reproductive success. Maize INDETERMINATE1 (ID1) is the only gene identified so far that specifically controls the transition to flowering in maize. ID1, which encodes a protein with a distinct arrangement of zinc finger motifs termed the ID domain (IDD), defines a family of transcription factor genes present in all higher plants. Comparison of IDD genes in the rice and Arabidopsis genomes, as well as those found in maize EST and genomic databases, reveals a total of 16 IDD genes in Arabidopsis, 15 in rice and at least 21 in maize. ID1 mRNA is expressed in immature leaves only and is not detected at the shoot apex or in mature leaves. In contrast, Northern hybridization experiments with gene-specific ZmIDD probes reveal a broad range of expression in all tissues tested, including mature leaves and tassel inflorescence. None of the ZmIDD gene tested had an expression pattern that is similar to ID1. Several unique features of ID1 protein suggest that it is a divergent member of the IDD family. Phylogenetic comparisons suggest that ID1 has two putative orthologs in related grass species - OsID in rice and SbID in sorghum. Western analysis with an antibody that cross-reacts with ID1 and the putative orthologs in rice and sorghum shows that all three proteins are detected exclusively in immature leaf. These findings suggest that ID1 and its putative orthologs in grasses may form a functionally distinct group within the IDD family.

P162

Comparative Profiling of the Sense and Antisense Transcriptome of Maize

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There are thousands of maize lines with distinctive normal as well as mutant phenotypes. To determine the validity of comparisons among mutants in different lines for a subsequent experiment, we first address the question of how similar the transcriptomes are in three standard lines at four developmental stages. Four tissues (leaves, 1 mm anthers, 1.5 mm anthers, pollen) from one hybrid and one inbred maize line were hybridized with the W23 inbred on Agilent in situ synthesized oligonucleotide microarrays with 21K elements drawn from the December 2003 maize EST assembly of MaizeGDB. Tissue-specific gene expression patterns were documented with leaves having the most tissue-specific transcripts. Haploid pollen expresses about half as many genes as the other samples. High overlap of gene expression was found between leaves and anthers. Anther and pollen transcript expression showed high conservation among the three lines while leaves had more divergence. A subset of these microarray results were validated via quantitative real time PCR and by hybridization to the NSF sponsored Maize Microarray (Univ. Arizona) 70-mer spotted microarray platform. Antisense transcripts represented ~6 to 14 percent of total transcriptome varying by tissue type but were similar across lines. Enrichment in GO terms related to cell cycle functions was found for the identified antisense transcripts. Conclusions: Despite high polymorphisms and structural differences among maize lines, the transcriptomes of the three lines examined displayed high similarities, especially in both reproductive samples (anther and pollen). We also identified potential stage markers for maize anther development. A large number of antisense transcripts were detected and implicated in important biological functions given the enrichment of particular GO classes. Supported by the National Science Foundation (98-72657). The Stanford Biomedical Informatics Training Program supported J.M. D.S. Duncan provided invaluable help with dissections.

P163

Comparative sequence analysis of orthologous Adh1/Adh2 regions within the genus Oryza

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The genus Oryza comprises rice (O. sativa), one of the most important food crops in the world and a model system in plant biology, and 23 wild species. These include both diploid and tetraploid species, with ten recognized genome types. Characterizing the genomic repertoires across the species of this taxonomic group can provide fundamental information regarding plant genome organization and evolution. In this context, a comparative genomics consortium, the "Oryza Map Alignment Project", was created to carry out genome-wide comparative genomic analysis at both the physical map and DNA sequence levels. Here, we present our results on comparative sequence analysis of orthologous Adh1/Adh2 regions on chromosome 11 across Oryza species. Bacterial artificial chromosome (BAC) clones covering the colinear and orthologous regions from eight diploid species and homoeologous regions from four tetraploid species were isolated, and approximately 2 Mb of sequence data has been finished, with nearly 2.1 Mb of additional sequence in various stages of finishing. Our initial results on the comparative analysis of these sequences show dynamic variation across the genus, by a variety of genetic mechanisms.

P164

Conserved physiological and regulatory processes in maize and sugarcane seeds

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Maize and sugarcane are important for the tropics agriculture. Despite their phylogenetical relationship, these two species developed particular processes to metabolize and store carbon. Maize accumulates and store carbon into seeds mostly as starch and storage proteins, while sugarcane accumulates carbon in the stem, mostly as sucrose. Carbon accumulation in maize seeds reflects on its size and economical importance, while in sugarcane, which is clonally propagated, the seeds importance have been extremely reduced. Indeed, breeders select sugarcane varieties against flowering. We asked if the small sugarcane seed has similar genomic composition and expression profiles of the big maize seed. We made a genomic comparison between maize and sugarcane storage proteins and seed-transcription factors. The complete set of zein genes and seed-expressed transcription factors of MAIZEST (www.maizest.unicamp.br), a database containing over 60,000 maize endosperm ESTs were TBlastX SUCEST (http://watson.fapesp.br/sucest.htm.), a database containing over 16,000 sugarcane seed ESTs. We found that sugarcane seeds processes and express orthologs comprising almost all zein classes found in maize seed. From the 414 transcription factors expressed in maize seeds, orthologs for 348 were found expressing in sugarcane seeds. In maize, of the 414 seed-expressed TFs, 113 show endosperm-preferred expression while in sugarcane, of the 347 seed-expressed TFs, only 30 appear to be seed-specific. There are 9 seed-preferred TFs common to sugarcane and maize. The results show a high genomic similarity between maize and sugarcane seeds, as related to the storage proteins. However the level of expression of the storage protein genes may be explained by the absence of expression of key TFs, that can be involved in seed development

P165

Construcion and Genetic Analysis of the Maize Mutator-transposon Insertional Mutant Pool

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Crosses were made between maize inbred lines Hz85,Mo17 and W328 (as recipient parent) and active mutator maize lines Q105,WW51, 115F, V26-2 and 919J (as donor parent). Mutant phenotypes of 29,000 F1 seed- lings and plants were recorded in field and mini-extraction DNA of 5,000 F1 seedling leaf was used to detect the flanking sequence of transposition via Mu-Tail PCR. 4,000 F1 plants were self / sister-pollinated to harvest F2 seeds for future study. Form our study, we found the mutant phenotypes observed included splitted/clustered leaf, tillering seedling, degenerate ear/tassel, apical ear, binate ear, purple anther, male sterility, dwarf plant height, susceptible to rust/smut and aphid/borer. Among 22,950 F1 plants of a cross, five male sterile plants appeared. Their pollen were checked and showed sterile or abortive. Cytoplasm of these five plants was proved to be S type. Among the F2 seeds of other crosses, the transposition frequency of mutator was calculated. It showed that the transposition frequency was 0.101763 under the two different recipient back-ground. Opaque endosperm mutants with high lysine content were screened from the mutator insertional mutant pool. The new mutant gene, o16, was mapped at 2.0-3.0cM distant from the marker UMC1141 on the chromosome 8 of maize with two different F2 mapping populations. Pyramided o16 with o2, the content of lysine in the endosperm was 32.51% higher than that of the o2o2 kernel. 107 Mutator insetion flanking sequences were obtained through the MuTail-PCR. Among them, only 70 sequences are unique. There were 40 sequences after BLASTN using an expectation score cut off

P166

Effects of cis-trans-Regulation on Allele-Specific Transcript Expression in the Meristems of Maize Hybrids

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Gene regulation involves complex molecular interactions. The phenotype of transcript expression is an ultimate result of cis- and trans-acting regulation. But their respective contribution to the divergent gene expression remains unknown in plants. In this study we present data showing the roles of cis-trans-effect on global gene regulation in maize hybrids during developing stages of the shoot apical meristem and ear inflorescence meristems. We analyzed allele-specific expression using the Massively Parallel Signature Sequencing (MPSS), an open-ended mRNA profiling technology of Solexa, Inc. The MPSS produces signature sequence tags of 17 bases of expressed genes, which allows identification of signature tag pairs with a single nucleotide polymorphism and correspond to the two alleles of an expressed gene in the hybrid. Using nearly 400 allelic signature tag pairs we found 60% of the genes expressed in the hybrid meristem were significantly different in allele-specific transcript level. Allelic expression during developmental stages of the meristems exhibited heterochronic regulation. The data suggest the abundance of cis-regulatory polymorphisms affecting hybrid meristem gene expression because both alleles are subjected to the same trans-acting factors in the hybrid. When comparing expression of the same allele in the hybrid vs. inbred parents, we found 50% of the genes expressed at a significantly different level between the genotypes. Such allele expression difference is likely attributed to the effect of trans-acting factors that differ between the hybrid and inbreds. While cis-regulatory variation predicts allelic additive expression, trans-regulation may result in non-additive expression in the hybrid, although cis-trans-interaction complicates the regulation. Study of transcript regulation on an allele-specific level provides a different level of understanding of gene regulation than focusing on overall expression in the hybrid. In addition, knowledge of allelic regulation in the meristem may have an impact on comprehending plant development.

P167

Endosperm-preferred expression of transcription factors involved in maize endosperm development

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Starchy endosperm cells constitute the bulk of the cereal endosperm and represent the major storage site for protein and starch. We created an endosperm-enriched ESTs database (MAIZESTdb) containing over 60,000 endosperm ESTs and used it to identify genes preferentially expressed in developing endosperm. To identify sequences encoding transcription factors (TFs) 29,206 maize assembled sequences (MASs), clustered from ~220,000 ESTs of the MAIZESTdb, were compared to TF sequences from TRANSFAC, Pfam and GenBank databases. This analysis resulted in 1,233 MASs (4,2% of the MAS set) representing TFs, 414 of which expressed in endosperm. Zinc-finger domain was the most represented family (12,1% of the TFs), followed by the Homeodomain (9,2%) and the bZIP family (6,7%). By comparing the level of expression and library source, we identified 113 TFs with endosperm-preferred expression. The NAC family was one of the most representative endosperm-preferred TF families (10,6%). NAC is a plant-specific multigenic family of TFs found to play roles in diverse developmental processes, including developmental programmes, defense and stress responses. The expression pattern of ESN-1(Endosperm-Specific NAM-1), one of the endosperm-preferred NAC family TFs, was further investigated. It is preferentially expressed in the aleurone layer, and may play a regulatory role during seed maturation and germination transition processes.

P168

Estimating the Abundance of Polymorphic Gene Deletions in Maize

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An estimated 40-50% of the genes in duplicated regions have been lost (Ilic et al., PNAS 100:12265-12270, 2003; Lai et al., Genome Research 14:1924-1931, 2005). Gene deletion has occured frequently during the evolution of maize, and it is therefore likely that some gene deletions are polymorphic in maize. We have begun to search for gene deletion polymorphisms with an in silicoanalysis of A188 EST sequences. Approximately 14,000 A188 EST sequences were downloaded from GenBank. These sequences correspond to 5420 EST contigs and 1994 EST singletons based on the TIGR EST assemblies. BLAST searches of the EST singleton sequences against maize B73 GSS sequences determined that 56 EST sequences did not hit a GSS sequence with a threshold of e-10; these ESTs may identify A188 genes that are not present in B73. Forty-seven sequences have been tested by PCR analysis, and 26 were not detected in at least one of the 12 maize lines tested. Fifteen sequences were not detected in B73. We have begun re-testing a sample of sequences by Southern blot analysis to verify their absence in B73. We are beginning a second analysis using EST sequences from W64A. The examination of a second maize line will help us estimate that frequency of gene deletion polymorphism in maize. Such estimates may be of use in predicting the fraction of genes in maize that are not in the B73 line.

P169

Full-length cDNA Maize Sequencing plus Functional Annotation

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Funded by the NSF PGRP (PI Yu) starting in May 2005, our goal is 30,000 distinct maize cDNAs in 3 years. Two normalized B73 libraries were built by Invitrogen in the Gateway pSport1 vector. Library 1 contains 16 organs dissected by the Maize Gene Discovery Project for the 20,000 ESTs in project 3530. Now 5' and 3' ESTs from 80,000 more clones yielded ~72,000 paired reads that identify nearly 30,000 apparently distinct genes. A strategic innovation after EST assembly is to analyze gene and protein models from other plants to estimate likely cDNA length. One candidate FLcDNA is picked from each assembly; finishing clones are consolidated by size into 384 plates, and bidirectional primer walking then starts. Library 2 contains seedlings, 6 abiotic seedling treatments, juvenile leaf, and anther stages that fill in key reproductive stages. Bi-directional EST sampling of 50,000 clones is in progress; a final round of EST sampling will go deeper into the better library. Transcriptome profiling annotation will be available for the 12 tissues in Library 2 from in situ synthesized Agilent 60-mer arrays (22K or 44K elements) or to Arizona spotted arrays (58K elements, NSF Array Project Univ. of Arizona). qRT-PCR will be used to certify alternatively spliced variants for selected genes. When full-length clones are finished, expression data will be part of clone annotation. EST data are deposited in GenBank and batches of FLcDNAs will be deposited after verification; data assemblies, sequencing primers, and expression data will be posted at http://www.maizecdna.org/ Materials include clones, high density filters printed with sequenced and unsequenced clones, and at a later stage amplified aliquots of the libraries, available from the Arizona Genomics Institute http://www.genome.arizona.edu/orders/

P170

Functional Genomics of Maize Chloroplast RNA Binding Proteins

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The nuclear genome of Zea mays encodes hundreds of different putative RNA-binding proteins (RBPs) that are likely participants in the maturation, modification and processing of organelle encoded RNA. We are interested in both identifying the RNA substrate(s) of chloroplast targeted RBPs and demonstrating their roles in mediating post-transcriptional modifications of chloroplast genome encoded RNA. By establishing RBP-RNA interactions of chloroplast targeted proteins whose mutagenesis is associated with a non-photosynthetic phenotype, the role of a particular RBP in producing a phenotype related to chloroplast biogenesis and metabolism may be determined. To characterize a variety of different RBPs in this manner, three key technologies have been developed and implemented by our research group: Photosynthesis Mutant Library [PML]: comprised of more than 2,500 Mu-induced chloroplast defective, photosynthesis impaired maize mutants; PlantRBP: a relational database that integrates maize genomic sequences with predicted orthologous groups among the complete rice and Arabidopsis proteomes, and that includes a web interface to facilitate searches based on targeting predictions, protein domain content, and other criteria (see Abstract from Nigel Walker, et al.); and RIP-chip: a micro-array based assay that reveals *in vivo* associations between specific chloroplast RNA ligands and specific chloroplast-localized RBPs. We have successfully employed these approaches to study a variety of plant-specific RBPs, including those harboring PPR, CRM, RNAse III, and helicase domains. We are currently expanding our studies to include additional proteins in these classes, and to explore other protein classes. The PML and PlantRBP web sites can be accessed at http://chloroplast.uoregon.edu and http://plantrbp.uoregon.edu, respectively.

P171

Generation and characterization of fluorescent protein tagged maize lines for cell biology and genomics applications

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We are generating maize fluorescent protein reporter lines to define expression domains and sub-cellular compartments for use in developmental, physiological, cell biology and genomics studies. Our long-term goal is to define and label target proteins for each sub-cellular compartment in different cell types, tissues and developmental stages. To maintain native expression levels and localization of tagged genes, we developed a semi-high throughput method to tag genes using triple template overlap PCR (Tian, et al., Plant Physiology, 135, 25-38.). Tagged genes are cloned using Gateway technology, and sent to the Maize Transformation Facility at Iowa State University for transformation. After validation of expression and bulking up of seed, all lines will be available through the Maize Genetics Stock Center. Currently, we have generated tagged lines for a nuclear protein, ABPHYL1, and a membrane associated protein, FASCIATED EAR2. Both tagged constructs show the expected localization. Transient expression of a third construct, RAB2A1, tagged with CFP, shows a punctate pattern consistent with predicted vesicle-associated location. We are in the process of tagging several cytoskeleton, vesicle transport and hormone signaling proteins with different color fluorescent tags. Progress on these will be presented. We request suggestions from the maize community for genes to tag and will provide guidelines for candidate gene selections. Our goal is to generate up to 100 stable transgenic lines of maize, which will be useful for characterizing mutants identified in forward and reverse genetic screens, for proteomics and gene profiling studies, and for diverse applications in cell and developmental biology. Characterization of the expression pattern and sub-cellular localization of these gene reporter constructs will provide a resource for choice of tissue specific promoters, sub-cellular localization targeting signals and research and teaching in cell biology. The data will be presented at http://maize.tigr.org/cellgenomics/.

