47th ANNUAL MAIZE GENETICS CONFERENCE

PROGRAM
&
ABSTRACTS

10 - 13 MARCH 2005

GRAND GENEVA RESORT
LAKE GENEVA, WISCONSIN

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Participants 170

General Information

Meals

All meals will be served buffet style in the Evergreen Ballroom, the Loramoor Room, and the Newport Grill. Breakfast will be served from 7:00 to 8:00 AM on Friday, 7:00 AM to 8:30 AM on Saturday, and from 7:30 to 9:00 AM on Sunday. Lunch will be served from 12:30 to 1:30 PM on Friday and Saturday. Dinner will be served from 6:00 PM to 7:00 PM on Thursday, Friday, and Saturday. Coffee, tea, and soft drinks are available at no charge during beverage breaks.

Talks and Posters

All talks and workshops will be presented in the Forum convention center.

All posters will be presented in Grand Ballroom. Posters should be hung Thursday evening and should stay up through Saturday evening, but should be removed by 9 AM on Sunday.

Hospitality

After the evening session on Thursday and Friday there will be informal socializing and poster gazing in the Grand Ballroom and nearby lobby. On Saturday evening there will be informal socializing in the Evergreen Ballroom, with music and dancing. Refreshments will be provided each night until 1 AM. After 1 AM, rooms 6102/6104 are available for continued socializing. These are "private party rooms" and alcoholic beverages may be brought in; however, you must stay in these rooms if you are carrying drinks and dispose of your trash and bottles in the party room.

Steering Committee

Please share your suggestions and comments about the meeting with the 2005 Steering Committee:

Martha James, Chair (mgjames@iastate.edu)
Jay Hollick, Co-chair (hollick@nature.berkeley.edu)
Mike Scanlon (mjscanlo@plantbio.uga.edu)
Daniel Grimanelli (daniel.grimanelli@mpl.ird.fr)
Wes Bruce (wes.bruce@pioneer.com)
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Richard Schneeberger (rschnee@ceres-inc.com)
Ed Buckler (esb33@cornell.edu)
Marty Sachs, local organizer, ex officio (msachs@uiuc.edu)
Karen Cone, treasurer, ex officio (ConeK@missouri.edu)
Mary Polacco, abstract coordinator, ex officio (PolaccoM@missouri.edu)

Acknowledgements

Many thanks go to Trent Siegfried and Mary Polacco 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. Special thanks are extended to Marty Sachs for his superb efforts in organizing the numerous details of this meeting. The meeting registration was outsourced to the MU Conference Center, and professionally handled by Amy J. Sheets.

Next Maize Genetics Meeting

The 48th Annual Maize Genetics Conference will be held March 9 - 12, 2006 at the Asilomar Conference Center, Pacific Grove, California. Local organizer is Jay Hollick (hollick@nature.berkeley.edu).

Schedule of Events

Thursday, March 10

3:30 PM – 5:30 PM	**POSTER HANGING**	
5:30 PM – 7:00 PM	**DINNER**	
7:15 PM – 9:00 PM	**SESSION 1 – PLENARY TALKS**	Chair: Mike Scanlon
7:15 PM	**David Jackson, Cold Spring Harbor Laboratory** *Genetic and Cellular Analysis of Signaling, Branching, and Phyllotaxy in the Maize Shoot Meristem*	
8:15 PM	**Dorothy Shippen, Texas A&M University** *Unraveling the Ends: Telomere Function and Dysfunction in Arabidopsis*	
9:00 PM	**POSTER HANGING, INFORMAL POSTER VIEWING, & HOSPITALITY**	

Friday, March 11

7:00 AM – 8:00 AM	**BREAKFAST**	
8:15 AM – 8:30 AM	**ANNOUNCEMENTS**	Martha James
8:30 AM – 10:25 AM	**SESSION 2 – BIOCHEMICAL GENETICS & CELL BIOLOGY**	Chair: Monika Frey
8:30 AM	**Klaas van Wijk, Cornell University** *Functional Specialization of Chloroplasts in Mesophyll and Bundle Sheath Cells in Zea mays: A Comparative Proteome Analysis*	
8:50 AM	**Richard Kowles, Saint Mary's University of Minnesota** *Expression of C4 Photosynthetic Characteristics in Oat-Maize Addition Lines*	
9:10 AM	**Tiffany Langeswich, Purdue University** *Fourier Transform Mid-range and Near-infrared Spectroscopies in Maize Genomics*	
9:30 AM	**L. Curtis Hannah, University of Florida** *A Modified Shrunken-2 Allele Enhances Maize Yield*	
9:50 AM	**Amy Cooke, University of Oregon** *Involvement of an RNAse III Homolog in Group II Intron Metabolism in Chloroplasts*	
10:10 AM	**Jorg Degenhardt, Max Planck Society** *The Terpene Synthase Family in Zea mays and Its Role in Defense Against Herbivory*	

10:25 AM – 10:50 AM **BREAK W/ BEVERAGES**

10:50 AM – 12:25 PM **SESSION 3 – EPIGENETICS & QUANTITATIVE TRAITS** Chair: Ed Buckler

10:50 AM **Jenelle Meyer, University of Missouri**
The Genetic Basis of Increased Maize Silk Maysin Levels Through Recurrent Selection

11:10 AM **Silvio Salvi, Università di Bologna**
The Maize QTL Vgt1 Corresponds to a ca. 2.7-kb Non-Coding Region Upstream of an Ap2-like Gene

11:30 AM **Christiane Belele, University of Arizona**
Identification and Characterization of DNA Sequences Required for Paramutation of the b1 Gene in Maize

11:50 AM **R. Keith Slotkin, University of California - Berkeley**
Heritable Silencing of the Mutator Transposon Family is Initiated by the Naturally Occurring Inverted Repeat Mu killer

12:10 PM **Khalil Kashkush, University of Georgia**
Readout Transcripts from the Dasheng Retrotransposon Alter the Expression of Adjacent Rice Genes

12:30 PM – 1:30 PM **LUNCH**

1:30 PM – 5:30 PM **POSTER SESSION**
Presenters should be at even numbered posters from 1:30 PM to 3:30 PM.
Presenters should be at odd numbered posters from 3:30 PM to 5:30 PM.

6:00 PM – 7:15 PM **DINNER**

7:30 PM – 9:30 PM **SESSION 4 - WORKSHOP** Chair: Jay Hollick
Strategies for Cloning Maize Genes

7:30 PM **Thomas Brutnell, Cornell University**
Ac as a Tool for Forward Genetics in Maize

7:55 PM **Donald McCarty, University of Florida**
Steady-State Transposon Mutagenesis and Cloning of Tagged Mutants in silico

8:20 PM **Esteban Bortiri, University of California - Berkeley**
Chromosome Walking in Maize: Cloning and Expression Analysis of ramosa2

8:45 PM **Matthew Evans, Stanford University**
Cloning The Indeterminate Gametophyte1 Gene Of Maize Using Tagging And Comparative Genomics

9:10 PM **Bailin Li, DuPont**
Map-Based Cloning in Maize: What Have We Learned?

9:30 PM **INFORMAL POSTER VIEWING & HOSPITALITY**

Saturday, March 12

Time	Event	
7:00 AM – 8:15 AM	**BREAKFAST**	
8:15 AM	**ANNOUNCEMENTS**	National Corn Growers Association
8:30 AM – 10:25 AM	**SESSION 5 – DEVELOPMENTAL GENETICS**	Chair: Wes Bruce
8:30 AM	**Paula McSteen, Penn State University** *Genetic and Hormonal Regulation of Inflorescence Development*	
8:50 AM	**Feng Zhang, Iowa State University** *Organ-specific Regulation and Molecular Evolution of the Maize pericarp color1 Gene*	
9:10 AM	**Olga Danilevskaya, Pioneer Hi-Bred** *Identification of Two Maize MADS Box Transcription Factors that Promote Floral Transition*	
9:30 AM	**Nick Lauter, University of Illinois – Urbana / Champaign** *microRNA 172 Downregulates glossy15 to Promote Vegetative Phase Change in Maize*	
9:50 AM	**Anne Sylvester, University of Wyoming** *RAB GTPases and the Maintenance of Developmental and Cellular Compartments During Leaf Development*	
10:10 AM	**John Doebley, University of Wisconsin** *The Maize Domestication Gene, tga1, is Member of a Family of Transcriptional Regulators*	
10:25 AM – 10:50 AM	**BREAK W/ BEVERAGES**	
10:50 AM – 12:25 PM	**SESSION 6 - GENOMICS**	Chair: Anne Sylvester
10:50 AM	**Steve Rounsley, Massachusetts Institute of Technology** *Evaluating Sequencing and Assembly Strategies for the Maize Genome*	
11:10 AM	**Volker Brendel, Iowa State University** *Estimating and Annotating the Maize Gene Space*	
11:30 AM	**Baltazar Antonio, National Institute Agrobiological Sciences** *Characterization of the Rice Genome Based on the Complete Sequence*	
11:50 AM	**Rita Monde, Purdue University** *The Maize TILLING Project: A Progress Report*	
12:10 PM	**A. Mark Settles, University of Florida** *Isolating Maize Kernel Composition Mutants by Near Infrared Spectroscopy*	

12:30 PM – 1:30 PM	**LUNCH**
1:30 PM – 3:30 PM	**POSTER SESSION**

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.

3:30 PM – 5:30 PM	**SESSION 7 - WORKSHOP** *Site-Specific DNA Integration*	Chair: Martha James
3:30 PM	**David Ow, USDA/ARS, Plant Gene Expression Center** *Recombinase-Mediated Plant Transformation*	
4:00 PM	**Qiudeng Que, Syngenta** *Position Effect of Targeted Integration at Different Transgene Loci in Higher Plants*	
4:30 PM	**Dan Voytas, Iowa State University** *High Frequency Homologous Recombination in Plants Mediated by Zinc Finger Nucleases*	
5:00 PM	**Vibha Srivastava, University of Arkansas** *Cre-mediated Site-Specific Gene Integration for Consistent Transgene Expression in Rice*	
6:00 PM – 7:15 PM	**DINNER**	
7:30 PM – 9:15 PM	**SESSION 8 - PLENARY TALKS**	Chair: Daniel Grimanelli
7:30 PM	**Zac Cande, University of California - Berkeley** *Unraveling the Meiotic Chromosome Condensation Network Using Maize Mutants*	
8:30 PM	**Detlef Weigel, Max Planck Society** *Function and Specificity of Plant microRNAs: Insights from Whole-Genome Studies*	
9:30 PM	**INFORMAL POSTER VIEWING, HOSPITALITY, & DANCE**	

Sunday, March 13

7:30 AM – 9:00 AM	**BREAKFAST**
9:00 AM – 10:55 AM	**SESSION 9 – GENOME ARCHITECTURE** — Chair: Marja Timmermans
9:00 AM	**Rachel Wang, University of California - Berkeley** *A High Resolution Cytogenetic Map of Maize Chromosome 9 Constructed by Single Copy FISH*
9:20 AM	**Brandon Gaut, University of California - Irvine** *The Genomic Extent of Artificial Selection*
9:40 AM	**Stephan Brunner, DuPont** *Evolution of DNA Sequence Non-Homologies Among Maize Inbreds*
10:00 AM	**Jinsheng Lai, Rutgers University** *Intraspecific Gene Movement in the Maize Genome*
10:20 AM	**Christiane Fauron, University of Utah** *Sequence Comparisons of Seven Mitochondrial Genomes from Maize and Teosinte*
10:40 AM	**Wade Odland, University of Minnesota** *Simplifying the Grass Genome to Evaluate the Maize Genome*
10:55 AM	**ANNOUNCEMENTS** — Jay Hollick
11:00 AM	**BREAK W/ BEVERAGES & ADJOURNMENT**