P172

Genome-wide isolation and mapping of resistance gene analogs

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Conserved domains or motifs shared by most known resistance genes (R genes) have been extensively exploited to identify unknown resistance gene analogs (RGAs). In an attempt to isolate all potential RGAs from maize genome, three approaches were adopted in the present study, including the modified AFLP (amplified fragment length polymorphism), modified RACE (rapid amplification of cDNA ends), and data-mining methods. A total of 23 and 12 RGAs were obtained in the modified AFLP and RACE methods, respectively; while, as many as 109 unigenes and 77 singleton ESTs that showed highly homologous with the known R genes were recovered via data-mining method. A mapping population consisting of 294 recombinant inbred lines (RILs) has been developed from the cross "87-1"/"Zong3" after self-pollination for ten generations. With an aim to map all RGAs on maize genome, STS, CAPS, and SNP markers tagged to RGAs were developed based on RGA sequence divergences between two mapping parent lines "87-1" and "Zong3". Up to now, 68 markers tagged to RGAs have been obtained and used to map the corresponding RGAs. On analysis of the RGA linkage map, we found that RGAs were not evenly distributed on maize genome. The number of RGAs located on chromosome 1, 4, & 10 are far more than those on chromosome 5 & 7. These results are in accordance with the distribution of major resistance genes or resistant QTLs on maize genome reported so far by other investigators. Development of markers for the remaining RGAs is in progress. After all RGAs being mapped on maize genome, we will provide the maize community a complete RGA linkage map for accelerating isolation of maize resistance genes.

P173

Global gene expression profiling of maize cms-T tapetal cells

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Fertility of the T-cytoplasm male sterile (cms-T) lines can be restored by the sporophytic action of dominant alleles of the rf1 and rf2 nuclear genes. Even though the rf2a gene is known to encode a mtALDH, the metabolic pathway involved in cms-T induced male sterility and fertility restoration is not well understood. Identified enhancers and suppressers of the rf2a gene suggest it is a complex pathway and essential for anther development even in normal cytoplasm plants. Efforts to understand this pathway have been hindered in part by the difficulty in analyzing tapetal-specific transcripts. The tapetal cells of the anther play a crucial role in microspore development. Indeed, male sterility in cms-T is associated with the premature degeneration of the tapetum . Expression studies conducted on anthers to date have made use of mixed populations of anther cells. In this study, we used laser-capture microdissection to specifically isolate tapetal cells from the lower floret of cmsT sterile and fertility-restored plants at the dyad/tetrad stage of microspore development, which is immediately prior the premature degeneration of the tapetum in cmsT plants. Isolated tapetal mRNA was used for global expression profiling. The resulting data comprise a useful resource for cloning genes involved in anther development, including those involved in fertility restoration.

P174

High-throughput BAC DNA preparation method towards rapid identification of candidate BAC clones

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Bacterial artificial chromosome (BAC) libraries are valuable tools for many areas of genome research such as physical analysis of large chromosomal regions, map-based cloning of genes and generation of integrated genetic and physical maps. BACs can also be used for the development of molecular markers for marker assisted breeding. In addition, BAC libraries from small genomes can be used for the identification of syntenic regions in large genomes. A crucial requirement of a BAC library screening project is rapid and accurate identification of clones of interest. Often it is required to prepare DNA samples of BAC clones identified in initial screening towards testing and identification of candidate clones. The availability of commercial BAC DNA miniprep kits simplifies the DNA preparation process, however, testing of several kits revealed highly variable yields and required elaborate troubleshooting or multiple preparations for a given clone making it uneconomical. In order to have an economical BAC DNA miniprep method for rapid screening of preliminary library screening hits, we have a developed a semi-automated, 96-well method for DNA extraction. This procedure is based on a boiling method and was developed by combining key steps from limited, existing protocols, adding new steps and by adapting it to high-throughput format. This improved method allowed us to rapidly screen preliminary library hits and efficiently identify candidate BACs for choromosome walking and marker development. Details on the development of the method and results obtained from the screening of a corn BAC library will be presented.

P175

It's a knockout: Massively parallel mapping of Mutator insertion sites from the MTM population.

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Insertional mutagenesis of genes discovered by sequencing has been attempted in Drosophila, C.elegans and mouse, as well as in Arabidopsis, and rice. In maize, Robertson.s Mutator (Mu) is the transposon of choice for studies of this type. The major advantage of using multiple copy insertions, such as Mu, for functional genomics is that the number of individuals in the population can be substantially reduced compared to single insertion systems. Transposons target hypo-methylated regions of the genome, reducing the target size to about 300Mb. Still, this target still requires at least 500,000 insertions for 2x coverage, or 90% probability of recovering an insertion in any given gene. Consequently, there is a critical requirement for novel methodologies that lessen the cost of sequence-indexing of insertions. The massively parallel DNA sequencing platform developed by the 454 Corporation is an emerging technology that is particularly powerful for applications such as transposon indexing. The Maize Targeted Mutagenesis (MTM) system (http://mtm.cshl.org) is based on a population of Mu plants, crossed with .Mu-killer, a dominant negative regulator of Mu activity. In this way, somatic activity has been substantially reduced. Individual grids consisted of 48 rows and 48 columns, with each plant represented in a grid as a row-column intersection. 4 DNA pools from intersecting columns and rows, in 2 distinct grids were produced by PCR representation and normalization. For each pool, over 65,000 reads, averaging 100 bp in length were sequenced using 454. We compared each column against its corresponding row to identify unique or near-unique insertions in the plant at the row-column intersection, and we found that each plant has on average 480 unique insertions. These include sequences corresponding to vp1 (Viviparous) and sde1 (RNA-dependent RNA polymerase), which had been previously identified at these intersections by conventional PCR. These results suggest that a single grid (96 samples, 2304 plants) will yield sufficient transposon insertion sequences for 3x coverage of the genome, while only 3 grids are required for 10x coverage, in a manageable population of plants

P176

Large-scale EST sequencing from developing ovules of maize undergoing megasporogenesis and female gametogenesis.

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As a complement to large EST sequencing efforts undertaken by the maize genetic community, we have developed new initiatives to functionally characterize the transcriptome of the maize ovule at early developmental stages. We have generated 4 cDNA librairies from developing ovules of B2000RL14, a drought resistant genotype from Celaya, Mexico. Ovules were hand-isolated at different stages and verified by monitoring female gametophyte development through whole-mount ovule clearing. Our first library includes ovules containing a megaspore mother cell prior to meiosis. Our second library include ovules undergoing megasporogenesis. The third and fourth libraries were generated with ovules undergoing female gametogenesis or containing fully differentiated embryo sacs. We have sequenced more than 12,500 ESTs, in which 5000 correspond to transcripts present in ovules prior to megaspore mother cell division, 4,000 to transcripts present in ovules undergoing female meiosis, and 3,500 to ovules undergoing female gametogenesis. These EST collections have been grouped using the ESTs annotation by Gene Ontology to assign molecular functions. We have initiated a bioinformatic analysis to identify ESTs that are exclusively expressed at specific developmental stages. We will present a summary of these global expression assessment.

P177

Large-scale Identification of Maize ESTs Important for Mexican Agriculture

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As part of the activities of the newly created National Genomic Laboratory for Biodiversity of Mexico (Langebio), and as a complement of the current sequencing initiatives in the USA, we are functionally characterizing the genome of maize landraces important for Mexican agriculture. Our initial efforts focused on the identification of genes important for drought tolerance, nutritional uptake, ovule formation, and seed development. Germplasm includes selected tropical and subtropical genotypes generated at INIFAP-Celaya (Mexico), and ancient native landraces originating in Central Mexico. To identify genes differentially regulated during conditions of abiotic stress, plants were grown under drought conditions or low phosphorous availability. Standard and subtractive cDNA libraries were generated and systematically sequenced. To identify genes involved in ovule and female gametophyte development, hand-dissected developing ovules were collected and staged after clearing. Three different collections (ovules undergoing meiosis, ovules undergoing megagametogenesis, and mature ovules) were used to generate complete cDNA libraries. Overall, a total of 5 standard and 8 subtracted libraries with an average insert size of 525 bp were generated and sequenced. More than 20,000 clones were clustered and blasted using publicly available databases as a reference. Automated assembling and manual annotation yielded a total 10,468 ESTs. The comparison of specific gene families sharing similar molecular functions suggests important changes in gene activity both during conditions of abiotic stress and ovule development. A summary of these comparisons will be presented.

P178

Linkage mapping of 1454 new Maize candidate gene loci

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In the frame of the French Genoplante consortium for plant genomics, we have genetically mapped 1454 new loci from cDNA sequences, to look for co-localization with QTL. These sequences were previously identified as candidate genes for several agriculturally important traits, based on annotation and bioanalysis. For linkage mapping, we used two populations of intermated recombinant inbred lines (IRILs), which allow a higher map resolution than non-intermated RILs. The first panel (IBM), derived from B73/Mo17, is publicly available from the Maize Genetics Cooperation Stock Center. The second panel (LHRF) was developed from F2/F252 to map loci monomorphic on IBM. We built framework maps of 237 loci from the IBM panel and 271 loci from the LHRF panel. Both maps were used to place 1454 loci (1056 on map IBM_Gnp2004 and 398 on map LHRF_Gnp2004) that corresponded to 954 cDNA probes previously unmapped. RFLP was mostly used, but PCR-based methods were also performed for some cDNAs to map SNPs. Unlike in usual IRIL-based maps published so far, corrected meiotic centiMorgan distances were calculated taking into account the number of intermating generations undergone by the IRILs. The corrected sizes of our framework maps were 1825 cM for IBM_Gnp2004 and 1862 cM for LHRF_Gnp2004. All loci mapped on LHRF_Gnp2004 were also projected on a consensus map IBMconsensus_Gnp2004. cDNA loci formed clusters near the centromeres except for chromosomes one and eight.

P179

Maize Domestication Block: a teacher's view from teosinte to table.

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How do you represent the diversity and developmental history of maize to high school students whose only contact with the living plant is shucking an ear of store-bought sweet corn? For the MU Plant Genomics Research Experience for Teachers, the answer is to take their teachers for a walk through the Maize Domestication Block. Starting with a planting of the most familiar variations available: hybrid field corn, popcorn, and sweet corn; the walk continues through a selection of regional inbred lines and native landraces. Finally teachers meet *Teosinte parvaglumis* and the maize mutants: *Cg1* (corngrass), *Tp2* (teopod), *tb1* (teosinte branched), *tlr1* (tillered), and *Ts6* (tassel seed), that represent the necessary morphological transitions for the conversion from wild grass to food source. This project was just one of the laboratories in the two day workshop "Mutants We Know and Love". The full two week long NSF funded summer workshop series for high school science teachers, covered DNA structure and function, hormones, biotechnology, and genome structure and function from the plant's point of view.

P180

Maize Sequence Indexed Knockouts using the UniformMu Transposon-Tagging Population

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Gene knockouts are an essential resource for functional genomics. In Arabidopsis, comprehensive knockout collections were generated by systematically amplifying and sequencing the genomic DNA flanking T-DNA insertion mutants. These Flanking Sequence Tags (FSTs) anchor each mutant to a specific locus within the genome. In maize, FSTs have been generated using DNA transposons. Both Activator and Mutator transposons can generate unstable or somatic insertions that are difficult or impossible to analyze for simple knockout phenotypes. Here we show that FSTs from the UniformMu population create easy to use knockout resources. The genetic markers in the UniformMu population allow for the selection of stable transposition events. We have sequenced MuTAIL-PCR products from 130 UniformMu seed mutants to generate 1,750 non-redundant FSTs. One-hundred transposon insertion sites unique to individual mutants were tested for inheritance by locus-specific PCR. We confirmed 90% of the FSTs to be stable, germinal Mutator transposon insertions. Only one or two locus specific primers were required to develop a confirmatory marker for each FST. These markers were used for rapid tests of co-segregation between FSTs and seed mutant phenotypes. Several seed mutants were found to co-segregate with specific FSTs, and results for one of these mutants will be presented. These data indicate that the UniformMu FSTs nearly exclusively represent germinal insertion mutants. Importantly, the clone name for each FST relates directly to a single seed stock making individual knockouts simple to identify from database searches.

P181

Maize Ultra High-Density Gene Map for Genome-Assisted Breeding

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Map-assisted gene cloning and marker assisted breeding require a high-density genetic map. The fine resolution in the target region could improve the precision in map-based cloning thus shorten the distance for chromosome walking; and identify accurate markers that tightly linked to the trait to reduce linkage drag of undesirable genes. Gene-based markers have the advantage of tight linkage to the trait, more conserve among related species, and possible lead for the function gene that influence or responsible for the trait. Despite of recent efforts, the mapped gene markers in maize are still limited. Previously, we mapped 4,368 genes in a widely used high resolution mapping population, intermated B73 and Mo17 (IBM) using a custom designed maize GeneChip microarray. Using a pattern match method, we genetically mapped 34, 034 SFPs representing 11,427 unique genes or EST clusters. The mapped genes are validated by sequence analyses, and supported by the macro synteny relations between rice and maize. Integration of these gene markers with other types of genetic and physical markers will facilitate marker assisted breeding and identification of genes that controls complex traits. The approaches and methods to develop this ultra-high density map will be reported.

P182

Maize gamete ESTs to identify cell-specific promoters in Arabidopsis

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Double fertilization in angiosperms was discovered in 1898, but the proteins that mediate gamete recognition and fusion are largely unknown. To develop tools for such studies, we constructed a cDNA library from Zea mays sperm cells (Engel et al., Plant Journal 34:647-707) and sequenced 5000 randomly selected ESTs. For the female, we constructed two libraries, one from embryo sacs and one from eggs; so far we've sequenced ~7000 ESTs from embryo sacs and ~4000 from eggs. All these libraries are useful for gene discovery; many ESTS correspond to hypothetical proteins, while others encode ORFs not yet annotated in fully sequenced genomes. Using RT-PCR we identified 5 transcripts that appeared to be present only in the sperm cells, and 19 transcripts that appeared to be embryo sac-specific. Then, using in situ hybridization, we identified a synergid cell-specific transcript, one expressed in both the synergids and in the central cell, and one specific to the central cell and egg cell. We identified Arabidopsis homologs for several of these sperm or embryo sac genes, and tested their promoters for specificity using GFP in transgenic Arabidopsis plants. Two sperm promoters worked; the GFP-marked sperm can be visualized entering the pollen tube during in vitro germination (Engel et al., Plant Physiology 138: 2124-2133). So far we have one Arabidopsis promoter that gives egg expression, one that gives synergid expression, and one that gives central cell expression. These promoters can be used for imaging and for testing whether candidate proteins play roles in double fertilization. We are currently carrying out functional analyses of some of the ESTs that appear specific to sperm or to embryo sacs; for example, in a knockout line for one of these Arabidopsis genes, the pollen tubes are not attracted to the mutant ovules.

P183

Maize genes involved in Western Corn Rootworm feeding response.

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Western corn rootworm (Diabrotica virgifera virgifera Le Conte) is a significant pest of maize causing losses of up to $1 billion annually. A number of insects comprise the rootworm complex. Among these the main pest in the MidWest is the western corn rootworm (WCRW). Feeding damage to maize roots by WCRW interferes with nutrient and water uptake making the plants more susceptible to other biotic and abiotic stress and increases lodging. Current control measures include costly insecticide application or crop rotation. These control measures are insufficient as WCRW have demonstrated the ability to overcome both chemical and rotation control measures. Transgenic corn expressing Bt in the root system is another alternative for control of WCRW, however, given the ability of this pest to overcome other resistance strategies, a polygenic resistance mechanism may be preferable. The objective of this study is to identify maize genes and associated biochemical pathways associated with WCRW feeding. V2 stage plants of a maize line with resistance to WCRW were infested with 50 neonate larvae (feeding) or mechanically wounded with a scalpel (wounded). Root tissue adjacent to the site of feeding or wounding was collected subsequently. Microarray hybridizations were conducted using the Maize Oligonucleotide Array with the methods described at www.maizearray.org. Comparisons evaluated were control vs. feeding, control vs. wounding, and wounding vs. feeding. The data were analyzed using a mixed model in SAS. Differentially expressed genes include cell patterning, hormone response, ion transport, chromatin remodeling, and post-translational gene silencing. More specifically, genes regulating meristem activity, root hair formation, and auxin response where identified. Further studies are underway to assess the role of changes in chromatin structure and gene silencing in the plant response to WCRW feeding.