Poster List

Biochemical Genetics

P1	**Xiquan Gao** xgao@ag.tamu.edu	*A 9-lipoxygenase gene functions in maize germination and development*
P2	**Martha Schneerman** schneerm@uiuc.edu	*A Functional Genomics Program for the Illinois Protein Selection Strains*
P3	**Brent Buckner** bbuckner@truman.edu	*An international scientific and cultural experience for undergraduate students*
P4	**Mark Williams** mark.e.williams@cgr.dupont.com	*An orange endosperm mutation co-segregates with and is associated with mutations in the lycopene epsilon cyclase gene of maize*
P5	**Alessandra Frizzi** alessandra.frizzi@monsanto.com	*Analysis of zeins in mature wild-type and transgenic maize kernels by Matrix-Assisted-Laser-Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS)*
P6	**Rachel Huegel** rcmiller@iastate.edu	*Analyzing the structure and function of maize GBSS and SSI*
P7	**Katia Wostrikoff** clw32@cornell.edu	*Assembly state dependent regulation of Rubisco LS translation: Mechanism and physiological relevance*
P8	**Prem Chourey** pschourey@ifas.ufl.edu	*Bigger endosperm size in maize relative to other cereal grains is due to cumulative action of two cell wall invertase genes in developing seeds*
P9	**Hugh Young** hyoung@purdue.edu	*Bridging the maize - Arabidopsis divide: Mutants of the latter to a fungal HDAC inhibitor that confers disease in the former*
P10	**Jeffrey Church** jbchurch@uiuc.edu	*Candidate Genes for N Utilization Differences in the Illinois Protein Strains*
P11	**Taijoon Chung** taijoonc@email.arizona.edu	*Characterization of a Maize Opaque Mutant mto38*
P12	**Ling Bai** lb226@cornell.edu	*Characterization of lycopene-beta-cyclase and manipulation of carotenoids biosynthesis*
P13	**Akiko Kubo** akiko@iastate.edu	*Characterization of maize su1, isa2, and isa3 mutants indicates formation of distinct starch debranching enzyme complexes*
P14	**Rena Quinlan** Rena_Q@hotmail.com	*Characterization of the P450 Carotene Hydroxylase Gene Families in Cereal Crops*
P15	**Lacey Strickler** lace_j@yahoo.com	*Characterization of the Zebra Lesion Mimic (zl1) Mutation in Maize*
P16	**Tahhan Jaradat** TJarad@Yahoo.com	*Characterization of the maize gene family encoding beta-carotene hydroxylase (di-iron monoxygenase type)*
P17	**Salvador Moguel** smoguel2@unlnotes.unl.edu	*Codon optimized fluorescent marker genes for maize*
P18	**Paula Casati** pcasati@stanford.edu	*Differential accumulation of maysin & rhamnosylisorientin in leaves of high altitude landraces of maize after UV-B exposure*

P19	**Mingxu Zhang** zhmx@iastate.edu	*Direct and indirect effects of altered Du1 gene expression on starch structure determination*
P20	**Carlos Harjes** ch20@cornell.edu	*Diversity in maize kernel carotenoids content*
P21	**Antoine Harfouche** alh283@ra.msstate.edu	*Ethylene Signaling Contributes to the Insect-Induced Defense Response in Maize*
P22	**Ryan Dierking** rmdq44@mizzou.edu	*Evaluation of water-stress associated changes in root architecture of viviparous (vp) maize mutants.*
P23	**Deborah Groth** dgroth@purdue.edu	*High-Throughput Screening for Slowly Digesting Starch in an EMS Mutagenized Maize Population*
P24	**Mo-Ju Cao** caomj@sicau.edu.cn	*Identification and genetic analysis of maize male sterile mutant obtained by space flight*
P25	**Charles Hunter** ibe@ufl.edu	*Identifying stable Mu-induced knockouts of specific genes for cell wall biosynthesis in the UniformMu maize population using reverse genetics*
P26	**Nick Georgelis** gnick@ufl.edu	*Investigation of ADP-glucose pyrophosphorylase protein-protein interactions and post-translational modification by phosphorylation*
P27	**John Gray** jgray5@uoft02.utoledo.edu	*It's Not Easy Being Green! - Insights into Chlorophyll Detoxification from Lesion Mimic Mutant Studies*
P28	**Logan Brown** lrbrown1@express.cites.uiuc.edu	*Lepidopteran preference testing of Glossy mutants and Glossy15 alleles for maize resistance.*
P29	**Andrea Descheneau** descheneaua@missouri.edu	*Maize Mitochondrial DNA Binding Proteins*
P30	**Ada Ching** ada.s.ching@cgr.dupont.com	*Maize brittle stalk 2 (bk2) Gene Determines Mechanical Strength of Tissue by Mediating Cellulose Deposition in Secondary Cell Walls*
P31	**Diego Fajardo** diegof@ufl.edu	*Molecular and Genetic Analysis of rgh3 Endosperm Mutant*
P32	**Kristyn Dumont** kdumont@smcvt.edu	*Oligopeptide transporters show differential gene expression during germination*
P33	**Heather Cartwright** heatherc@biomail.ucsd.edu	*Pangloss Genes are Required for the Asymmetric Divisions that Give Rise to the Subsidiary Cells of Stomata in Maize*
P34	**Matthew Meyer** mrmwc2@mizzou.edu	*Photosystem II genes effect Lepidopteran damage in maize*
P35	**Lisa Haney** lhaney@uiuc.edu	*Role of Opaque2 in Regulation of Zein Production in the Illinois Protein Strains*
P36	**Ruairidh Sawers** rjs47@cornell.edu	*Semi-dominant alleles of Oil yellow1 contain single amino acid substitutions in a magnesium chelatase subunit*
P37	**Howard Rines** rines001@umn.edu	*Sexual transfer of B-chromosomes from maize, Zea mays L., into oat, Avena sativa L.*
P38	**Jorge Nieto-Sotelo** jorge@ibt.unam.mx	*The 5íUTR region of maize Hsp101 mRNA contains an IRES.*

P39	**Udo Wienand** udo.wienand@uni-hamburg.de	*The Etched 1 gene of Zea mays encodes a plastid protein with a zinc ribbon-like domain and similarity to eucaryotic transcription elongation factors.*
P40	**Dana Bush** dlw3f9@mizzou.edu	*Two Flavonones and Two Flavonols effects on Aspergillus flavus Growth*
P41	**Jonathan C. Lamb** jclp59@mizzou.edu	*Use of abundant retroelements to cytologically distinguish Tripsacum and Zea genomes*
P42	**Wanchen Li** aumdyms@sicau.edu.cn	*mRNA Differential Display of Drought Tolerant Inbred Lines Under Water Stress in Maize*
P43	**R. Frank Baker** rfb11@psu.edu	*tie-dyed2 is impaired in sugar movement out of leaves*

Bioinformatics

P44	**Christopher Maher** maher@cshl.edu	*A pattern matching approach to identify microRNAs in plant genomes*
P45	**Yong Rhee** yrhee@students.wisc.edu	*Analysis of the origin of a small inverted repeat sequence found in a tissue culture induced allele of C2*
P46	**Nick Lauter** nickl@uiuc.edu	*Bioinformatic, phylogenetic and gene expression analyses of maize microRNA172 genes and their APETELA2- like gene targets*
P47	**Yucheng Feng** yfeng@danforthcenter.org	*Examine the synteny between maize and rice*
P48	**Edward Buckler** esb33@cornell.edu	*Gramene Diversity Module: Sharing the Data Behind Germplasm, QTL, and Breeding Studies*
P49	**Carolyn Lawrence** triffid@iastate.edu	*MaizeGDB Community Curation Tools*
P50	**Trent Seigfried** devolver@iastate.edu	*MaizeGDB In A Nutshell: A Tour Of The Maize Community Database*
P51	**Ling Guo** guol@iastate.edu	*Multi-K means Clustering - Capturing the Natural Shape of Data*
P52	**Karthik Viswanathan** vkaru@iastate.edu	*PathBinderH - a tool for Linnaean Taxonomy-Aware Literature Searches*
P53	**Nigel Walker** nigel@chloroplast.uoregon.edu	*Plant RNA Binding Proteins: Tools for functional genomics and application to chloroplast biogenesis*
P54	**Snehalata Nadiger** snehagururaj@yahoo.com	*Study of the unorthodox breeding behavior of shrunken 1 Bombay*
P55	**Darwin Campbell** darwin@iastate.edu	*Technical Aspects of the MaizeGDB Project*
P56	**Karthik Viswanathan** vkaru@iastate.edu	*The MAGI Web Site: a resource for Maize Genome Assembly, Annotation and Mapping*
P57	**Mary Polacco** PolaccoM@missouri.edu	*The Plant Ontology Consortium: Comparing phenotypes and gene expression among angiosperms.*
P58	**Alexandra Ciungu** andra_ioana@msn.com	*Two Novel Arginine/Serine (SR) Proteins in Maize are Differentially Spliced and Utilize Non-Canonical Splice Sites*

P59	**Carolyn Lawrence** triffid@iastate.edu	*Use PGROP to locate educational activities, programs, and resources about plant genomics*

Cell Biology

P60	**Kan Wang** kanwang@iastate.edu	*Agrobacterium-mediated stable transformation of multiple maize inbred lines using a standard binary vector system*
P61	**Hong N. Nguyen** hnguyen@email.arizona.edu	*Characterization of Cyclins and Cyclin-Dependant Kinases in Developing Maize Endosperm Cells*
P62	**Fengling Fu** ffl@sicau.edu.cn	*Drought Tolerant and Male Sterile Material Screening from Maize Callus Mutated by Gamma Ray and Sodium Azide*
P63	**Anoop Sindhu** asindhu@purdue.edu	*Functional loss of a COBRA family protein in the maize brittle stalk2 (bk2) mutation*
P64	**Olivier Hamant** olivier@nature.berkeley.edu	*Maize Shugoshin is required for Centromeric Cohesion during Meiosis*
P65	**Christine Chase** ctdc@mail.ifas.ufl.edu	*Mitochondrial biogenesis and function in developing pollen of normal and S male-sterile maize*
P66	**Xuexian Li** xli@plantbio.uga.edu	*Phosphoserines on Maize CENTROMERIC HISTONE H3 and Histone H3 Demarcate the Centromere and Pericentromere during Chromosome Segregation*

Cytogenetics

P67	**Inna Golubovskaya** innagol@uclink4.berkeley.edu	*AFD1, a maize REC8 homolog, displays dose-dependent functions during meiosis*
P68	**Fangpu Han** hanf@missouri.edu	*Behavior of minichromosomes derived from the B chromosome of maize*
P69	**Margarida L .R. Aguiar-Perecin** mlrapere@carpa.ciagri.usp.br	*Breakage-fusion-bridge cycles, chromosome healing and unequal recombination events in maize callus cultures*
P70	**Weichang Yu** wy593@mizzou.edu	*Characterization of a Maize Isochromosome 8S:8S*
P71	**Ferdinand Amarillo** feamarillo@bio.fsu.edu	*Construction of a High-Density Cytogenetic Map of Maize Chromosome 9*
P72	**Debbie Figueroa** figueroa@bio.fsu.edu	*Development of a Pachytene Cytogenetic FISH Map of the 90 Core Bin Marker Loci*
P73	**Matthew Bauer** mjbc4b@mizzou.edu	*Endoreduplicated Chromosome Structure*
P74	**Fangpu Han** hanf@missouri.edu	*Inactivation of the B chromosome centromere in an A-B translocation*
P75	**Ashley Lough** AshNLaw@aol.com	*Mitochondrial DNA Insertion into Nuclear Chromosomes of Maize*
P76	**Kelly Dawe** kelly@plantbio.uga.edu	*Progress from the Maize Centromere Consortium*
P77	**Carolyn Lawrence** triffid@iastate.edu	*The Effects of Monosomy on the Rate of Transmission of Abnormal Chromosome 10*

P78	**Hank Bass** bass@bio.fsu.edu	*The Maize-10-Maze project, a public field replica the maize pachytene karyotype, decorated with mutants.*

Developmental Genetics

P79	**Philippa Barrell** pbarrell@botinst.unizh.ch	*nrm2, a mutant defective in cytokinesis during meiosis*
P80	**Colin Shepherd** coshep@iastate.edu	*A Maize Chimeric Promoter Drives High Level GFP Expression in Endosperm and Embryo*
P81	**Gregorio Hueros** gregorio.hueros@uah.es	*A PCR-based forward genetics screen to identify mutants in endosperm transfer cell development.*
P82	**Enrico Magnani** emagnani@berkeley.edu	*A reverse genetic approach to find new members of the ERF family of transcription factors involved in maize inflorescence development.*
P83	**Gregorio Hueros** gregorio.hueros@uah.es	*A search for upstream signals controlling the promoter of ZmMRP-1, an endosperm transfer cell specific transcriptional activator.*
P84	**Andrea Gallavotti** agallavotti@ucsd.edu	*An Investigation of the Branching Pathways of Maize Inflorescences: A Screening for Enhancers/Suppressors of the ramosa1 Mutant*
P85	**Koy Saeteurn** kiomy15@berkeley.edu	*Analysis of the Dominant Maize Mutant Corngrass1 (Cg1)*
P86	**Hector Candela** hcandela@berkeley.edu	*Axis formation in the maize leaf ñ inside, out, upside, down.*
P87	**Matthew Evans** mmsevans@stanford.edu	*Basal Endosperm Development In Maize Depends On Maternal Baseless1 Activity*
P88	**Chang-deok Han** cdhan@gsnu.ac.kr	*Characterization of members of a zinc finger 'ID domain' protein family in Rice (Oryza sativa L).*
P89	**Solmaz Barazesh** sxb944@psu.edu	*Characterization of the bif1 mutant in maize*
P90	**George Chuck** gchuck@nature.berkeley.edu	*Chromosome walking to the tasselseed4 locus of maize*
P91	**Jacqueline Weiss** c2217@truman.edu	*Cloning and tissue-expression studies of a KANADI4-like gene from Zea mays*
P92	**Jiabing Ji** jiabing@plantbio.uga.edu	*Cloning of narrow sheath/Pressed Flower homologue in tomato and its evolutionary implication in lateral organ development*
P93	**Ryan Douglas** rndouglas@gmail.com	*DNA sequence diversity at an ANGUSTIFOLIA-like gene among inbred lines and open-pollinated landraces of maize*
P94	**Nick Lauter** nickl@uiuc.edu	*Defining the modules of epidermal phase transition and identifying their genetic regulators*
P95	**Simon Malcomber** malcombers@msx.umsl.edu	*Diversification of SEPALLATA genes in grasses*
P96	**Mona Rezapour** mona_rezapour@hotmail.com	*Double Mutants at the thick tassel dwarf1 and fasciated ear2 loci reveal complex interactions affecting vegetative and inflorescence meristems*