P184

Mapping a major QTL against head smut and enriching this region with newly-developed markers in maize

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Head smut is one of the most serious diseases in maize, causing severe yield loss worldwide. Resistance against head smut was believed to be quantitative trait, acting in an additive and dominant effective manner. Here we present our results towards mapping of the resistance gene (R) and saturation of the R-region with the developed markers. The 314 BC1 plants and 120 BC1:2 families derived from Ji1037 (resistant)/Huangzao4 (susceptible) were evaluated for their resistances against head smut after artificial inoculation of pathogen in 2004 and 2005. Two QTLs on bin 2.09 and bin 5.03 have been detected by using 120 polymorphic SSR markers. The major QTL on bin 2.09 was bordered by SSR markers bnlg1893 and umc2184, and could explain 36% of phenotypic variation; while the other one on bin 5.03 could only account for 8.98% of phenotypic variation. The anchored ESTs, IDPs, RGAs, and BAC-end sequences on bin 2.09 were exploited to develop markers to saturate the R-gene region. As a consequence, we have successfully developed six markers, including one CAPS, two SNPs, and three STS markers. Of them, one CAPS, one SNP, and one STS have been mapped in the QTL region. With the newly-developed markers and the BC1:2 mapping population, the major QTL conferring resistant to maize head smut was eventually delimited into 7.7 cM, covered by ~16 BACs according to the physical map in the Maize Mapping Project. Fortunately, a resistance gene analog showing highly homologous with the rust resistant genes has been identified in the mapped region, and this would greatly accelerate cloning of the gene conferring resistant against maize head smut.

P185

Mapping and characterization of the sorghum pc gene: a host disease resistance gene corrupted to assist infection by a fungal necrotroph

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Several pathogenic fungi excrete phytotoxins that selectively affect specific host plant genotypes. Although our understanding of plant-microbe interactions has increased tremendously in recent years, little is known about host-selective toxin resistance genes in plants. In contrast to structurally similar "gene-for-gene" type resistance genes, the known toxin resistance genes are highly variable in their genetic properties. The pc gene confers resistance against root and crown rot in sorghum caused by the soil-borne fungus Periconia circinata. The Pc gene is genetically unstable, generating recessive toxin-resistant alleles (pc) at a meiotic rate of about 1 in 4000. A high-density genetic map including the Pc locus was constructed using an RFLP marker and 18 newly generated PCR-based markers. The Pc gene was confined to a 0.9cM segment on the chromosome 9, which corresponded to about 110 kb on the physical map. This region was sequenced and ten gene candidates were identified. Isogenic wild type and Pc-pc mutant sorghum lines were screened using single stranded conformational polymorphism (SSCP) to detect mutations in the gene candidates. The SSCP analysis revealed a consistent decrease in the copy number of a tandem duplicated NBS-LRR gene in the pc mutant lines as compared to the wild type lines. All other genes were identical between the isogenic lines. Frequent unequal recombinations resulting in copy number variations have been reported for other tandem duplicated NBS-LRR genes. The data suggest that the loss of functional NBS-LRR gene copies provides peritoxin resistance in sorghum. These findings suggest a novel virulence strategy, wherein plant pathogens exploit the host defense system to disrupt and invade plant tissues.

P186

Microarray Resources for Maize

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The Maize Microarray Project (http://www.maizearray.org) was initiated to provide comprehensive, low-cost, public sector long-oligonucleotide microarrays for gene expression analysis in Zea mays L. The overall objectives are to produce an array with 70-mer oligonucleotides for ~30,000 identifiable unique maize genes, provide a website to distribute microarray information & document expression data generated by the project, perform expression profiling with a subset of maize tissues, utilize the flexible NimbleGen system to experimentally refine oligonucleotide design, and develop design tutorials and web based experimental design and analysis tools. An array with 57,452 70-mer oligonucleotides is available as a slide pair for $125. As of January 2006, 2200 slide sets have been ordered &1950 array sets have been distributed to researchers in ten countries. A Sybase relational database, Zeamage, has been constructed to store the multiple types of data generated from this project. Replicated baseline expression profiles have been generated for 18 tissues and deposited in Zeamage as have other expression studies. NimbleGen arrays have been have been used to orient unoriented ESTs, validate AZM transcription, and assess antisense transcription. A set of standardized cross-platform protocols has been developed to allow performance of oligos on NimbleGen arrays to be predictive of performance on spotted arrays. Optimized experimental protocols, design tutorials for the more common types of microarray experiments, & MIAME compliant data curation tools are available on the project website. Four workshops have been held at the University of Arizona and a total of 45 maize researchers have participated.

P187

Microarray-based Pathways Analysis of Maize Kernels with Drought Tolerance and Low Aflatoxin Contamination

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Aflatoxin contamination caused by Aspergillus fungi is a greatest concern in corn production world wide. Pre-harvest A. flavus infection usually happens when corns suffer drought stress at late developmental stages. However, drought resistant lines displayed less aflatoxin contamination under same situation. Although mechanisms adapted to water stressed environments are widely reported in the maize kernels, but the majorities are focused on the early stages of kernel development. It is still unclear about the molecular mechanisms of drought tolerant lines with low aflatoxin contamination under water deficit. One hypothesis is that some metabolic pathways in the developing kernels are affected differently in drought tolerant and sensitive lines under water deficit stress. The objective of this study is to test this hypothesis and to better understand the changes of gene expression in response to drought stress during the late stages of seed development, and to identify the related biochemical pathways and resistant genes. The maize inbred lines of Tex6 and B73 were used in this study. Tex6 has been reported to have resistance to Aspergillus infection and reduced preharvest aflatoxin contamination. B73 is used as a susceptible control. Transcriptional profiles of kernels at the 25, 30, 35, 40, 45 day after pollination were compared under normal and water deficit conditions using the 70-mer maize oligonucleotide arrays (version 1, ~58,000 sequences) from Maize Oligonuleotide Array Project. Several inbred lines with different tolerance of drought stress and A. flavus infection were used in the research to validate the microarray study using real time PCR.

P188

Molecular and Functional Diversity of the Maize Genome

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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 are performing SNP discovery in 4000 genes. The sequence data from our SNP discovery panel are being used to determine the proportion of genes influenced by artificial selection during the domestication and crop improvement phases of maize evolution. The SNPs will be 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 relative (teosinte, Zea mays ssp. parviglumis) for traits of evolutionary, developmental and agronomic importance. Our project will generate several key resources for the maize research community: (i) validated SNP markers from 4000 genes, (ii) a maize QTL mapping population of 5000 fully genotyped RILs from crosses between diverse maize inbred lines and B73, (iii) a maize association mapping population consisting of 281 diverse maize inbred lines, (iv) a teosinte association mapping population, and (v) a maize-teosinte QTL mapping population of backcross RILs. We expect the maize QTL and association mapping populations to be of greatest interest to the maize community. Seed will become available from all of our germplasm resources, and collaborators are invited to contribute candidate genes and trait data for association mapping. For further information see the project website, www.panzea.org.

P189

Nearly Identical Paralogs (NIPs) and their evolutionary implications

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As an ancient segmental tetraploid, the maize genome contains large numbers of paralogous gene pairs that are expected to have diverged by a minimum of 10% over time. NIPs (Nearly Identical Paralogs) are defined as paralogous genes that exhibit >98% identity. Sequence analyses of the "gene space" of B73 genome, coupled with wet lab validation, have revealed that at least ~1% genes have a NIP. In most instances (20/26) both members of NIP pairs are expressed and are therefore at least potentially functional. In some instances (8/10), members of a given NIP pair exhibit differential patterns of gene expression. The finding that some families of NIPs are closely linked genetically while others are genetically unlinked is consistent with multiple modes of origin. NIPs provide a mechanism for the genome to circumvent the inherent limitation that diploid genomes can carry at most two "alleles" per "locus". As such NIPs may have played important roles during the evolution and domestication and may contribute to the success of long-term selection experiments in this important crop species.

P190

Noncollinear cytidine deaminase genes in maize

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Cytidine deaminase is an enzyme conserved from bacteria to man and provides critical functions of RNA editing and can even catalyze the deamination of C on DNA. Based on such a conserved function, we investigated the organization of corresponding gene copies in the maize genome. Interestingly, the number of sequences and loci encoding a putative cytidine deaminase in maize is quite variable. Moreover, we found that it might represent an example of self-catalytic gene modification and adaptation. When the z1C locus of maize is compared between B73 and BSSS53, BSSS53 contains an extra copy of this gene via a 5.2kb long helitron insertion, named Hel-MG1. There are two additional copies in the BSSS53 genome that are absent in B73 and differ in coding regions by an insertion and deletion of extra amino acids. The B73 genome has a single copy not on chromosome 4 like BSSS53 but on chromosome 7 (Contig #299). Comparison with other inbred lines also shows that the gene copy present in the two locations in BSSS53 (chromosome 4) and B73 (chromosome 7) is absent in W64A. Transcripts for cytidine deaminase vary in tissue specificity between inbred lines, indicating that the variability observed could be locus dependent. Therefore, noncollinear genes might represent a rapid adaptation for changes in gene expression and function. Interestingly, a comparison of two copies of a cytidine deaminase gene between BSSS53 and B73 shows the removal of an aminoterminal region by introducing a new start codon based on a single nucleotide change of C to T, which could have the potential to provide new enzyme specificity.

P191

Optimized Target Preparation Method for Single-Feature Polymorphism Detection in Maize

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We describe the detection of sequence validated single-feature polymorphisms (SFPs) between maize inbred lines by hybridizing RNA or complexity-reduced genomic DNA to an Affymetrix GeneChip expression array. Direct hybridization of labeled total genomic DNA to oligonucleotide expression arrays for SFP detection was initially demonstrated in organisms with relatively small genomes, such as ~12Mb yeast (Winzeler et al., 1998) and ~130Mb Arabidopsis (Borevitz et al., 2003). For larger genomes such as ~2500Mb maize, however, obtaining robust hybridization signals requires a sample preparation method optimized to reduce repetitive or high-copy sequences in the target prior to array hybridization. In this study, we evaluated the efficacy of four different complexity-reduction methods for sensitive SFP detection in maize: cDNA, methyl filtration, high-Cot selection, and AFLP. These four methods were applied to four diverse maize inbred lines (B73, Mo17, CML69 and Tzi8) with 3 replications per line (48 GeneChips). Our results indicate that all genome reduction methodologies offer modest power to detect SFPs with the commercially available GeneChip Maize Genome Array. Moreover, the detection of non-SNP genetic polymorphisms by probes, such as allelic non-homologies and copy number of genes, may produce some of the apparent Type I error rates. The AFLP technology is covered by patents and patent applications owned by Keygene. AFLP is a registered trademark of Keygene.

P192

Positive Selection in CMS-associated Regions of Maize Mitochondrial Genomes

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There are three cytoplasmic-male-sterile (CMS) mitochondrial genomes in maize, named T, S and C, after where they were discovered. The CMS-determining factor in CMS-T is Turf-13, a chimera of sequences from the rrn26 coding and downstream regions, that has the upstream region of atp6 as its upstream region. Immediately downstream of Turf-13 is atp4. We have aligned the Turf-13 region with its homologous sequences from all five maize cytotypes, as well as those of Z. mays ssp. parviglumis, Z. luxurians, Z. perennis, Tripsacum dactyloides and Sorghum bicolor (taken from our whole-genome sequences of each), as well as the previously published Oryza sativa. The 1-kb Turf-13/atp4 region accounts for nearly a fifth of all of the nucleotide differences between the 535,825-bp CMS-T genome and any of the fertile Z. mays genomes. The nucleotide changes preferentially result in amino acid changes. Similarly, the 2-kb orf355/orf77 region in CMS-S accounts for about a tenth of all of the nucleotide differences between the 569,553-bp CMS-S genome and the fertile Z. mays genomes. Whereas the sequences forming Turf-13 are present in all the taxa, those forming orf355/orf77 are present only sporadically and are of incomplete length. Reversing this process and looking for highly divergent regions, another region was found that accounted for more than half of the 92 nucleotide differences between the 569,630-bp NB (the predominant fertile cytotype) genome and the 680,603-bp ssp. parviglumis genome. This 5-kb region includes an 1159-amino-acid ORF that is implicated in CMS-S, although both of these cytotypes are male fertile. Again, the substitutions preferentially induce amino acid changes. These three examples argue for positive selection in these CMS-associated genes.

P193

Preliminary Analysis of Differential Gene Expression between a Maize Superior Hybrid and Its Parents Using the 57K Maize Gene-specific Long-oligonucleotide Microarray

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Heterosis, a term used to describe the superiority of hybrid performance over the mean of its parents or of the better parent, is a universal, economically extremely important phenomenon in plants, animals, and humans. Application of heterosis in agriculture has revolutionized production of many agricultural crops, the most notable ones including maize, rice, and sorghum. Heterosis of maize hybrids is estimated to contribute to grain yield in U.S. at a rate of 77 kg per hectare per year. The genetic basis of heterosis has been extensively studied genetically; however, little is known about which genes are responsible for heterosis and how those genes respond. To answer these questions, we are studying the molecular basis underlying heterosis in maize using several combined approaches. We have generated or obtained all essential materials, including two elite inbred lines (B73 and Mo17) that have been most widely used in maize hybrid variety breeding and production, B73 x Mo17 hybrid, Mo17 x B73 hybrid, 303 RILs of the maize B73 x Mo17 IBM population, and BC hybrids between the RILs and Mo17 or B73. We collected tissues of roots, leaves and developing ears or filling kernels at four developmental stages (V4, V14, R1 and R3) of a selected group of the parental lines and hybrids. Expression of genes in the tissues is currently being profiled and comparatively analyzed, between maize inbred parents and their hybrids, and between the super maize hybrid and a super rice hybrid, using the 57K maize gene-specific long-oligonucleotide microarrays. Preliminary analysis of an array containing ~ 32,000 genes indicated that at least 800 genes were more actively expressed by 2- to 10-fold in hybrid than in its parents. The results of these experiments and analyses will be presented.

P194

RICEATLAS: a transcriptional profile database of rice cell types

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We are constructing a cell type transcriptional atlas of rice, with whole-genome expression data for each individual cell type, normalized to permit comparisons between and among cell types. Cell types are isolated by laser capture microdissection from paraffin sections of acetone-fixed tissues. RNA is isolated, amplified, and profiled on whole genome microarrays, along with a common reference RNA that is profiled on each array for normalization. To date approximately 50 cell types have been profiled, with a goal of 135. A web-based database (http://plantgenomics.biology.yale.edu/riceatlas) permits downloads and a variety of views and filters of the data, including metabolic pathways. The atlas permits cell comparisons to identify cellular signatures, motifs and hierarchies, as well as identification of genes associated with cell characteristics. Housekeeping and co-expressed genes are particularly simple to identify.

P195

Retrotransposon polymorphisms in intergenic regions strongly affect recombination within adjacent genes

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The recent discovery of extensive retrotransposon polymorphisms in maize raises questions regarding their effect on recombination in adjacent regions. Fu and Dooner (2002) found that retrotransposons, which comprise most of the repetitive DNA in maize, differed markedly in make-up and location relative to the genes in the bz genomic region. In particular, they found that the 1.5-kb bz-stc1 intergenic segment in McC, a genetic line, is replaced in inbred B73 by a 26-kb retrotransposon block. In order to assess the effect of retrotransposon heterozygosity on recombination in the adjacent bz and stc1 genes, we have examined recombination within the same genetic interval in the presence and absence of the 26-kb retrotransposon block. To accomplish this, we introgressed all haplotypes into the common background of the inbred W22. We then compared recombination in the bz gene, the bz-stc1 intergenic region and the stc1 gene in two heterozygotes: one between the McC and B73 haplotypes, which contains the retrotransposon block, and one between the McC and W22 haplotypes, which doesn't. From populations involving more than 50,000 individuals from each heterozygote, we isolated and sequenced 240 recombinants. We found the following: (1) Heterozygosity for the retrotransposon cluster has a strong suppressing effect on recombination. The effect is most pronounced in the adjacent regions: four-fold in bz and two-fold in the closest stc1 segment. Overall, the size of the same genetic interval is twice as large in the McC/W22 heterozygote than in the McC/B73 heterozygote. (2) No recombinants were obtained in the bz-stc1 intergenic region of either heterozygote, although these shared about 900 bp of homology, suggesting that recombination in the interval is restricted to genes. Our finding implies that haplotype structure will profoundly affect the correlation between genetic and physical distance for the same interval in maize.

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SNP genotyping using allele competitive PCR (AC-PCR)

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More and more Single Nucleotide Polymorphism (SNP) information is becoming available in plant and animal species, from extensive sequencing projects like EST or even genomic sequencing. Moreover, new sequence polymorphisms are more and more being found within some target genes by re-sequencing approaches, for instance for association genetics or phylogeny studies. It is very useful to be able to genotype such SNPs on more individuals without having to use sequencing. This is particularly true for instance for linkage or association mapping. The ideal genotyping method should be efficient with any SNP, irrespective of there being a restriction site or not containing the substitution. A lot of different techniques have already been proposed for this purpose. The simplest and cheapest of them is the allele-specific PCR, in which the 3' end of one of the primers is on the SNP, so that there will be presence or absence of amplification product depending of the allele at the SNP position. However, false positives are frequent, so that reliability remains a problem in many cases, and the method gives dominant markers. We have developed and optimized an alternative approach called Allele-Competitive PCR (AC-PCR) in which the competition effect between primers specific to each alternative allele greatly improves reliability of scoring as compared to allele-specific PCR. Codominant scoring also becomes possible with AC-PCR.