P97	**Michael Zanis** mzanis@ucsd.edu	*Fate and Consequence of the Zag1/Zmm2 Gene Duplication Across Grasses*
P98	**Kazuhiro Ohtsu** kazohtsu@iastate.edu	*Functional analyses of genes involved in meristem organization and leaf initiation*
P99	**Ambika Chandra** auc135@psu.edu	*Fungal Induced Sex Change in Male Buffalograss Mimics Tasselseed-2 Mutant*
P100	**Xiujuan Wang** xiujuan@iastate.edu	*Gene expression profile analysis of maize (Zea mays L.) pericycle cells*
P101	**Andrea Eveland** aeveland@ufl.edu	*Invertase-Mediated Sucrose Utilization during Early Reproductive Development in Maize and Arabidopsis*
P102	**Christopher Spencer** spencer@truman.edu	*Isolation and expression studies on a Fasciata1-like gene in maize*
P103	**Luca Pasini** adriano.marocco@unicatt.it	*Isolation and mapping of defective endosperm mutants by using AFLP markers*
P104	**Clinton Whipple** cwhipple@biomail.ucsd.edu	*Maize C-class homeotic genes control floral meristem and reproductive organ identity*
P105	**Graziana Taramino** graziana.taramino@cgr.dupont.com	*Map based cloning of RTCS, a gene controlling crown and seminal root formation in maize*
P106	**Joshua Strable** joshua-strable@uiowa.edu	*Microarray analysis of vegetative phase change in maize*
P107	**Andrew Doust** adoust@umsl.edu	*Millets, maize, and the control of morphological diversity*
P108	**Kanok-orn Srilunchang** srilunchang@botanik.uni-hamburg.de	*Molecular analysis of the ubiquitin system in the maize egg cell*
P110	**Elene Valdivia** erv105@psu.edu	*Pollen beta-Expansins in Maize*
P111	**Ivan Acosta** ivan.acosta@yale.edu	*Positional Cloning of the Maize Sex Determination Genes tasselseed1 and silkless1*
P112	**Mark Cigan** mark.cigan@pioneer.com	*RNAi-mediated transcriptional silencing of anther-expressed genes results in male sterile maize*
P113	**Paolo Sabelli** psabelli@ag.arizona.edu	*Specific features of the RB/E2F pathway in cereals: RBR1 regulates RBR3 and controls endoreduplication during maize endosperm development*
P114	**Steven Runo** smruno@ucdavis.edu	*Striga KNO1 (KNOTTED1-like homeobox) RNAi as a Resistance Mechanism in Maize*
P115	**Ada Wong** awong01@uoguelph.ca	*Sucrose affects ID1 protein expression in developing maize leaves*
P116	**Yi Ma** yum105@psu.edu	*Sugar and Starch Accumulation Patterns Are Altered in tie-dyed1 Leaves*
P117	**Calin Marian** cmarian@bio.fsu.edu	*The terminal acidic SANT1 (Tacs1) gene is expressed in meristem-enriched tissues & encodes an acidic SANT domain similar to some chromatin-remodeling complex proteins*

P118	**Zhennan Xu** zhennan@waksman.rutgers.edu	*The maize aberrant pollen transmission 1(apt1) gene is homologous to the Arabidopsis SABRE gene and encodes a Golgi protein required for pollen tube growth*
P119	**Xianting Wu** xzw104@psu.edu	*The role of polar auxin transport in inflorescence development in maize*
P120	**Masaharu Suzuki** msuzuki@mail.ifas.ufl.edu	*Toward in silico transposon tagging of genes controlling aleurone development*
P121	**Xueyuan Cao** caoxy68@iastate.edu	*Transcriptional regulation of the maize vp1 gene*
P122	**Mingshu Huang** muh147@psu.edu	*camouflage1 patterning results from a defect in the chlorophyll biosynthetic pathway*
P123	**Gabriella Consonni** gabriella.consonni@unimi.it	*empty pericarp (emp) Mutants As A Tool To Study Embryo-Endosperm Interaction In Maize*
P124	**David Henderson** davidh@plantbio.uga.edu	*ragged seedling2 Leaves Fail to Expand Despite Retention of Adaxial/Abaxial Polarity*

Epigenetics

P125	**Jennifer Stonaker** jenne@berkeley.edu	*A putative histone methyltransferase is a candidate gene for rmr2, a modifier of pl1 paramutation*
P126	**Rajandeep Sekhon** rss222@psu.edu	*Differential CG and CNG methylation modifications of maize pericarp color1 epialleles in the presence of an epigenetic modifier Ufo1*
P127	**Julio Ramirez** bacillusman@yahoo.com	*Elucidating the transcriptional regulation of the class I knox gene knotted1 in maize*
P128	**Karen Cone** conek@missouri.edu	*Functional genomics of maize chromatin*
P129	**William Haun** haunx003@umn.edu	*Maize Sdg118 is the Functional Ortholog of Arabidopsis thaliana Kryptonite*
P130	**Susan Parkinson** sep@uclink.berkeley.edu	*Mutations in Required to maintain repression6 suggest evolutionary processes mechanistically related to paramutation*
P131	**Lyudmila Sidorenko** lyudmila@ag.arizona.edu	*Mutations that Affect Maintenance of Paramutation at b1 and pl1 Have Little or No Effect on Maintenance of Silencing in p1 Paramutation*
P132	**Maike Stam** mstam@science.uva.nl	*Paramutation: long-range epigenetic interactions in maize*
P133	**Karen McGinnis** mcginnis@ag.arizona.edu	*Role of chromatin proteins in paramutation at the B gene in maize*
P134	**Kyungju Shin** ksgw3@mizzou.edu	*Suppressor of plant blotching1 (Spb): an epigenetic modifier of Pl-Blotched*
P135	**Mieke Van Lijsebettens** milij@psb.ugent.be	*The Elongator histone acetyl transferase complex has a function in cell proliferation during organ growth in plants*
P136	**Stephen Gross** smgross@berkeley.edu	*The nature of allelic interactions affecting pl1 paramutation*

P137 **Lyudmila Sidorenko** lyudmila@ag.arizona.edu — *cis-acting Sequence Requirements for Establishment and Maintenance of Silencing in Maize p1 Paramutation*

Genome Structure / Synteny

P138 **Theresa Musket** muskett@missouri.edu — *A Maize Root Transcriptome Map*

P139 **Marna Yandeau-Nelson** myandeau@iastate.edu — *Analysis of unequal sister chromatid and homolog exchange at a tandem duplication of the a1 locus: rates and patterns of recombination breakpoint resolution*

P140 **Luca Gianfranceschi** luca.gianfranceschi@unimi.it — *Cloning of GaMS-1 male-sterile mutant: Linking genetic and physical maps*

P141 **Michael Gore** mag87@cornell.edu — *Genome-wide scan for structural polymorphisms in diverse maize germplasm*

P142 **Tsui-Jung Wen** tjwen@iastate.edu — *The Maize Genome Contains A High Frequency Of Nearly Identical Paralogs (NIPs)*

Genomics

P143 **Tsui-Jung Wen** tjwen@iastate.edu — *A High-Density Genetic Map Of Maize Transcripts*

P144 **Ron Okagaki** okaga002@umn.edu — *A radiation hybrid system for the genetic and physical mapping of the maize genome*

P145 **Nick Lauter** nickl@uiuc.edu — *Agronomic and basic science utilities of the intermated NC89 x K55 RIL (INKRIL) population*

P146 **Jiong Ma** jiongm@stanford.edu — *Analysis of Maize Anther Development Using the 21K Maize Oligo Array*

P147 **Nathan Springer** springer@umn.edu — *Applications of oligonucleotide microarrays for polymorphism detection and comparative genomic hybridization*

P148 **Baltazar Antonio** antonio@nias.affrc.go.jp — *Biological materials from the Rice Genome Resource Center*

P149 **Antonio de Oliveira** acostol@terra.com.br — *Brazilian Maize Genome Initiative*

P150 **Mary Polacco** PolaccoM@missouri.edu — *CIMDE: A community IBM genetic mapping service*

P151 **Heather Yates** hey2@cornell.edu — *Candidate gene association for genes influencing kernel starch composition in maize*

P152 **Wilfred Vermerris** vermerris@purdue.edu — *Cell wall genomics update: High-throughput screening for secondary cell wall defects using NIR spectroscopy*

P153 **Moises Cortes-Cruz** moises@waksman.rutgers.edu — *Collinearity of the Alpha Zein Genes Between Different Inbred Lines of Maize (Zea mays, L.)*

P154 **Susan Latshaw** latshaw@ufl.edu — *Confirmation and Analysis of Germinal Insertions Identified by Mutail Blast Clusters*

P155	**Brad Barbazuk** bbarbazuk@danforthcenter.org	*Consortium for Maize Genomics: Progress Towards an Effective Large Scale Whole Sequencing Strategy for the Maize Genome*
P156	**Agnes Chan** achan@tigr.org	*Consortium for Maize Genomics: Towards Assembly of the Maize Genome*
P157	**Anne-Celine Thuillet** athuillet@wisc.edu	*Detection of genes under selection during the domestication of maize from sequence data*
P158	**Peter Rogowsky** peter.rogowsky@ens-lyon.fr	*EST analysis of transition stage maize embryos*
P159	**Pietrantonio Costrini** pietrantoniocostrini@hotmail.com	*Effects of phosphorus on the growth of root and shoot in diverse maize inbred lines*
P160	**Owen Hoekenga** oah1@cornell.edu	*Functional and comparative genomic analysis of aluminum tolerance in Zea mays*
P161	**Masanori Yamasaki** yamasakim@missouri.edu	*Genomic Screening for Domestication and Improvement Genes in Maize*
P162	**Moises Cortes-Cruz** moises@waksman.rutgers.edu	*Genomic Structure of the Dicer-1 Homologue Gene in Maize (Zea mays, L.)*
P163	**Xiaolan Zhang** xzhang@plantbio.uga.edu	*Global expression analyses of genes involved in meristem organization and leaf initiation*
P164	**Joachim Messing** messing@waksman.rutgers.edu	*High Resolution Physical Map, Sequence Composition, and Genome Organization of Maize*
P165	**Carletha Blanding** crb1988@uncw.edu	*Identification of genes involved in UV-B stress responses by transcriptome profiling*
P166	**Randall Wisser** rjw29@cornell.edu	*Identification of loci under selection in a maize population recurrently selected for quantitative resistance to northern corn leaf blight*
P167	**Yan Fu** yanfu@iastate.edu	*Identification of ultraconserved sequences in grass genomes*
P168	**Catherine Svabek** cxs455@psu.edu	*Isolation and functional characterization of a flavonoid 3í hydroxylase corresponding to the red aleurone 1 locus of maize*
P169	**Ron Okagaki** okaga002@umn.edu	*Junction-linked primers for mapping repetitive elements*
P170	**Thomas Ruff** thomas.g.ruff@monsanto.com	*Large Scale Transformation and Trait Screening in Corn*
P171	**Matthieu Falque** falque@moulon.inra.fr	*Linkage mapping of 1454 new maize candidate gene loci*
P172	**Jack Gardiner** gardiner@ag.arizona.edu	*Microarray Resources for Maize*
P173	**Ruairidh Sawers** rjs47@cornell.edu	*Microarray analysis of maize bundle sheath and mesophyll photosynthetic differentiation*
P174	**Ratnakar Vallabhaneni** ratplant@hotmail.com	*Mining genome data to reconstruct gene families controlling plastid isoprenoid biosynthesis in the Poaceae*
P175	**Amanda Jones** 17431@udel.edu	*Molecular Marker Development for Map-based Cloning in Maize*