P197

Sequence analysis of the alpha-zein gene family in two maize inbred lines

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The maize prolamins, also known as zeins, represent a major source of essential amino acids for livestock as well as for humans. They are expressed exclusively during endosperm development. The prolamins are divided into two classes: the first one has high levels of sulfur-rich amino acids and proline, and is encoded by one to two gene copies, while the second one, called alpha-zeins, contains high levels of leucine and glutamine, and is encoded by a larger gene family. Therefore, the latter serves as a model for gene amplification and movement in the maize genome. The alpha-zeins include 80% of all zeins and are encoded by four subfamilies, z1A, z1B, z1C and z1D, in six different loci of the genome. To obtain molecular data from these loci, their DNA sequences were compared between two inbred lines, B73 and BSSS53. Haplotype variation at each locus indicates that chromosomal regions represent a combination of recombination events between different haplotypes. Further divergences by transposition, sequence amplification, and gene insertion events arose either before or in addition to these recombination events. Given these diverse sources of haplotype and sub-haplotype formations in all these unlinked loci, the sequence features found so far indicate that the maize genome consists of a set of conserved ancestral genes that serve as anchor points for combinatorial genetics.

P198

Sequence indexing and mapping of germinal Mu insertions in UniformMu maize using massively parallel DNA sequencing technology

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Resources for comprehensive insertional mutagenesis based on insertion sites that are mapped in the genome and searchable by DNA sequence (i.e. indexed by sequence) are an extraordinarily powerful tool for functional genomics. Toward this end we have developed large inbred transposon populations that provide comprehensive coverage of the maize genome. We estimate that the UniformMu inbred transposon population (40000 M2 lines) contains at least 400,000 independent germinal Mu insertions providing 3-4X coverage of the maize genome. To facilitate molecular analysis of insertion sites, we have developed an optimized MuTAIL PCR protocol for efficient amplification and sequencing of Mu flanking sequences. Heretofore we have focused on analysis of specific lines carrying seed mutants. We have shown that using high throughput sequence data to identify allelic insertions is a powerful method for forward genetics applications. For reverse genetics applications, however, developing a comprehensive sequence index of the insertions in the full population using conventional high throughput sequencing technology is cost prohibitive. The massively parallel sequencing technology recently developed by the 454 Corp promises to revolutionize DNA sequencing especially for applications that can utilize large numbers of relatively short (110 nt) sequence reads. We demonstrate a highly efficient protocol (TIR Direct) for sequencing Mu insertion sites that takes specific advantage of the scale afforded by the massively parallel sequencing platform recently developed by the 454 Corp. Custom multiplexed sequencing adaptors enable pooling of multiple sub-libraries in a single sequencing library allowing accurate de-convolution sequences from pooled samples to individuals. All sequences are precisely anchored with respect to the TIR end to maximize efficiency and yield of flanking sequences. We have used this protocol to map Mu insertions in two pools comprised of 96 independent *empty pericarp* (*ep*) and 96 *embryo specific* (*emb*) mutants, respectively. The data enable large scale comparisons of two mutant classes. In silico detection of allelic insertions in the dataset further enhanced identification of candidate genes. We successfully mapped < 2000 independent Mu insertions by BLASTN analysis of the MAGI 4 genome assembly. These data indicate that the 454 instrument will reduce the cost of sequence indexing and mapping of Mu insertions by at least an order of magnitude making comprehensive coverage of the genome a realistic near-term goal.

P199

Signaling plant DNA repair: An NBS1 homolog in Arabidopsis

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Double stranded breaks (DSBs) in DNA due to trauma or the natural processes of replication present a threat to genomic stability; however, DSBs are also a normal part of meiotic recombination. The MRN complex, composed of the Mre11, Rad50 and Nbs1 proteins, is of significant interest because it is involved in processing both mitotic and meiotic DSBs and is implicated in much of the signaling associated with repair of DNA breaks. Two moieties of this complex, Rad50, and Mre11 have been described previously in plants. By data mining for sequence homology to the important, functional protein domains of Nbs1, we have recently identified strong candidates for Nbs1 homologs in plants, including maize and Arabidopsis. In striking contrast to animal systems, null alleles of Nbs1 in Arabidopsis are viable and fertile. Nbs1 deficiency in Arabidopsis leads to hypersensitivity to the DNA damaging agent MMS, confirming a role for this gene in DNA repair.

P200

The MAGI Web Site: a Resource for Maize Genome Assembly, Annotation and Mapping

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~3,100,000 maize genomic sequences primarily composed of gene-enriched GSSs, random Whole Genome Shotgun (WGS) sequences, and BAC shotgun reads were assembled into MAGIs (Maize Assembled Genomic Islands, Emrich et al., 2004). Similarly ~550,000 methyl filtered (MF) sequence reads from Sorghum bicolor (BTx623) were assembled into SAMIs (Sorghum Assembled genoMic Islands). A web-resource is available at http://magi.plantgenomics.iastate.edu/ to access these assemblies. To identify genomic contigs associated with particular genes, MAGIs and SAMIs may be searched using the Blast tool. GBrowse, a component of GMOD, is used to display annotated assemblies. Segregation data in the IBM RIs have been generated for ~5,000 MAGIs and ESTs. A new genetic map based on these data and generated using MultiMap, including linkages to AGI's physical map , can be viewed via CMap. Detailed annotation regarding all ISU genetic markers is available on the MAGI website. Members of the community can request that specific MAGIs be genetically mapped. The MAGI website therefore serves as a community resource for map-based cloning projects as well as for analyses of genome structure and comparative genomics.

P201

Transcript Profiling of the Illinois Protein Strains and Derived Germplasm

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The Illinois Protein Strains are the result of the longest continuous genetic selection experiment in higher plants. Initiated in 1896, 105 cycles of divergent recurrent selection for grain protein concentration have produced populations with the known phenotypic extremes in maize for grain protein concentration and a number of correlated traits. To gain insights into gene expression responses to long-term selection, we have conducted mRNA expression profiling of the Illinois Protein Strains, using Ceres' proprietary long-oligo microarray. Microarray comparisons of developing seeds from IHP- and ILP-derived inbreds, as well as the reference inbred line B73, reveal significant expression differences in many genes, with the seed storage protein genes exhibiting the most dramatic changes. Another study compared transcript profiles of seed tissues from lines with high or low starch concentration, which were derived from a backcross population of (ILP x B73) x B73. This experiment identified consistent gene expression differences among lines, across developmental stages, and between two different evaluation years. Continued characterization of mRNA expression profiles within these unique genetic resources will provide additional insights into how transcriptional regulatory networks respond to breeding and selection.

P202

Transcriptional analysis of the differentiated ovule and female gametophyte of Arabidopsis thaliana by Massively Parallel Signature Sequencing (MPSS)

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In flowering plants the female gametophyte develops inside the ovule within the pistil. In Arabidopsis thaliana, the female gametophyte is composed of 7 cells: 2 synergids, the egg cell, three antipodals, and a binucleated central cell whose nuclei fuse before fertilization. We have developed a micro-aspirator that allows the massive isolation of ovules of Arabidopsis for total RNA extraction. Ovules are collected from dissected gynoecia without tissue contamination from the placenta or the carpel wall. Using this technology we can obtain 1.5 micrograms of total RNA in 2 hours of work (approx. 40 micro-aspirated gynoecia). We have isolated RNA from wild-type and mutant ovules of Arabidopsis and generated a large-scale collection of short mRNA-derived and non coding RNA(ncRNA)-derived MPSS tags. Our first 2 MPSS signature collections are derived from wild-type fully differentiated ovules containing cellularized female gametophytes prior to pollination, and fully differentiated homozygous sporocyteless/nozzle (spo/nzz) ovules that do not initiate female meiosis and in which the female gametophyte is absent. After determining the penetrance of the spo/nzz mutation, we developed a bioinformartic approach to identify all annotated genes that are candidates to be specifically expressed in the female gametophyte. We have used complementary microarray data generated by the group of V. Sundaresan (UC. Davis) to validate our results and assess the parameters necessary to identify a large portion of the female gametophyte transcriptome. We will present a summary of this analysis and a subsequent comparison to global expression profiles generated by microarrys in vegetative tissues.

P203

Transcriptome Analysis of the Low-Phosphorus Responses in Roots and Shoots of a Phosphorus-Efficient Zea mays Line Identifies Alterations of Several Metabolical and Physiological Processes

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In response to low-phosphorus (low-P), maize (Zea mays) develops biochemical and morphological processes to optimize and to increase the internal concentration of this element. This work identifies and details these adaptations through a comprehensive analysis of the changes in gene expression in response to P deprivation. In order to identify genes involved in phosphate uptake and utilization efficiency and to define the expression profiles of such responses, we constructed several subtracted cDNA libraries from P-deprived plants. Tissue was isolated from roots at day 1, 3, 6 and 10 after the beginning of the stress (ABS), and from shoots at day 3, 6 and 10 ABS. Such times match the onset of many biochemical and morphological changes. Resulting ESTs were assembled and functionally annotated, obtaining 633 unigene clusters. Differential expression was confirmed using a cDNA microarray with representative sequences of each unigene cluster. According to the results, we identified groups of genes that showed early, continuous or late differential expression. When the functional annotation of the genes identified was revised, we found that these genes reflect that under low-P conditions, modifications in several cellular processes are occurring. Furthermore, in order to discover more genes up or down regulated under low-P conditions, we used the maize oligonucleotide array (www.maizearray.org) to perform hybridizations with RNAs from different time points. We identified and defined with more detail the physiological adaptations and the transcriptional regulation of Zea mays roots under low-P stress. With these approaches, and, although the potential function of all unigenes is still under analysis, it is possible to conclude that Zea mays performs a wide range of responses, starting from hormone and transcriptional signaling events to metabolic adaptations which mainly include: 1. Stress related responses; 2. Carbohydrate mobilization, 3. Lipid degradation and exchange, and; 4. Enzyme production and transporting outward the rizosphere.

P204

Transcriptome Profiling of Pre-meiotic Cell Fate Using Three Male-sterile Mutants in Maize

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To study genetic regulation of cell fates during maize flowering, we employ a classical genetic approach. The mutants we study fail to achieve key cell identities that would normally occur before the maturation of the anther and the onset of meiosis. Currently our work focuses on three mutants: msca1, ms23, & mac1, in which the affected cells fail to achieve anther organ, tapetal, & tapetal/middle layer identities, respectively. By comparison to fertile siblings, transcriptome profiling of the mutants allows us to determine the transcriptional "signature" of cell types at various developmental stages, and more generally to begin to separate the transcriptome of the committed pre-meiotic cell lineage from the background of vegetative development. Transcriptome profiling results using Agilent 22K in situ synthesized arrays will be reported for 4 stages (spikelet and dissected 1 mm, 1.5 mm, or 2.0 mm anthers). In addition to the data generated, we are learning how best to classify male-sterile mutants in anticipation of studying many additional cases in which cell differentiation is abnormal. Other approaches to classifying anthers include proteomics and forward and reverse genetics analysis. RescueMu or other tags in genes of interest are identified through bioinformatics after the transcriptome and proteomics results are analyzed. Mu tagging populations were screened in summer 2005 for new alleles of mac1 and "putons" are being analyzed now. We plan to tag 2-3 additional key genes per year using this approach. The long-term study of anther development is a collaboration with the Cande lab, UC-Berkeley. DSD supported by an undergraduate grant from Stanford; research was supported by the NSF. Mutant stocks were supplied by W. Sheridan and P. Bedinger.

P205

Utilizing microarrays and mutants to dissect photosynthetic differentiation in the maize leaf

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In maize, C4 photosynthetic activities are partitioned between two morphologically and physiologically distinct cell types, the bundle sheath (B) and the mesophyll (M). Carbon is initially fixed as a C4 sugar in the M cells and then shuttled to the B where it is decarboxylated and fixed into C3 sugars in the Calvin cycle. This metabolic cooperation is dependent on an, as yet, undefined communication network between these two cell types. To dissect photosynthetic differentiation in maize, we have utilized oligonucleotide microarrays to examine gene expression in the B and M cells. To isolate M cells we performed an enzymatic digestion of leaf tissues and to isolate B cells a mechanical separation method was employed. We developed an ANOVA based model to allow statistical elimination of stress effects caused by the isolation of M cells. We are now using microarray analysis to define gene networks that are perturbed in the B- and M- cell-specific mutants, bsd2 and hcf136, respectively.

P206

Verification of Domestication and Crop Improvement Candidate Genes in Maize by Extended Sequencing

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In maize (Zea mays ssp. mays), both domestication and crop improvement have involved selection of specific alleles at genes controlling key morphological and agronomic traits, resulting in reduced genetic diversity relative to unselected genes. We have conducted genomic screens for artificial selection to identify candidate agronomic genes reflecting their roles in domestication and plant breeding (Wright et al. 2005 Science 308:1310; Yamasaki et al. 2005 Plant Cell 17:2859). One limitation to our approach is that the short length of the alignment (average length: 2,000 bp) demonstrated that the false-positive rate appears to be low in our initial short sequence screening. The selected genes have functions consistent with agronomic selection for growth regulation, nutritional quality and maturity.

P207

Wide-scale survey of transcriptional heterosis in F1 maize immature ear

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The genetic and molecular mechanisms underlying heterosis are still unclear. Recent data suggest that regulation of gene expression might play an important role in determining hybrid vigor. As a contribution to uncover regulatory mechanisms possibly causing or being influenced by heterosis, here we present data on the transcription profiling in immature ears between inbred lines B73 and H99, and their F1 heterotic hybrid using cDNA microarray technology and real-time RT-PCR. Relative expression of 4,905 ESTs represented in triplicate on each slide, corresponding to about 1,900 maize genes, was investigated simultaneously by five replicated hybridizations per inbred-hybrid contrast. Relative variation of gene expression generally did not exceeded a 1.5-fold value. However, by means of robust statistical approaches applied to data quality check and significance analysis, we were able to identify genes expressed at a significantly different level between both inbred lines and their hybrid. Validation by real-time RT-PCR largely confirmed microarray data from both contrasts. Both up and down regulated genes were found, B73 vs. F1 comparison showing a higher number of differentially expressed genes than H99 vs. F1. Regulatory hierarchies of expression levels were also estimated, allowing the establishment of a conceptual bridge between molecular regulation events and quantitative genetics concepts generally employed for illustrating heterosis. In fact, both dominance and over-dominance components were found involved in non-additive gene expression variation in the studied ear developmental stage, encompassing a wide variety of biological processes. We discuss the possibility that heterotic phenomena observed at the transcriptional level might reflect the general mechanisms of hybrid vigor establishment in maize.

P208

Widespread Maize Natural Antisense Transcripts are Frequently Located in UTR Repeat Regions and Conserved in other cereal Transcriptom using Strand-specific Microarray Hybridized with two maize Inbreds

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By virtue of their ability to form double-stranded RNA duplexes, Natural Antisense Transcripts (NATs) can regulate gene expression patterns. To investigate NATs in maize transcriptome labeled cDNA samples from two inbred lines (B73 and Mo17) were hybridized with a strand-specific oligonucleotide microarray containing 736 oligo pairs for candidate NATs identified from public EST databases and 3951 oligo pairs derived from various sources for survey. More than 3400 oligo pairs yielded detectable signals, indicating that NATs are prevalent in the maize transcriptome. The first large scale analysis about NATs in randomly chosen UTR and selected UTR repeat regions were performed and the results showed NATs were dramatically widespread in UTR and UTR repeat regions. In the category of rice NAT homologs, 683 out of 898 rice NATs homologs showed expression in maize transcriptom, which suggested the conservation for many NATs between rice and maize transcriptom. In addition, 48 NATs from potential pseudogenes were firstly identified via large scale analysis in transcriptom. Two new methods to identify potential NATs were created and validated in this study. More importantly, more than 3% NATs were expressed in only one of the two inbreds and the interaction between transcription orientation and genotypes showed significance statistically, which could be one potential alternative hypothesis for heterosis molecular mechanism.