P176	**Qiong Zhao** qiongzhao@wisc.edu	*Molecular Population Genetics of Maize Domestication*
P177	**Wei Zhao** zhaow@cshl.edu	*Molecular and Functional Diversity in the Maize Genome*
P178	**Bi Irie Vroh** biv2@cornell.edu	*Physiological and molecular analysis of nitrogen uptake and utilization in maize*
P179	**Yan Fu** yanfu@iastate.edu	*Quality Assessment Of Maize Assembled Genomic Islands (MAGIs) And Experimental Verification Of Predicted Novel Genes*
P180	**Jianxin Ma** jma@uga.edu	*Recombination, Rearrangement, Reshuffling and Divergence in a Centromeric Region of Rice*
P181	**Georgia Davis** davisge@missouri.edu	*Root Growth Maintenance During Water Deficit: Physiology, Genomics and Proteomics*
P182	**William Briggs** whbriggs@wisc.edu	*SNP Genotyping for Diversity and Mapping Studies in Maize and Teosinte*
P183	**Wolfgang Goettel** goettel@waksman.rutgers.edu	*Sequence analysis of the P1-wr gene cluster*
P184	**Brent Kronmiller** bak@iastate.edu	*Sequencing a 1.3 Mb contig spanning the rf1 fertility restorer locus as a prototype to assess complex-genome coverage strategies*
P185	**Enrico Pe** enrico.pe@unimi.it	*Thirty putative MIR genes of maize and their relationship to rice and sorghum sequences*
P186	**Wesley Marchione** wmmarchione@dow.com	*Towards identification of a platform for routine SNP genotyping in corn*
P187	**Darren Morrow** djmorrow@stanford.edu	*Transcriptional Expression Profiling During Maize Anther Development in Normal and Male Sterile Mutants*
P188	**Wenjing Tao** taow@missouri.edu	*Transcriptome characterization of apical and basal regions of maize root growth zone under water deficit condition*
P189	**A. Leonardo Iniguez** aliniquez@wisc.edu	*Use of high density maskless 70mer NimbleGen microarrays to determine transcriptional orientation of 9364 unique maize sequences*
P190	**Nadine Hocker** nadine.hoecker@zmbp.uni-tuebingen.de	*Young primary roots as a model for heterosis studies in maize*

Quantitative Traits / Breeding

P191	**Junjian Ni** jn66@cornell.edu	*An Update on Gramene QTL Data Module*
P192	**Lucia Gutierrez** luciag@iastate.edu	*Association Between Molecular Markers Diversity and Phenotypic Diversity in Barley Species*
P193	**Godfrey Asea** asea.1@osu.edu	*Comparison of Phenotype and Genotype-based Selection Procedures for Multiple Disease Resistance in Maize*
P194	**Natalya Kozhukhova** natavolk@rambler.ru	*DNA Markers for Maize Genetics, Breeding, and Seed Production*

P195	**James Holland** james_holland@ncsu.edu	*Detecting epistasis with double-introgression near-isogenic lines of maize*
P196	**Stephen Szalma** stephen_szalma@ncsu.edu	*Diversity-based approaches to exploring genetic phenomena and quantitative traits in maize*
P197	**Emily Dunn** d1665@truman.edu	*Epicuticular wax components affect Lepidopteran-feeding behavior*
P198	**Diaa Al-Abed** dalabed@utnet.utoledo.edu	*Expression of CBF3 under the stress inducible promoter Rd29A using split-seed explant to enhance drought and cold tolerance in maize*
P199	**Sherry Flint-Garcia** Flint-GarciaS@Missouri.edu	*Genetic Diversity and Selection for Amino Acid Genes and Content in Diverse Maize*
P200	**Michael Lee** mlee@iastate.edu	*Genetic Mapping and Analysis of QTL Affecting Tassel Branch Number in Maize*
P201	**Tina Wambach** twambach@uoguelph.ca	*Genetic Variation for Maize Seedling Root Growth under Water-Deficit Stress*
P202	**Jai Dev** jdhp@rediffmail.com	*Genetic diversity in corn inbreds and its relationship to heterosis in single cross hybrids using RAPD markers*
P203	**Nicole Riddle** riddlenc@missouri.edu	*Genetic variation for the phenotypic effects of ploidy change*
P204	**Leilani Robertson** larobert@unity.ncsu.edu	*Identification of QTL Responsible for Resistance to Fusarium Ear Rot and Fumonisin Contamination in Maize (Zea mays)*
P205	**Paul Mason** pam20@cornell.edu	*Identification of Quantitative Trait Loci Important for Maize Aluminum Tolerance Using the Intermated B73 x Mo17 Population*
P206	**Asheesh Singh** asheesh@uoguelph.ca	*Identification of maize grain yield QTLs using an identical-by-descent limited recombinant inbred line population*
P207	**Guri Johal** gjohal@purdue.edu	*Identifying a natural suppressor of cell death in maize: implications for gene discovery, diversity evaluation and beyond*
P208	**Patrick Brown** pjb34@cornell.edu	*Inheritance of panicle architecture in sorghum*
P209	**Jennifer Jacobs** jacob155@umn.edu	*Inhibition of Pathogenic E. coli O157:H7: Analysis of Transgenic Maize Expressing Colicin E7*
P210	**Megan Stewart** mstewart@uoguelph.ca	*Mapping QTLs associated with Late-Season Cold Tolerance in Maize*
P211	**Ann Stapleton** stapletona@uncw.edu	*Mapping When Phenotype Measurements Are Not Well Behaved: Comparison of Recursive Partitioning with Composite Interval QTL Mapping*
P212	**Daisuke Fujita** dfujita@agr.kyushu-u.ac.jp	*Mapping of genes for antibiosis to green rice leafhopper, Nephotettix cincticeps Uhler, in exotic germplasm of rice*
P213	**Roberto Lizarraga Guerra** robertol@ag.arizona.edu	*Mappingof opaque 2 modifier genes in maize endosperm*
P214	**Elizabeth Lee** lizlee@uoguelph.ca	*Novel Mapping Population Structure - Identity-by-Descent Limited Recombinant Inbred Lines*

P215	**Amber Brown** brown@bio.fsu.edu	*QTL Mapping of Telomere Length-Regulating Factors*
P216	**Michael Gerau** mjgf36@mizzou.edu	*QTL analysis of root traits under two different water regimes*
P217	**Peter Bommert** bommert@cshl.edu	*QTL and phenotypic analysis implies a role for the fasciated ear2 gene in controlling ear seed row number*
P218	**Mercedes Murua** mercedes.murua@syngenta.com	*QTL mapping of resistance to Ustilago maydis in sweet corn*
P219	**Thomas Brooks** tbrooks@msa-msstate.ars.usda.gov	*Quantitative Trait Loci Associated with Resistance to Fall Armyworm and Southwestern Corn Borer Leaf Feeding Damage*
P220	**Kristen Leach** kalp55@mizzou.edu	*Response of Maize Primary Root Growth to Varying Degrees of Water Stress.*
P221	**Gael Pressoir** ghp5@cornell.edu	*Significant association between CLE genes and inflorescence architecture in maize*
P222	**Dana Bush** dlw3f9@mizzou.edu	*Survey of Aflatoxin Levels among Diverse Maize Germplasm*
P223	**Peter Balint-Kurti** peter_balintkurti@ncsu.edu	*The Identification and Analysis of Maize QTLs for Southern Leaf Blight and Gray Leaf Spot Resistance.*
P224	**Jianming Yu** jy247@cornell.edu	*The Power of the Joint Linkage and Association Mapping in Plants*
P225	**Mark Jones** jones.390@osu.edu	*The effects of combining regions of chromosomes 3, 6 and 10 on resistance to Maize dwarf mosaic virus (MDMV) and Sugarcane mosaic virus (SCMV)*
P226	**Joshua Meyer** jimeyer@uiuc.edu	*Using Diverse Maize Germplasm to Investigate NUE*