P209

Aflatoxin Levels Survey among Diverse Maize Germplasm

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Aflatoxin accumulation in maize presents a risk to mammalian health and is a source of considerable economic loss to producers. *A. flavus* is a soil-borne fungus that produces aflatoxin. Water stress, high temperature, and insect damage can all contribute to increased aflatoxin accumulation. Once grain is found to be infected with aflatoxin few decontamination alternatives are available. The FDA legislates the allowable toxin level to be 20 parts per billion (ppb) in grain for human consumption and 0.5 ppb in milk products. The optimum strategy to aflatoxin accumulation prevention is the development of aflatoxin resistant inbreds. The objective of this experiment was to conduct a survey of aflatoxin levels in maize germplasm lines. Mp313E, Va35, Tex6, Ab24E, Mp420, Sc212M, Mo18W, Lo1016, Lo964, Os420, B97, Polj17, F2, FR697, B73, and Mo17 were chosen based on previous knowledge about aflatoxin accumulation or stress response. A subset of 86 lines from the Maize Diversity Project was also examined for aflatoxin accumulation. Two replications of each line were grown over two years. Inoculation of *A. flavus* NRRL 3357 by the nonwounding silk channel technique was performed 19 days after pollination. Ears were harvested at maturity, shelled, bulked, and ground for aflatoxin analysis. Aflatoxin analysis was done using a competitive binding ELISA. With the completion of this germplasm survey we hope to identify highly resistant and susceptible lines and set the stage for associative analysis of candidate genes for aflatoxin reduction in maize.

P210

Association Analysis of Candidate Genes for Aluminum Tolerance in Maize

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Aluminum (Al) toxicity is a major constraint to maize productivity on acidic soils throughout the world. Al becomes soluble at low pH, inhibiting root growth and severely reducing yields. While Al toxicity is a worldwide problem, it is a major limitation to production in tropical developing countries where maize is a staple crop and soil amelioration is not an economically feasible option. Breeding for tolerance would provide an inexpensive, sustainable solution to the problem. Maize expresses considerable genetic variation for tolerance to Al toxic soils, which can be useful in determining genes important to Al tolerance. We are using association analysis to determine significant correlations between genetic and phenotypic variation. These analyses were done using a panel of 288 lines that represent much of the diversity in maize. Al tolerance was estimated as seedling root growth over five reps in an Al-containing hydroponics solution. Root growth in the assay varied over seven fold between lines. Candidate genes were selected using results from microarray experiments and comparative genomic analysis of maize genes similar to known Al tolerance genes in other grass species. Twelve candidate genes were analyzed in the association panel. Several significant associations were identified, with root growth differences of up to 20% between alleles. This work is supported by NSF Plant Genome Award DBI #0419435 (PI: Kochian).

P211

Characteristics of Hexaploid Maize Derived from Inbred Oh43

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An inbred ploidy series (e.g., 1N-6N) is useful for molecular analysis of gene dosage effects. Maize inbred monoploid to tetraploid series are now available for several inbred lines. To generate inbred hexaploid, tetraploid Oh43 ears were pollinated using pollen from tetraploid Oh43 tassels that had been treated with trifluralin. The anti-microtubule herbicide trifluralin can cause failure of the second pollen mitosis. Hence, tetraploid pollen could be produced from the treated tetraploid plants. Screening ~1300 progeny from crosses described above via chromosome counting of root tip cells revealed five plants at the hexaploid level. One is a putative euploid while others were aneuploids with 61 or 62 chromosomes. Surprisingly, an octoploid plant was also identified. The hexaploid plants are morphologically distinct from the diploid and tetraploid Oh43 plants. The plant stature and growth rate decrease as the ploidy level increases, such that the flowering times are more delayed in plants of higher ploidy. The pollen and stomata sizes increase with the ploidy levels. Tassels of the hexaploid plants are much less branched than those of the diploid and tetraploid plants but the main spike is larger. Pollen shedding of the hexaploid tassels is normal and the pollen is of normal shape. Development of the ears is delayed in hexaploid plants. Some hexaploid plants produced silks but not all. The male and female fertility of the hexaploid plants is being investigated.

P212

Colicinogenic Maize: Inhibition of Pathogenic E. coli O157:H7

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Escherichia coli O157:H7 is a highly virulent pathogenic bacterium that naturally resides asymptomatically in the digestive tract of cattle. Contamination of foods and water via manure has resulted in multiple food-borne outbreaks. Pre-slaughter control strategies using probiotic E. coli strains capable of inhibiting pathogenic E. coli have previously been explored but their success is highly dependent on the colicinogenic bacteria's ability to colonize and produce the colicin in the gastrointestinal tract. A novel approach to circumvent these limitations is being investigated. A transgenic maize line that produces colicin (E7) was developed, the seed of which will be tested as a feed. Two constructs were designed and used for biolistic bombardment into HiII maize callus tissue. The first construct included the colicin E7 (1763 bp) gene driven by a constitutively expressing promoter (CaMV35), whereas the second construct included only the immunity E7 (263 bp) gene driven by the same promoter. The immunity E7 protein binds the colicin E7 protein (a DNase) to prevent nonspecific activity, thereby when the immunity E7 is expressed in conjunction with the colicin E7, higher levels of expression are expected. HiII callus was transformed and plants were regenerated. All plants were analyzed for transgene insertion (PCR analysis) and copy number (Southern Blotting). RT-PCR was performed to determine if more plants expressed the colicin E7 gene when it was present with the immunity E7 gene.

P213

Detection of Donor QTL Alleles in Illinois Low Protein for Enhanced Starch Levels and Ethanol Production Efficiency

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There is interest in modifying levels of protein, oil and starch on maize grain for specific end uses. An example is breeding for higher levels of starch to enhance starch fermentation efficiency to produce ethanol. QTL associated with kernel composition traits would facilitate the development of maize inbreds and hybrids with desirable levels of components enabling a better and more efficient conversion to secondary products. Protein, starch, oil, and kernel weight were measured on grain samples harvested from two replications of a backcross population in five environments (1993, 1994, 2003, 2004 and 2005). By evaluating in a (ILP/B73)B73 background we are assessing for donor alleles that would improve inbreds and hybrids for increased starch. A molecular map with 144 markers was used to detect QTL associated with kernel weight, protein, oil and starch concentration. Using composite Interval mapping, ten QTL were identified for starch in bins 1.03, 1.05, 1.07, 1.11, 2.08, 3.06, 5.04, 6.01, 6.05 and 9.03; eleven QTL were identified for protein in bins 1.03, 1.05, 1.07, 1.11, 2.03, 2.08, 4.08, 6.01, 6.05, 8.02 and 8.05; six QTL were identified for oil in bins 1.05, 1.11, 2.04, 5.04, 6.00 and 9.04; and eleven QTL were identified for kernel weight in bins 1.05, 1.06, 1.08, 2.04, 3.03, 3.04, 3.06, 4.09, 5.07 and 7.03. The QTL explained 38.3%, 40.2%, 34.2%, and 40.4% of the total phenotypic variation for starch, protein, oil, and kernel weight, respectively. Most alleles for higher starch came from ILP which has higher starch concentration than B73. Most QTL alleles for higher protein came from B73. Five of ten QTL associated with starch concentration were also significant for protein. Two QTL identified for starch were also significantly associated with oil concentration. QTL do not appear to map to starch structural biosynthetic genes, suggesting unknown regulatory loci may influence quantitative variation for starch concentration.

P214

Detection of QTL Alleles for Pericarp Thickness and Ear Inflorescence Architecture in South Korean Germplasm

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Consumption of fresh waxy corn is widespread among Asian people for its tenderness, sweetness, and stickiness. Due to an increasing Asian-American population and market, genetic research and breeding on waxy corn with the goal of providing for U.S. fresh consumption is needed. Quantitative Trait Loci(QTL) for mapping taste quality characteristic from Asian varieties followed by Marker Assisted Selection(MAS) into more adapted U.S. backgrounds would enhance breeding success. Objectives of this study were to detect QTL for: pericarp thickness which affects tenderness; and ear inflorescence architecture traits relevant to consumer preference and yield. The analyses were conducted on five pericarp thickness and nine ear inflorescence architecture traits measured on 264 F3 families from cross between BH20 with very thin pericarp thickness (40.53 +/- 2.96 microm) and BH30 with moderate thickness (66.89 +/- 5.28 microm), both waxy corn inbreds from South Korea. 100 SSR markers were mapped on the population. A total of 41 individual QTL were detected on pericarp thickness traits, which are different portions of the kernel. QTL for different pericarp thickness traits explained phenotypic variation ranging from 31.7 to 42.3%. Most of the alleles for thin pericarp thickness traits came from the BH30 parent. Principal Component Analysis (PCA) showed the first principal component (PC) explained a major portion (87.60%) of pericarp trait variation. Eight PC-QTL were detected in bins 1.07, 1.10, 2.06, 3.00, 4.03, 4.08, 6.05, and 9.03. A total of 42 individual QTL were detected on ear inflorescence architecture traits. QTL for ear inflorescence architecture traits explained the phenotypic variation ranging from 8.7 to 32.8%. Four PCs were identified and twenty PC-QTL were detected. Notably, QTL regions significant for two or more individual ear architecture traits and a PC-QTL were detected in bins 1.08(id1), 3.04(ts4, lg3), 4.05(fea2), 7.02(ra1) and 8.05(knox5) which have known inflorescence mutants and genes. This information will be used in further study and breeding efforts.

P215

Genes involved in cell identity and patterning play a role in brace root morphology.

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In many maize lines, the temporary seminal root system senesces and the permanent adventitious root system takes over the primary role of soil and nutrient acquisition and terrestrial anchorage. The prohibitive nature of studying mature root systems has led to a deficit in the understanding of their genetic basis. Brace roots constitute of a major part of the below ground portion of the permanent root system and are easily observed in the field. Our goal was to reveal the genetic nature of brace root architecture by performing quantitative trait loci (QTL) analysis using the high resolution Intermated B73 x Mo17 (IBM) mapping population. During the summers of 2004 and 2005, a subset of 94 IBM mapping lines were grown in two replications at the south farm location in Columbia, Missouri. Each line was evaluated for number of nodes with brace roots, the total number of brace roots at each node, root angle, total number of nodes, and plant height. A genetic linkage map was constructed using Mapmaker Experimental 3.0 with 643 markers evenly spaced throughout the genome at approximately 10 cM apart. Composite interval mapping was performed using QTL cartographer 1.16 for Unix. The number, location and effect of QTL differed between years. QTL for brace root traits were located in proximity to QTL for vertical root pulling force identified in other populations, as well as QTL for temporary root traits in the IBM mapping population. Several candidate genes were identified using the IBM neighbors map. Among the candidates, comparison of mutant and wild-type siblings supports *barrenstalk2* and *ramosa1* as candidates affecting root angle and *dwarf10* and *hairy-sheathed-frayed1* as candidates for the number of brace roots per node. Association analysis is currently under way for *barren stalk2*.

P216

Genetic Analysis of Ear Shoot Development in Maize

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The number of ears per plant (prolificacy) and the stability of ear shoot production have been targets of selection during maize domestication. At least 24 QTL have been associated with prolificacy in maize. Also, several loci identified by alleles with mutant phenotypes have been cloned or characterized. Ear shoot development is being investigated in 2 populations. The first population was created by crossing C103 with an isogenic line, C103AP. C103 is single-eared inbred that is prone to abort its ear shoots under stressful environmental conditions. C103AP, a prolific inbred, was produced by four generations of backcrossing C103 to a highly prolific popcorn population (AP) as the non-recurrent parent. C103 was crossed to C103AP and a mapping population of 218 recombinant inbred lines (RILs) was developed. The objective of this project is to detect QTLs for prolificacy and ear shoot abortion. The second population was created by crossing inbred B104 and 95:2, a prolific popcorn inbred line. The F2 and F3 generations of that population have been evaluated in several environments to detect QTL with consistent effects.

P217

Genetic Approaches to Enhance Provitamins A and Total Carotenoids in Maize Grain

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Micronutrient deficiencies affect millions of people worldwide. HarvestPlus (www.harvestplus.org), a CGIAR Global Challenge Program, aims to increase micronutrient content in staple crops through bio-fortification. Our goal is to perform genetic research to facilitate breeding to increase provitamin A and carotenoid concentrations in maize (http://cropsci.uiuc.edu/faculty/rocheford/). 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). Vitamin A deficiency is estimated to affect 25% of preschool-aged children worldwide, with as many as 33% in Southeast Asia & Africa affected (HarvestPlus). American diets are sometimes deficient in xanthophylls, which can lead to macular degeneration. To assess variation in carotenoid content and identify breeding materials, the Buckler/ Goodman diversity lines were surveyed using HPLC. Mapping populations of W64a x A632 and IHO x B73 were used to identify QTL that affect provitamin A and total carotenoid concentrations, which will be useful in marker-assisted selection. Synthetic populations are being used to quantify effectiveness of visual selection for total carotenoids. Near-isogenic populations for a mutation at phytoene synthase (y1) were developed for nutritional studies. Considerable variation was found within diversity lines, values ranging from 0.0-8.1ug/g, 0.02-1.2ug/g, 0.0-6.7ug/g, 0.02-19.4ug/g, 0.01-26.3ug/g, and 0.05-39.5ug/g for beta-carotene, alpha-carotene, beta-cryptoxanthin, lutein, zeaxanthin, and total carotenoids, respectively. CI7 and DEexp are the best lines, showing 10-14ug/g beta-carotene in some environments, and show transgressive segregation in F2:3 families, with values of over 15ug/g beta-carotene. QTL mapping identified two regions in two studies affecting carotenoids on chromosomes 6 and 8, with candidate genes y1 and epsilon cyclase, and supported through significant association analysis tests. Selection for total carotenoids has produced materials in the 45-65ug/g range, pertinent to our strategy to visually select for orange color; use favorable alleles of phytoene synthase and epsilon cyclase; and use QTL to be identified from SC55, an inbred which slows flux at beta-carotene, to breed higher levels of provitamins A.

P218

Genetic Diversity for Nitrogen Use Efficiency in Maize

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With supplemental nitrogen fertilizers applied to nearly every acre of maize grown, environmental issues are raised & the agriculturalist's economic viability is impacted. Improving the nitrogen use efficiency (NUE) of maize would not only benefit the producers by reducing their fertilizer input costs, but would also benefit the environment by reducing excess nitrogen run-off. To gain an understanding of the range of physiological strategies & their genetic control, we have characterized NUE & its component traits in diverse maize germplasm. The core set of 25 "Diversity" inbred lines, selected to represent much of the allelic variation present in maize, was crossed to B73 and the resulting hybrids were evaluated over two years at different N rates in an N-response nursery. There is considerable genetic variation within these hybrids for NUE & N-responsive traits, including biomass and grain yield, plant N accumulation, N remobilization during grain filling, & grain composition. NUE phenotypes were consistent in both years for a given genotype, and hybrids were identified that have contrasting phenotypes at low N and show either strong or weak yield responses to N. We find strong correlations among some traits, such as grain yield & kernel number (r= +0.86), N uptake & NUE (r= +0.61), and grain starch & protein concentration (r = -0.97). The relationships among these traits suggest distinct genetic pathways controlling maize N responses and possible regulatory mechanisms for NUE. To further investigate these mechanisms, the results of a microarray study comparing nitrogen responses in the B73 x Mo17 hybrid will also be presented.

P219

Genetic Diversity of Maize Primary Root Growth and Abscisic Acid Content to Water Stress.

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Drought is the most limiting factor to crop production worldwide. Water deficit conditions cause a decrease in plant productivity reducing plant size and potential yield. Maintenance of root elongation is an important adaptive response to drought conditions. In addition, abscisic acid (ABA) accumulation is required for root growth maintenance under water deficits. The objectives of this study were to: 1) evaluate the extent of genetic variation for root growth rate under water stress in maize; 2) assess the degree to which the material used in this study represents the breadth of genetic diversity in maize; 3) determine whether ABA levels were correlated with root growth rates. Twelve maize inbred lines were evaluated. Primary root growth rate was determined under three different water regimes: -0.03 (well-watered), -0.3 (mild stress), and -1.6 (severe stress) MPa. Primary root length was measured at three time points over 24 hours (well-watered) or 72 hours (water-stressed). Statistical analysis of average rate and the root growth rates as a percentage of well-watered revealed that P2 maintains root growth best under water stress. Conversely, B97 was the most affected by water stress. Eighty-seven simple sequence repeat (SSR) markers were used to genotype the twelve lines. These data were analyzed with data from 260 maize lines from Liu et al (2003) to assess the extent to which these twelve lines span the diversity in maize. The resulting dendrogram indicates that the twelve lines represent the US, European, and some semi-tropical material; however, the most diverse tropical germplasm is not represented in this material suggesting that additional genetic diversity in root growth rates is available in maize. Significant differences were observed among lines within treatments for root ABA levels, however, there was no significant correlation between root growth rate and ABA concentration. This suggests that ABA-independent pathways may also play a role in root growth maintenance in maize and that other hormones, such as auxin and ethylene, may determine growth rate.
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P220

Genetic Mapping And Analysis Of Panicle Morphology In Maize

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The number of tassel branches is an important determinant of tassel size in maize. The objective of this study was to identify regions in the maize genome which affect tassel branch number in a maize population developed by crossing inbreds derived from the Iowa Long-ear and Short-ear cycle 24 sub-populations. The inbreds, LE-37 (long ear) and SE-40 (short ear), have approximately 2 and 11 tassel branches, respectively. F2 plants were genotyped at 179 DNA marker loci. TASSEL branch number data were collected on F2 plants, and on their F3 lines in replicated trials in four environments. QTL were mapped in both F2 and F3 generations, using composite interval mapping (CIM). Multiple interval mapping (MIM) was also used to map QTL and to assess epistasis among QTL. Four QTL explaining 23% of the phenotypic variance were detected in the F2 generation . In the F3 generation, 10 QTL accounting for 70% of the phenotypic variance in the combined analysis were detected, seven of which were detected in all four environments. Three of the QTL were consistently detected in the F2 and F3 generations. The MIM model with epistasis accounted for 89% of the phenotypic variation in thecombined analysis. MIM generally detected the same QTL as CIM, but with some changes in position and effect. QTL x environment interactions were significant (P

P221

Heterotic segments transferred to a maize flint line by a balanced lethal system with high grain yield.

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In order to increase the efficiency in the production of maize hybrid seeds it is necessary to reach high grain yield with a relative low cost of production. It needs an expansion of the basic knowledge of the heredity of the characters that may be develop new breeding tool on experimental materials with hard endosperm. The balanced lethal systems allow the study of the relative contribution of different heterotic segments to hybrid vigor due to the heterocigozity of certains chromosomic segments, while the rest of the genome tend to homocigozity for continuos selfing. Furthermore, it allow the commercial use of these lines due to the fixation of the heterotic segment. In this way, inbred lines of the seed companies with a good combinatory ability, but low grain yield or pollen production, impossible to use at the commercial level production, may be improvement. In this way, a balanced lethal system was transferred to a corn flint line proceeded of the single commercial hybrid by backcrosses. The grain yield of the backcrosses was higher than the same commercial inbred lines per-se.

P222

Identification and Analysis of Quantitative Trait Loci for Southern Leaf Blight and Gray Leaf Spot Resistance.

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Quantitative resistance is the dominant form of resistance utilized in cultivated maize. However, very little is known about the molecular-genetic or phenotypic basis of quantitative resistance in maize or any other crop. Our work has two major aims; working with the foliar diseases Gray Leaf Spot and Southern Leaf Blight, we are identifying and mapping new sources of resistance from diverse germplasm, and we are developing materials and methods for the detailed characterization and fine-mapping of selected QTLs. We will present the following aspects of our work: a summary of our work mapping disease QTL in 10 different segregating populations, including fine mapping using the IBM population; results from a meta-analysis of published maize disease QTL papers; an update on the production and analyses of near-isogenic lines differing for disease resistance phenotypes; and an evaluation of the disease resistance phenotypes of 300 diverse lines taking into account interactions with flowering time.

P223

Identification of Genomic Regions Affecting Quantitative Resistance to Common Rust (Puccinia sorghi Schw.) and Vegetative Phase Change in a Sweet Corn (Zea mays L.) Population

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Previous studies conducted by the UW-Madison sweet corn breeding program have described a relationship between vegetative phase change and quantitative resistance to common rust in at least one genetic background. The objectives of this study were to i) evaluate each of the 130 S3 families and the parents of population 21B x 11B for quantitative resistance to common rust and vegetative phase change traits; ii) determine if there is a relationship between quantitative resistance to common rust and vegetative phase change traits in population 21B x 11B; iii) identify QTL (quantitative trait loci) that are associated with quantitative resistance to common rust, vegetative phase change traits, or both. There were highly significant genotypic effects for percentage common rust and all vegetative phase change traits. Percentage common rust was positively but weakly correlated with vegetative phase change traits; first leaf with adult wax (r=0.21), last leaf with juvenile wax (r=0.25), and transition zone (r=0.27). In this study, no QTL were associated with percentage common rust in population 21B x 11B. A single QTL, associated with marker bnlg127, was significantly associated with each of the vegetative phase change traits evaluated in this population. This QTL mapped to the long arm of LG9, to a similar position as Gl15, a locus known to have a role in vegetative phase change. It is possible that Gl15 is the gene associated with this QTL, and differential genetic background effects on epidermal characters are to a great extent mediated by allelic differences at the Gl15 locus.

P224

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

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Nitrogen (N) is often a yield-limiting nutrient in the production of maize. The identification of genes involved in nitrogen use efficiency (NUE) and the use of such genes to develop hybrids with greater NUE could have both economic and environmental benefits. The objective of this study was to identify QTL controlling NUE and its component traits. The mapping population used was a set of hybrids developed by crossing lines from the IBMRI population to the Illinois High Protein (IHP1) inbred. This population takes advantage of the high mapping resolution of the IBMRIs and the high N uptake phenotype of IHP1. The results presented here are from the first year of a field study completed in 2005. A core set of 92 IBMRI*IHP1 hybrids were grown at three N rates (0, 84, and 252 kg/ha) and scored for NUE and its components and grain quality traits, while a set of 260 IBMRI*IHP1 hybrids were grown at a single N rate and used to evaluate grain quality traits and flowering time. In this preliminary analysis, QTL were detected for all but two of the traits studied. QTL differences were observed between high N and low N, suggesting G x N interactions.

P225

Linkage Disequilibrium in the IBM and IBM Syn 10 Populations of Maize

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The public IBM and IBM Syn 10 populations are the result of the cross between inbred lines B73 and Mo17 followed by 4 and 10 generations of random intermating. The IBM RILs were produced by continuous generations of self-pollination, while the IBM-10 RILs were produced from doubled haploids. The populations are being used to refine assessments of physical and genetic mapping and to test methods that connect genotypic and phenotypic information for several traits. The IBM-10 RILs are under development as another resource for the public domain. Linkage disequilibrium (LD) is present in a population when non-random association of alleles occurs at pairs of loci. Because LD is reduced by recombination, and recombination occurs at each meiosis, the level of LD should decrease with each generation of random mating. We hypothesized that IBM-10 would have a lower level of LD than IBM due to these extra generations of intermating. IBM (n=283, where n is the number of lines in the population) and IBM-10 (n=360) were screened with 2140 SNP loci. Estimates of two-locus LD (D, D', and r2) were calculated for each pair of loci, and comparisons between the two populations were made. LD was lower in IBM-10 than in IBM at the whole genome and individual chromosome levels. Patterns of LD across chromosomes are discussed, and genetic maps and allele frequencies are presented.

P226

Mapping Quantitative Trait Loci for Southern Leaf Blight Resistance in a B73 x NC250 F2:3 Mapping Population and Determination of Introgressions in Sister Lines NC292 and NC330

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Southern Leaf Blight (SLB) is an important pathogen of maize grown in warm humid regions, causing yield losses of 20-30% on occasion. The commonly used stiff stalk line B73 is susceptible to SLB, while NC250, a line derived from tropical germplasm, shows extremely high resistance. Quantitative trait loci (QTL) controlling resistance to SLB have been identified on chromosomes 1, 3, 5, 6, and 9 in a B73 x NC250 F2:3 mapping population. Two SLB-resistant B73 near isogenic lines were derived from a B73 x NC250 cross, followed by successive backcrosses to B73. In each generation the most SLB-resistant plants were selected. The resulting sister-lines, NC292 (BC3) and NC330 (BC4), are B73-like agronomically, but have retained most of the SLB resistance from NC250. Screening with molecular markers confirmed that these lines are >90% B73-like and has identified the regions introgressed from NC250. The introgressed regions correspond to regions where QTL were identified in the B73 x NC250 F2:3 population. We are developing tools for detailed characterization of each of the major NC250-derived SLB QTL.

P227

Physiological mechanisms underlying grain yield QTLs

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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 using the same mapping populations. 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. All of the genetic variation for grain yield in this population is due to physiological differences from silking through physiological maturity. One of the grain yield QTLs appears to influence dry matter accumulation during the grain filling period, another grain yield QTL appears to influence partitioning to the ear, and the third grain yield QTL only appears to influence total dry matter accumulation. 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.

P228

Potential Impact of the Ab10 Chromosome on Preservation of Maize Genetic Resources

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More than 18,000 maize accessions are maintained by the North Central Regional Plant Introduction Station (NCRPIS). To ensure preservation of the original genetic profile of populations, stocks are propagated using the following methods. A balanced sample from 100 ears provides the seed for planting. For populations, those plants are non-reciprocally sibbed such that each plant is used once as a male or female. Inbred lines are typically self-pollinated. The Abnormal Chromosome 10 (Ab10) causes segregation distortion during female meiosis, preferentially transmitting knobs and linked loci to the next generation. Its presence could impact efforts to preserve a population dynamics status quo. We propose to screen populations for the presence of Ab10. In those populations where Ab10 is discovered, we will determine the proportions of individuals (1) homozygous for the normal chromosome 10 (N10), (2) heterozygous (N10/Ab10), and (3) homozygous for Ab10. We also will determine the chromosome 10 constitutions of samples from prior (and potentially future) generations. Results from these analyses will permit us to determine, for Ab10 containing populations, whether the propagation methods used ensure preservation of genetic profile over time or allow the proportions of knobs and linked loci to increase.

P229

Presence of maize grain yield QTL x Environment interactions: dilemmas for plant breeders and geneticists

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Plant breeders constantly strive to select and develop superior genotypes that have stable performance across environments. This selection is primarily phenotypic, but sometimes can be genotypic utilizing molecular markers. Objective of this study were to identify grain yield QTLs in hybrid condition from modern day inbred lines, and determine the presence of QTL x Environment interactions. Due to environmental differences observed in 2004 and 2005 in terms of weather and also plant physiology traits (photosynthesis difference of 40% between years), QTLs were identified in separate years. Significant variation was observed among Recombinant Inbred Lines in each year. Due to a majority of genome being homogenous between the parental inbred lines (~70% Identity by Descent), minor effect grain yield QTLs were identified. In 2004, four markers were retained in multiple locus model (MLM) for grain yield, while in 2005, six markers were included in grain yield MLM. Two markers were retained in MLM in both years; however, the favorable parental allele at that these marker loci were reversed between the two years demonstrating QTL allele x environment interaction. This presents plant breeders with interesting and challenging prospects for phenotypic and marker assisted selection and also poses challenges to our efforts at University of Guelph to define underlying physiological mechanisms governing grain yield at a genetic level.

P230

QTL Analysis of Rice Tungro Disease

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Molecular mapping of the resistance to rice tungro spherical virus (RTSV) was conducted with the goal of facilitating the development of tungro resistant varieties through marker-assisted selection and genetic transformation. A near-isogenic line, TI-11, carrying the RTSV resistance from ARC11554 in TN1 background was found to have 5 regions of introgression based on PCR-based markers namely, in chromosomes 2L, 4S, 5L, 11L and 12L. Using QTX, single-marker regression analysis showed significant association of the RTSV resistance with two markers on chromosome 4 with likelihood ratio statistics (LRS) of 9.6 ($P < 0.05$) at marker 5600 (15.7 cM) and LRS of 9.1 ($P < 0.01$) at marker 6100 (17.2 cM), but not with markers on other chromosomes. The QTL on chromosome 4 was then localized using an additional eight evenly spaced markers on chromosome 4S. Interval mapping analysis using QTX showed a strong QTL (LRS=15.2) at RM8213 (10.7 cM). This QTL explains 10% of the variance with $P < 0.001$. Preliminary marker validation corroborates the presence of the resistance QTL in the region, as evidenced by the consistent introgression of the ARC11554 allele at RM8213 in three resistant lines derived from crossing with IR64 accompanied by phenotypic selection.

P231

QTL Data at MaizeGDB: Curation and "then some".

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Discovery of candidate genes for agronomically important traits, such as disease or pest resistance often begins with one or more quantitative trait locus (QTL) experiments. Each experiment approximates the chromosomal locations sites (QTLs) that contribute to expression of the trait. These loci may not be detected in other QTL experiments, due either to environmental factors, and/or a lack of allelic diversity for a region in other mapping panels. Before devoting effort to marker assisted selections, or to candidate gene cloning, it would be helpful have facile access to systematized information about all known QTLs for traits of interest, coupled to other information about germplasm, nearby loci and sequence information. In the mid 90's, MaizeDB began curating QTL information from the literature. To permit this work to continue at MaizeGDB, a new, Web accessible curation interface has been designed and implemented. The new design accommodates a legacy trait hierarchy developed at MaizeDB and recently harmonized with the rice Trait Ontology at Gramene, and trait descriptors used by GRIN (the Germplasm Resources Information Network). It incorporates new utilities to facilitate and to control the quality of data entry. The curation module will be accessible to any maize cooperator wishing to add a new experiment to MaizeGDB. In addition to describing the curation tool, we will show a consensus map for several traits represented in MaizeGDB. We will report on our collaboration with Susan McCouch and staff at CIMMYT and GrainGenes towards a common template for entry of bulk QTL data for rice, maize, *Triticeae* and oats.

P232

QTL mapping of resistance to Ustilago Maydis in sweet corn

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We mapped and characterized quantitative trait loci (QTL) for resistance to Ustilago maydis (common smut). A sweet corn population of 330 F5:6 families was generated by crossing a suceptible inbred to a resistant line. F5:6 families were genotyped with 180 SSR and 20 SNP markers. The lines were evaluated in field trials under natural infection in Stanton, MN, Columbia Basin, WS and Nampa, ID in 2001-2003. Resistance to U. maydis was highly heritable (h2= 0.75). Several QTL for U. Maydis resistance were detected by composite interval mapping. The number and position of the QTL varied depending on the location and year, indicating the presence of genetic by environment interactions. Three QTL were detected in all locations. Based on results marker assisted selection seem to be a suitable strategy for improving the resistance of U. maydis.

P233

Quantitative Trait Loci Mapping for Starch, Protein, and Oil Concentrations with High-oil Maize by SSR Markers

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The objectives of this investigation were to map QTL controlling oil, protein and starch concentrations and to evaluate their genetic effects in maize grain with an F2:3 population by SSR markers. 298 of F2:3 family lines were derived from a cross between a high-oil line, By804, selected from Beijing High Oil germplasm (BHO C13) and a normal inbred line, B73. F2 individuals were genotyped with 183 SSR markers to construct a genetic linkage map, which spanned a total of 1605.7 cM with an average interval of 8.77 cM. Totally, six, six, and five QTL associated with oil, protein, and starch concentration were mapped in eight chromosomes respectively by CIM. The proportion of phenotypic variation for three traits explained by single QTL ranged from 4.14 to 13.13%. The mapped QTL for oil concentrations accounted for 86.23% of phonotypical variations while QTL for protein and starch concentrations only explained 35.89% and 27.08% of phonotypical variations. Four out of six QTL for oil concentrations detected among F2:3 lines were as the same as those simultaneously detected in both F2 and F3 seeds, indicating these QTL may be more stable than the others. Five out of six mapped QTL for oil concentrations in this investigation using BHO seemed to have similar chromosome locations to those identified using IHO. These results demonstrated that genomic response for long term selection to increase oil concentration only occurred in some similar or identical chromosome regions. Although QTL detected in different populations were identical or similar, more favorable alleles were different from one to another. The most favorable allele was in oilc1-1 located on chromosome 1 in BHO while one QTL located on chromosome 8 was more favorable. These suggested that more diverse germplasm should be necessarily to discover the more favorable alleles for chemical compositions in maize traits.

P234

Rapid and reliable purity identification of maize (Zea may L.) F1 hybrids using SSR marker

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Using a rapid and low costly DNA extraction method, genomic DNA extracted from two maize F1 hybrids and their parental lines was subjected to SSR analysis with 10 primer pairs. Four of them could detect polymorphism between the male parents and the female parents of the two hybrids, which could be used for purity assessment of the hybrids. Moreover, the purity of seed samples of the two F1 hybrids was identified using the SSR analysis with the four primer pairs and isozyme analysis respectively. For one hybrid, the results obtained using the two methods are consistent, but for the other one, isozyme analysis failed to detect any polymorphism between the parental lines because they are closely related. These results clearly demonstrate that SSR marker should be useful for assessing purity of maize hybrid, even if the hybrid derived from two related parental lines.

P235

Recurrent Selection Mapping in Two Maize Populations Suggests that Some Common Loci are Associated with Northern Leaf Blight Selection

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Recurrent selection (RS) is commonly used for trait improvement in crop species. Using RS, the International Maize and Wheat Improvement Center achieved significant gains for resistance to northern leaf blight (NLB), a disease of global significance, in eight diverse sub-tropical maize populations. In one of the populations putative QTL for NLB resistance were previously identified by RS mapping from significant changes in allele frequencies between different cycles of selection. The loci identified are unlikely to be the genes under selection, but instead in linkage with them. The purpose of this study was to evaluate to what extent common loci or chromosomal segments were associated with RS in a separate, similarly selected, population. Over 60 simple sequence repeat (SSR) loci were chosen in chromosomal segments previously shown to be associated with RS. Of these, allele frequencies at ten loci exhibited significant deviations from drift in both populations. Analysis of the alleles at these loci showed that there were 60% shared and 40% private alleles between the populations. Of the shared alleles 10% significantly increased in frequency in both populations while 60% increased in only one population. The results presented here suggest that there are several common loci associated with NLB selection between these populations, but there is little correlation between the SSR allele(s) that increased in frequency.

P236

Study on waterlogging tolerance at Maize seedling stage

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Waterlogging, a serious environmental stress, affects agronomic performance of maize (Zea mays L.). In order to facilitate the breeding process, we conducted this study. 20 common maize lines were subjected to successive artificial flooding, waterlogging tolerance coefficient was used to screen flooding tolerant gene, and assess their sensitive stage and the best treatment time to waterlogging. In the meantime, peroxidase (POD) activities and malondialdehyde (MDA) contents were measured using 6 lines of 20 lines, which were screened by waterlogging tolerance coefficient and phenotype. The results indicated that the second leaf stage (V2) is the most sensitive stage in three leaf stage. The best treatment time is 6 days after flooding. POD activities and MDA contents are negatively and significantly correlated. According to the changes of leaves after flooding and waterlogging tolerance coefficients, we screened out three highly tolerant inbred lines and 3 highly susceptible inbred lines. HZ32 and Jiao51 have highest waterlogging tolerance coefficients, K12 and Tzi9 have lowest coefficients. The result of phenotype, waterlogging tolerance coefficient,MDA contents and POD activities are identical. HZ32(highly tolerant) and K12(highly susceptible) were used as parents to construct F2 population. Root length, plant height, and dry weight were observed in a 332 F2:3families. After correlation analysis, shoot DW and total DW showed the highest correlation coefficient with 0.9492. On the other hand, the trend of waterlogging tolerance coefficients of root length, plant height and dry weight showed continuous variation. It suggests that this morphological trait was controlled by multiple genes and it accorded with normal distribution. The results indicated that waterlogging tolerance coefficient of shoot DW is the most suitable index for waterlogging tolerance screening. This study could increase the efficiency of material screening using the MDA content and POD activities to assistant selection.

P237

Using sorghum to study quantitative variation in grass inflorescence architecture

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The grass inflorescence is the primary food source for humanity, and has been repeatedly shaped by human selection during the domestication of different cereal crops. Of all major cultivated cereals, sorghum shows the most striking variation in inflorescence architecture traits such as branch number and branch length. We are using sorghum as a model to explore developmental variation in the grass inflorescence, using a combination of linkage mapping, candidate gene mapping, and association analysis. QTL mapping in a sorghum RIL population yielded two candidates that co-localize with major QTL for relevant traits. Dw3, the sorghum ortholog of brachytic2, co-localized with QTL for plant height, rachis length, and branch length. Sb-ra2, the sorghum ortholog of ramosa2, co-localized with a QTL for primary branch number. This population is being used to create NIL lines for candidate genes and QTL regions of interest. Two additional mapping populations are being created, and a panel of 282 diverse sorghum lines has been assembled for the detection of phenotype-genotype associations. Preliminary screening of an EMS-mutagenized sorghum population yielded two mutant with floral organ phenotypes, and a more extensive screen in 2006 promises to yield new inflorescence mutants of utility to the grass community.

P238

TED, a new autonomous transposon of the Mutator superfamily

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bz-m175, a new mutable allele of the *bz* locus, was isolated in stocks derived from Rhoades and Dempsey's original Hi Loss x Hi-Knob stocks. Other unstable mutants belonging to different systems, such as *Mrh* (for *Mutable Rhoades*) and *Mut* (for *Mutator*, different from *MuDR*), had been previously isolated in this background by Rhoades and Dempsey themselves. Genetic crosses indicate that instability of the new *bz* mutant resides at the *bz* locus, i.e., that the transposable element in *bz-m175* is autonomous. Molecular characterization of the element indicates that it is a member of the *MuDR* superfamily of transposons, which we have named *TED* (for *Transposon Ellen Dempsey*). *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, which is highly homologous to MURA, the putative *MuDR* transposase. However, unlike *MuDR*, *TED* does not encode a B function. Both parental lines carry an element very similar, if not identical, in sequence to *TED*, so either one could have contributed the transposon in *bz-m175*. Sequences homologous to *TED*, though shorter, are found in most maize lines and probably correspond to nonautonomous members of the *TED* family.

P239

A set of maize transgenic lines with *Ds* transposons for localized mutagenesis 1

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We are developing a set of transgenic lines for localized transposon mutagenesis in maize. The lines carry *Ds* elements, which transpose preferentially to linked sites, and will be useful to scientists interested in saturating specific regions of the maize genome with insertions. We are using two types of *Ds** constructs, modified to facilitate the PCR isolation of transposon-adjacent sequences. In one type, the *Ds** excision reporter is *bz*and the *Ds** marker is *R*. This construct is being used with a highly embryogenic *sh bz wx; r* inbred line (SBW) that we recently developed for this project. In the other type, the *Ds** excision reporter is c and the *Ds** marker is *R*. This construct is being used with the standard HiII hybrid (*c; r* in genotype). Both of these constructs will enable users to select and map transpositions based simply on seed color. We have already demonstrated that engineered elements can transpose germinally and that adjacent sequences can be readily isolated by IPCR from total genomic DNA. Now, we are in the process of generating a set of 124 lines that will carry a uniquely marked *Ds** element at regularly spaced locations in the genome. In this set, most genes in the genome will be within 7 cM of a launching platform and will be, therefore, targets for localized transposon mutagenesis. These lines will be deposited in the Maize Stock Center and will serve as starting materials for the generation of future insertion libraries by interested scientists. Supported by NSF-PGRP grant DBI-03-21494.

P240

Analysis of transposon transcription using tiled microarrays

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Transposable elements are ubiquitous members of all higher eukaryotic genomes and make up the majority of genetic material present in the maize genome. These mobile DNAs are critical elements in the creation of new genetic variation. The maize genome contains approximately 58% repeated, non-genic sequences, but little information is available on the transcription of these sequences. Repetitive sequences are usually masked and not analyzed in genomic expression studies because the exact locus associated with expression will not be known. The omission of repetitive sequences creates a gap in the knowledge of plant transcriptomes. The goal of this study was to evaluate sense and antisense transcription of maize repeats using a microarray array based approach. A custom microarray was developed that contained tiled 70-mers at 35bp intervals for 72 transposon and non-coding repeat sequences. This array was interrogated with RNA derived from tissue cultured cell lines and non-cultured plant tissues. This research indicates that many maize repeats have a low level of transcription across tissues, and that some are transcribed at a high level. Many of the tiled sequences did not detect transcription that would correspond to functional transposable elements. While transcription of repeats was generally similar in intensity and frequency across tissues, certain elements showed tissue or treatment specific sense and/or antisense transcription. Further analysis of the tiled repeats revealed transcription initiation within the LTR region of many LTR-retrotransposons and novel transposon transcripts. This study supports the hypothesis that repetitive sequences are active members of the plant transcriptome that are transcribed in both ubiquitous and tissue specific manners.

P241

Caught in the Act: Dramatic Amplification of a Rice Transposable Element During Recent Domestication

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Transposable elements (TEs) are the largest component of eukaryotic genomes and their accumulation has contributed to genome evolution and diversification. Among TEs, one type, called miniature inverted-repeat transposable elements (MITEs) are thought to be particularly important in the evolution of plant genomes where they are present in very high copy number (frequently >1,000 copies/TE family) and are preferentially located in or near genes. MITEs are non-autonomous DNA transposons that can only transpose when provided with transposase acting in trans from autonomous elements elsewhere in the genome. The first active MITE called mPing, was independently isolated from the rice genome by three groups. In this study we found that the copy number of mPing has increased over 1,000 fold since domestication, from a single copy in tropical japonica varieties to over 1,000 copies in the temperate japonica strain Gimbozu. We characterized 280 of the new insertions and found that 70% were within 5kb of annotated rice coding regions but that insertions into exons and introns were significantly underrepresented (compared with computer simulation controls) suggesting that detrimental insertions are very rapidly removed from the population. RT-PCR analysis of the RNA produced by genes with new mPing insertions in their introns or regulatory regions suggested that these insertions have a minimal impact on gene expression, thus providing one reason for why mPing elements can attain such high copy numbers. A second reason comes from our discovery that mPing elements are still actively transposing in strains that already have over 1000 copies. Based on an analysis of parent and progeny insertion sites, we estimate that there are over 20 new insertions per generation per progeny plant.

P242

Comparative Analysis of Transposable Elements in the Model Legumes Lotus japonicus and Medicago truncatula

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Transposable elements (TEs) are genetic elements that can move (transpose) from one genetic locus to another and, in doing so, frequently increase their copy number. To date, TEs have been found in all characterized eukaryotic genomes where they frequently are the most abundant component. The availability of large quantities of genomic sequences from several eukaryotic species has, in recent years, facilitated the study of TEs in their genomes. However, the approaches used to identify and analyze TEs are greatly influenced by the characteristics of particular sequence databases. For the completely sequenced genomes of rice (Oryza sativa) and Arabidopsis full-length TE sequences can be deduced and chromosomal locations defined. In contrast, for a partial and fragmented database consisting of short reads (such as Brassica oleracea), only TEs with coding sequences can be identified with confidence, and their copy numbers must to be extrapolated to the whole genome. In this study, we did a comparative analysis on a subset of sequences from the ~500Mb genome of model legumes Lotus japonicus (~44Mb) and Medicago truncatula (~94Mb). Lotus and Medicago are members of the Leguminosae family, which includes several agriculturally important crops such as soybean and garden pea. The Lotus and Medicago sequences represents a third type of database, where finished or nearly finished BAC sequences covering a significant fraction of the genome are available, with the chromosomal positions determined for Medicago but yet to be determined for Lotus. For the analysis of such a database, we devised strategies and methods to identify and characterize a wide variety of TE types to compare the TE abundance and diversity. Computational analysis provided an extrapolated estimate of genome-wide TE abundance and phylogenetic analysis provided a comparative relationship of TE lineages. Results show that both Medicago and Lotus contain all the TEs found in previous analyses of plant genomes, including transposase encoding elements, retrotransposons, Helitrons and MITEs (miniature inverted-repeat transposable elements), together accounting for >20% of their genomes.

P243

Constructing MightyMu-tagged lines for gene and enhancer trapping

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Several types of transgenic maize lines, namely the MightyMu lines, were made. These MightyMu lines contain different extended versions of RescueMu-type transgene, i.e., an engineered Mu1 element equipped with pUC-based plasmid components. There are 4 groups of Agrobacterium-transformed MightyMu transgenic maize plants. These include two versions of GUS enhancer trap, one GFP enhancer trap and one activation gene trap with the promoter from Cassava Vein Mosaic Virus (pCaVMV). A total of 131 GUS lines, 33 GFP lines, and 27 activation lines were obtained. After crossing with active Mutator plants, extensive screening for heritable, mobile MightyMu elements was performed over 3-4 generations. The first group of 83 GUS lines in Hi II background contained higher transgene copy number (up to 10) and many carried extra sequences outside of the T-DNA borders. After screening >2,500 individuals from 51 events, no heritable transpositions were found in this group. The second group of GUS lines, GFP lines and the activation lines were all in A188 background and contained 1-3 copies of transgene and carried no extra sequences outside the T-DNA borders. After screening >3,500 individuals from 14 GUS events in A188 background, one heritable transposition was confirmed. After screening >1,500 individuals from 6 activation events, at least 3 heritable transpositions were confirmed. GFP lines, only one generation after crossing with active Mutator plants, are currently being evaluated. A GUS enhancer trap tagging population and an activation tagging population will be established in summer 2006.

P244

Discovery of Helitrons in the Maize Genome Database

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Seminal studies from several laboratories have discovered that microcolinearity of genes may differ among different maize lines. This, so called "violation of genetic colinearity" or "+/- polymorphism" apparently is caused by Helitrons, a recently identified novel family of transposable elements. Despite their massive abundance and propensity to capture and mobilize host gene sequences, Helitrons remain virtually uncharacterized in the maize genome. Furthermore, captured genes described so far by maize Helitrons are small gene pieces that contain in frame stop codons. Identification of additional members is important to determine the impact of Helitrons on the evolution of maize genome. In this report, we have used the conserved termini of known maize Helitrons and Maize Genome Sequencing Project, and it associated, exponentially growing database to search for other Helitrons and genes contain therein. We will present the data generated from these studies.

P245

Effects of position on heritable silencing of MuDR elements

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Mu killer (Muk) is an inverted repeat version of a MuDR transposon that can heritably silence MuDR elements at one or several positions. Muk produces a hairpin transcript that triggers post-transcriptional and transcriptional silencing of full length elements, resulting in DNA methylation and the heritable loss of transcriptional activity of both of the two genes encoded by MuDR. We have isolated an exceptional chromosomal position that fails to heritably maintain this silenced state. An element at this position can be effectively silenced by Muk, resulting in the loss of mudrA expression and methylation, but once Muk is lost via segregation all aspects of MuDR activity are restored. We hypothesize that sequences flanking this particular insertion encode information that "resets" the heritable epigenetic signals that would normally maintain the heritable silenced state of MuDR. We suggest that the genome may be structured in such a way that there is local variation in the capacity to carry epigenetic information through meiosis, and that the heritability of MuDR silencing can be used to uncover such variation.

P246

Enhancer detection and activation tagging strategies in maize: research advances at Cinvestav Irapuato

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Different strategies for activation tagging and enhancer detection have been developed in our lab using maize. Although our goal is to study genes involved in reproductive development, the transposant population will be available for the scientific community. The artificial En/I-based activation tagging system includes a genetic construct with an immobile source of transposase under the control of the CaMV35S promoter, a mobile non-autonomous element comprising a tetramer of CaMV35S, and 2 selectable markers to identify stable unlinked transpositions (BAR gene conferring resistance to the herbicide basta and Su1 that converts the pro-herbicide R7402 into sulfonylurea, inhibiting plant growth). The enhancer trap strategy comprises a two-element system, in which a Ds transposon was engineered by adding a selection marker gene (C1), a reporter gene (uidA promotorless sequence), and a counter-selection gene (Bz1 located outside of Ds element and will indicate a linked transposition). From a modified Ac element that had an immobilized source of transposase under the control of a CaMV35S promoter, we inserted a counter-selection gene (Bz1) to obtain the second element of our system. For this case, the selection of transposant lines is going to be performed on a novel selection system based in grain color, as a result of the ability of complementing the recessive c1 and bz1 genes from 909B. Some expected results will include 1) brown grains, which result from the presence of C1, Bz1 genes, 2) bronze grains will result if C1 is absent, and Bz1 is not expressed, 3) purple grains will be obtained if C1 is present and Bz1 is absent. So far, we have performed several transformation experiments through biolistics and A. tumefaciens using embryogenic-organogenic callus derived from shoot tips of in vitro germinated seedlings. A first group of transposant lines from the activation tagging strategy has been generated and is currently under genetic and molecular analysis to determine single stable insertions. The progeny of self pollinated plants has been characterized through PCR and segregation analysis.

P247

Flanking-fragment display: a technique for efficient isolation of Mu-flanking fragments

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Transposon tagging has been proved to be a powerful and reliable tool for exploiting gene function in plant genomics research. Detection and isolation of flanking fragments for Mutator (Mu) insertion are the bottleneck in the insertion mutagenesis study. A new method called flanking-fragment display (FFD) has been developed to simultaneously display all possible flanking regions for both endogenous Mu and insert Mu in Mu-induced mutants. The technique involves three steps following the AFLP principle: 1) double-digestion of genomic DNA and ligation of adaptors; 2) pre-amplification and amplification of restriction fragments; 3) display of PCR bands on polyacrylamide gels and cloning of mutant-specific bands. After survey of Mu-sequence and multiple tries, a pair of restriction enzymes BglII and MspI were selected to cut genomic DNA. Unlike the Mu-AFLP in which an absorption technique was used to enrich the Mu-flanking fragments, a pre-amplification step was adopted here to effectively enrich a subset of double-digested fragments, including Mu-flanking bands. Due to enriched template DNA, all Mu-flanking fragments as well as other homologous fragments could be efficiently amplified by using a pair of primers designed respective on Mu-sequence and an adaptor. The PCR products were then displayed on polyacrylamide gels, and mutant-specific bands could be observed in mutant when comparing parental lines and Mu-induced mutant. Mutant-specific band was isolated and sequenced, and the flanking sequence was used to design primers. By using Mu-specific primer and flanking-sequence-specific primer, PCR products could only be observed in Mu-induced mutants, this confirmed that mutant-specific band was derived from flanking region of Mu insertion, but not endogenous Mutators.

P248

Function Characterization of MURB Protein in Maize

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The regulatory transposable element MuDR encodes two genes: mudrA and mudrB. mudrA encodes several forms of MURA transposase, and the mudrB encodes several forms of the MURB helper protein. The MURA proteins function as transposases, but little is known MURB function. Work from Lisch, Freeling and collaborators implicate MURB as a required partner in germinal insertion, because lines containing a single MuDR element with deletions of or epigenetically silenced mudrB lack new insertions. The same group reported that MURB is present in nucleic, however, we found that MURB lacks nuclear localization signals and that in transient expression assays is localized exclusively in the cytoplasm and does not interact with MURA transposase (Ono et al.). As a result of retention of potentially coding intron mudrB can theoretically encode 4 proteins; based on transcript abundance, only the 207 aa and 231 aa MURBs should be present in reasonable abundance. To investigate MURB function we are taking several new approaches. TAP-tagged MURB proteins were expressed from a gene copy in maize protoplasts and the interacting proteins recovered and subjected to Mass Spectrometry sequencing generating a list of putative partners for further investigation; transgenic maize expressing tagged MURB will be generated to recover in vivo partners from various maize tissues. We successfully expressed MURB207 in E. coli and insect cells (SF9) based on Western blotting results. Various biochemical analyses such as DNA-binding and ATP-binding assays are under way. WQ is supported by an NSERC Postdoctoral Fellowship. Research is supported by the NIH.

P249

Genome-wide Examination of the Relationship between Tripsacum and Zea: A Heartbreaker?

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Elucidating the origin of maize requires understanding its relationship not only to its putative closest relatives, the wild species of Zea, known as the teosintes, but also to the New World grass genus, Tripsacum. Although Zea and Tripsacum are widely considered to be sister taxa, the relationships among the eight taxa of Zea and the sixteen of Tripsacum remains to be fully elucidated. Given the difference in basic chromosome numbers (n = 10 vs. n = 18) and genome sizes between them, the divergence of Zea and Tripsacum likely involved genome-wide reorganization that would be best assayed by numerous unlinked markers rather than individual genes. The maize MITE (miniature inverted repeat transposable element) family Heartbreaker (Hbr) displays a number of features that make it a good candidate for use as a source of such markers, including high copy number, insertion preference for genic regions, high sequence identity, indicating a recent expansion in the genome, and, most importantly, stability in the maize genome. Hbr elements were previously detected in all of the teosintes and in two Tripsacum species. In this study we assayed a panel of 32 accessions, including 20 Tripsacum, 11 Zea and 1 Manisuris, with two primer combinations (HbrInt5/ Mse+CAT and HbrInt5/ Mse+CTC). The number of fragments per accession/primer combination ranged from 2 to 34. In addition to the two Tripsacum species for which Hbr insertions had already been described, we also confirmed the presence of Hbr elements in another 13 Tripsacum taxa, and, at a much lower frequency, in the more distantly related andropogonoid grass, Manisuris. Phylogenetic analysis using tree and network methods revealed little robust tree structure, suggesting close relatedness between Zea and Tripsacum species due to either shared ancestral polymorphisms or gene flow. Network analysis also confirmed the Zea x Tripsacum allopolyploid origin of Tripsacum andersonii.

P250

Helitron discovery and description in Arabidopsis and rice

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Helitrons comprise a recently-discovered class of eukaryotic transposable elements that are believed to transpose by a rolling circle mechanism. Helitrons have frequently acquired fragments of genes in maize, and have fused exons from different genes captured within a single Helitron. Hence, they may be major contributors to the creation of novel genes by exon shuffling. So far, Helitrons have been found in Arabidopsis, rice, maize, barley, wheat, Drosophila, C. elegans, fungi, and fish. Helitrons can be difficult to identify because they have few structural features, including the absence of a target site duplication. Helitrons contain 5' TC and 3' CTRR termini and a small hairpin near the 3' end, and they insert into the host dinucleotide AT. We have taken a novel approach to find Helitrons by using the 3'-terminal stem-loop structure and nearby CTRR to screen Arabidopsis, rice and other genome databases, followed by alignment of individual candidates to precisely identify Helitron ends. By this process, we have found 29 Helitron families (9 new families) in Arabidopsis and 55 families (53 new families) in rice. With this expanded data set, several novel aspects of Helitron amplification and evolution have been determined.

P251

Levels of activity regulation of the Bg-rbg system of transposons

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Activity of the Bg-rbg system of transposons can be assessed by frequency of reversion of the mutable o2-m(r) alleles as a result of excision from these alleles of nonautonomous rbg elements in presence of autonomous Bg elements. On the whole organism level, reversion frequency of such alleles is regulated by the specificity of their interaction with different Bg transposons and can strongly depend on dosage of these autonomous elements (Genetika (Moscow) 39: 769-774, 2003; Maydica 48: 275-281, 2003). Behavior of o2-m(r) alleles and Bg elements can be explained on the posttranscriptional level assuming that Bg-encoded protein(s): 1) act as oligomeric complexes; 2) can form inactive aggregates at their high concentrations; 3) may participate in transposition complexes together with rbg encoded products (Genetika (Moscow) 39: 769-774, 2003; Maydica 48: 275-281, 2003). Analysis of probable Bg-encoded proteins (MNL 79: 32-35, 2005) indicates on possibility of transcriptional level of Bg activity regulation by the interaction between Z-DNA forming regions of the Bg transposon and Z-DNA binding domains of its encoded proteins (MNL 80, submitted) explaining observed features of Bg dosage effects as well as the specificity of relationship between different o2-m(r) alleles and Bg elements.

P252

Origin and diversification of maize White cap haplotypes

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Transposon mediated rearrangements and gene amplification are essential processes of genome evolution. Recent studies have shown that helitrons have contributed to the extensive haplotype diversification in the recent evolution of the maize genome. We have previously shown that the dominant White cap allele identifies a unique haplotype that contains a large repeat structure harboring 24 copies of the ZmCCD1 carotenoid dioxygenase gene. Molecular analyses indicate that the array likely arose from a relatively recent concerted amplification event. Though apparently common, the mechanism(s) of concerted gene amplification in plant genomes is unknown. Further molecular analysis of the Wc amplification mechanism may yield clues to the mechanism. To derive the history of the Wc haplotype we compared the sequences of the Wc repeat region with the single copy ZmCCD1 locus of inbred B73 and the maize ancestor Teosinte ssp. parviglumis. Remarkably, both dominant and recessive Wc1 alleles found in modern maize are associated with haplotypes that are highly derived relative to teosinte. In parviglumis, the ZmCCD1 gene is located in a compact gene-rich region sandwiched between a glutamyl-tRNA synthase gene (Glu-tRNA Syn) and a ribosomal large subunit protein 21 gene (RPL21). Transposon sequences or other repetitive elements were not evident. The local gene order is conserved in an assembled B73 BAC sequence containing ZmCCD1, however, the distance separating the upstream RPL21 gene and ZmCCD1 is greatly expanded. Sequence alignments identified two large helitron-like sequences in the intergenic regions with insertion sites at positions -92 bp and -694 bp relative to teosinte ZmCCD1. Consequently the ancestral promoter of ZmCCD1 is displaced in yellow grained B73 (wc1). The repeat ZmCCD1 copy recovered in BAC clones from Wc1 contained an unrelated transposon insertion in the 5' UTR forming the apparent 5' boundary of the repeat. These data suggest that maize ZmCCD1 gene has undergone multiple cycles of selection for loss and for restoration of endosperm expression in yellow and white grain varieties, respectively. Helitrons and other transposons have played an active role in the generation of the genetic diversity at the locus. The potential role of transposons in mediating gene amplification is under investigation.

P253

Proteomic Analysis of *Mutator* Transposition

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The *Mutator* (*MuDR*/*Mu*) transposon family of maize contains an autonomous element (*MuDR*) and several types of slave elements. Transposition of *Mu* elements takes place almost exclusively during terminal cell divisions and occurs via one of two biochemical modes: conservative (cut-only or cut-and-paste) in somatic cells and net replicative in reproductive tissues. At the cellular level, transposon activity requires at least DNA and chromosomal repair processes. Aside from downstream studies on the consequences of transposon activity, relatively little is known about the proteins that modulate their upstream (excision and insertion) behavior *in vivo*. Using a proteomics-based approach, our goals are to: 1) determine whether *Mutator* activity changes the host proteome, 2) identify host proteins that are expressed at the same time that *Mutator* is activated, and 3) identify host proteins characteristic of cells where net replicative transposition is the major pathway. To investigate the effect of *Mutator* activity on the host proteome, protein was isolated from 1.0 mm (all mitotic cells) anthers of *Mu*-active and -inactive individuals, labeled with fluorescent dyes, mixed and separated first by isoelectric point (pH 4-7) and second by molecular weight. This two-dimensional DIGE separation technique allowed approximately 2,500 spots per gel to be visualized. *Mu*-active and -inactive individuals exhibited 48 differentially regulated spots ($p < 0.05$, fold change > 1.5). Interestingly, 47 out of the 48 spots were up-regulated in the transposon active lines. Thus far, thirty-four of the forty-seven spots have been sequenced via LC-MS/MS and determined to encode genes with mainly metabolic functions. Experiments are underway to identify the remaining proteins and to test additional pH ranges. In parallel, transcriptome experiments are being performed. This work is supported by a grant from the National Institutes of Health. D.S. is a trainee of the NIH Stanford Genome Training Program.

P254

RAD51 is required for the repair of Mu-induced DSBs in maize

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In late somatic cells rates of Mu insertion and excision are both high. In contrast, although high rates of insertion are also observed in germinal cells, germinal excisions are recovered only rarely. We, and others have hypothesized that development-specific differences in DNA repair processes explain this apparent contradiction. In yeast RAD51 is essential for homologous recombination that repairs double strand breaks (DSBs) using sister chromatids as the repair template. We have isolated deletion alleles of the two maize rad51 genes. In RAD51+ plants germinal revertants are recovered only rarely (2.6 X 10-5) from the MuDR-insertion allele a1-m5216. In contrast in RAD51- plants (i.e., rad51a, rad51b double mutants) the rate of germinal derivatives from a1-m5216 is at least 40-fold higher. Most of the germinal derivatives involve deletions of the MuDR insertion and/or the a1 gene. Our interpretation is that in wild-type germinal cells MuDR excisions are efficiently repaired via RAD51-directed homologous recombination with the sister chromatid, which replaces the excised MuDR, resulting in a low rate of germinal derivatives. In late somatic cells, MuDR excisions are not readily repaired in this manner, resulting in a high rate of somatic revertants. Two experiments suggest that RAD51 is also required for repairing Mu-induced DSBs in early somatic cells. First, a high proportion of Mu-active (but not Mu-inactive) RAD51- mutants exhibit severe developmental defects. Second, ear sectors of germinal derivatives were recovered at a higher rate from RAD51- mutants than from controls. Together these results suggest that RAD51 is required for repairing Mu-induced DSBs during early vegetative development. The high rate at which partial deletions of the a1 locus were recovered in this study indicates that the rad51 double mutant stock offers an attractive means to generate knock-out alleles for functional genomic studies.

P255

Recent Capture of Maize Cytochrome P450 Monooxygenase Gene by a Helitron

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Gene movement by Helitron family of novel transposable elements has apparently played a major role in gene duplication, exon shuffling and creation of gene non-colinearity among various maize inbred lines. Reported gene pieces captured by maize Helitrons lack significant coding capacity for biologically active proteins. In this report, we provide evidence that maize CYP72A27 gene represents an intact portion of a cytochrome P450 monooxygenase (P450) gene recently captured by a Helitron and transposed into an Opie-2 retroposon. Both terminal ends of this element share striking similarity to Helitron insertion discovered in maize mutants, sh2-7527 and ba1-ref. The four exons of the CYP72A27 gene contained within the element contain an open reading frame (ORF) for 435 amino acid residues with more than 95% sequence similarity to another maize P450 gene, CYP72A26. These data suggest that capture of CYP72A27 either happened recently or is under strong evolutionary selection. We also provide evidence that CYP72A27 is transcribed. These data provide evidence that Helitrons can capture and mobilize functional genes, a process that may contribute to molecular basis of heterosis or hybrid vigor in maize.

P256

Regional Ac mutagenesis of the ps1 locus generates novel allelic variation

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A large-scale *Ac* regional mutagenesis was conducted to isolate multiple *ps1* alleles utilizing a closely linked *Ac* insertion as a donor element. One thousand four hundred and seventy-eight *Ac* transposition events were selected. Screens of selfed progeny ears screened resulted in the recovery of 24 mutant *ps1* alleles. Twenty-one *ps1* alleles carried an *Ac* insertion, whereas 3 "footprint" alleles were induced by *Ac* excision. *Ac* insertions were dispersed throughout the 5' UTR and coding region. Despite the absence of introns in the *ps1* gene, phenotypic variation was detected that was correlated to *Ac* insertion site within the gene. Interestingly, a novel *Ac* read-out promoter *Ac*tivity was defined that mediates a low level of *ps1* transcription. A genetic scheme was developed to identify "footprint" alleles and 83 germinal *ps1* footprint alleles were recovered. Of these, 19 *ps1* excision alleles were unique. The stable footprint alleles are valuable in defining structural domains within the *ps1* protein and can be used in breeding programs to alter the carotenoid composition of the maize grain.

P257

Several Transposases Control Mutator Activity in Maize

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Mutator (*MuDR*/*Mu*) transposons are mobilized *in vivo* by multiple MURA proteins encoded by the *mudrA* gene of the autonomous element *MuDR*. These transposases mediate various aspects of Mutator activity. The translation product of the main *mudrA* open reading frame, MURA823 protein, supports only *Mu* excision, and its activity does not explain the high germinal insertion frequency of these elements. We found that following an alternative splicing decision involving the first intron, a slippery sequence at the exon:exon junction permits programmed (-1) frameshift translation to produce MURA854 protein. This protein has a novel N-terminus and catalyzes both *Mu* somatic excision and germinal insertion *in vivo*. Similar to *MuDR*-mediated events, there are characteristic 9-bp host target site duplications upon *Mu* insertion. Both MURA823 and MURA854 transposases program developmentally late somatic excisions. A third transposase, MURA736 is produced from transcripts prematurely terminated within or retaining the last intron; interestingly, it supports developmentally earlier excision events and a high frequency of somatic deletions. Thus, complex RNA processing events including the first example of translational frameshifting used in production of eukaryotic transposase diversify *mudrA* gene functions by generating three distinct transposase proteins. Research supported by the NIH.

P258

The Maize Mre11/Rad50/Nbs1 DNA repair and recombination complex

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We are only beginning to understand how plants protect and repair their DNA, and most of the information we have about plant DNA repair is limited to only one plant, Arabidopsis. Many components of human and plant DNA repair mechanisms are conserved but there are important and informative differences. For example, null mutations in most repair genes are associated with embryo lethality in mammals while Arabidopsis nulls deficient in the same genes are viable, even though the genes are single-copy. This and recent findings connecting environmental stress and DNA repair, make plants an extremely valuable system for studying DNA repair. Expanding these studies beyond Arabidopsis to maize has revealed that there is an informative diversity of responses to DNA damage even among plants. A DNA repair complex conserved between plants and animals contains the Mre11 and Rad50 proteins (MRN). This complex plays an important role in the processing of broken DNA ends as well as signaling to the cell that there has been damage. We have previously reported that maize is unusual in having two expressed copies of the Mre11 gene, Mre11A and Mre11B that produce similar but quite distinct proteins. In addition to further details about the expression patterns of Mre11A and Mre11B, we also describe our discovery of a plant homolog for the third, signaling component of the complex, the Nbs1 protein, in both maize and Arabidopsis. The predicted Nbs1 proteins include all five signature domains only found together in Nbs1 proteins of animals. We have cloned the maize Nbs1 gene and, by yeast two hybrid assay, show it interacts with Mre11A but, interestingly, not with Mre11B. Currently, we are overexpressing all three recombinant proteins for antibody production to test their interaction in vivo by coimmunoprecipitation. We have identified a nonsense mutant of maize Nbs1 by TILLING and are currently selfing and backcrossing this mutant to the W22 inbred line.

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