Transposable Elements

P227	**Bryan Penning** bpenning@purdue.edu	*A PCR based strategy to clone genes tagged by somatic mutagenesis*
P228	**Liza Conrad** ljc28@cornell.edu	*A two-component Activator/Dissociation tagging system in Maize*
P229	**Ken Naito** kanito@plantbio.uga.edu	*Amplification of mPing and Ping Transposable Elements in the rice cultivar Gimbozu.*
P230	**Yi Hou** houyi@iastate.edu	*Centromeric retrotransposons: potential role of the integrase C-terminus in determining genomic distribution*
P231	**Cagla Altun** caltun@purdue.edu	*Characterization of Maize Mre11 Genes*
P232	**Cesar Alvarez-Mejia** calvarez@ira.cinvestav.mx	*Establishing enhancer detection and activation tagging as strategies to reveal gene function in maize.*
P233	**Leta Tulu Bedada** letatb@yahoo.com	*Heterosis and Combining Ability of Selected East African Maize (Zea mays L.) Populatins*
P234	**Bao-Cai Tan** bctan@mail.ifas.ufl.edu	*Identification of Candidate Genes in Six Endosperm Mutants in Maize*

P235	**Hee Chung Ji** hji@hawaii.edu	*Inheritance of Husk Leaves in Maize*
P236	**David Skibbe** skibbe@stanford.edu	*Investigation of Mu transposition using proteomics*
P237	**Alan Smith** alansmith@wisc.edu	*Microarray-based transcription analysis of maize repetitive elements in tissue culture reveals sense and antisense transcription*
P238	**Zhennan Xu** zhennan@waksman.rutgers.edu	*Mx-rMx, a family of interacting transposons in the growing hAT superfamily of maize*
P239	**Damon Lisch** dlisch@uclink.berkeley.edu	*Position-dependent escape from establishment of silencing.*
P240	**Sonia Walia** walias@umich.edu	*Searching for Helitrons and Helitron- Related Transposable Elements in the Maize Genome*
P241	**William Eggleston** weggles@saturn.vcu.edu	*Sex-Specific Differences in the Frequency and Timing of Reversion of PIF-Associated r1 alleles*
P242	**Margaret Woodhouse** branwen@berkeley.edu	*Silenced Mutator elements become progressively and heritably reactivated through multiple generations in a mop1 (modifier of paramutation1) mutant background*
P243	**Dawn Holligan** dawn@plantbio.uga.edu	*Transposable Elements in the model Legume Lotus japonicus: abundance, diversity and intra-specific polymorphism*
P244	**Thomas Peterson** thomasp@iastate.edu	*Transposon-Induced Rearrangements*

Late Submissions

P245	**Doreen Ware** ware@cshl.edu	*The Gramene Database*
P246	**Zuxin Zhang** zux_zhang@263.net	*Fertility restoration mechanisms in CMS-S of maize (Zea mays L.) revealed through expression differences identified by cDNA microarray and suppression subtractive hybridization*
P247	**Zuxin Zhang** zux_zhang@263.net	*Revelation on early response and molecular mechanism of submergence tolerance in maize roots by microarray and suppression subtractive hybridization*
P248	**Tong Zhu** tong.zhu@syngenta.com	*Development of Maize Ultra High-Density Gene Map Using Single Feature Polymorphisms Detected by GeneChip Microarray*

Plenary Address Abstracts

Plen1

Genetic and Cellular Analysis of Signaling, Branching, and Phyllotaxy in the Maize Shoot Meristem

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Plant growth and development relies on pools of stem cells within specialized structures called meristems. How these stem cells proliferate and how their descendants are patterned will determine the overall form of the adult plant. We have taken a genetic approach to understanding genes and pathways that regulate maize shoot morphogenesis. Our studies have not focused on any particular molecular pathway, however all of the genes that we isolated appear to be involved in pathways for intercellular communication, highlighting the importance of cell to cell signaling in plant development. Different communication strategies will be discussed, from intercellular trafficking of homeodomain proteins to more canonical receptor-ligand type interactions. I will also discuss our discovery of a role for cytokinin signaling in meristem size and phyllotaxy, and cloning of the *RAMOSA3* gene that regulates inflorescence branching in maize.Despite many recent advances from forward and reverse genetics, we are still limited by the relatively low number of genes that give a detectable phenotype upon mutation. I will therefore describe approaches to tag plant genes in high throughput for localization or protein interaction studies. Such approaches can aid in understanding the functions of all plant genes in the absence of discernable knockout phenotypes.

Plen2

Unraveling the Ends: Telomere Function and Dysfunction in Arabidopsis

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Telomeres are nucleoprotein caps at the ends of eukaryotic chromosomes that facilitate terminal DNA replication by the telomerase reverse transcriptase, and provide stability to the genome. *Arabidopsis* is a useful model to explore aspects of telomere biology, as it is a genetically tractable organism with an extraordinary tolerance to genome instability. Our research is currently focused on understanding how telomerase and other telomere-associated proteins influence telomere dynamics and chromosome end protection. The length of the telomeric DNA tract is strictly maintained within a species-specific set point. We have recently shown that the set point is achieved by the intermittent action of telomerase on the shortest telomeres in the population. To learn more about telomere dynamics, we analyzed three POT (Protection Of Telomere) genes from Arabidopsis that encode single-strand telomere DNA binding proteins. Intriguingly, mammals have only a single POT gene. Arabidopsis *POT1* and *POT3* genes influence telomere length. Disruption of *POT1* leads to progressive telomere shortening, although telomerase activity is unperturbed, implying that *POT1* is a positive regulator of telomere length involved in telomerase recruitment. *POT3* plays a role in establishing the set point for telomere length, since a point mutation in this gene results in shorter, but stable telomeres. The predominant function of *Pot2* is not in telomere length regulation, but rather in chromosome end protection, as over-expression of a dominant negative allele of *AtPOT2* leads to end-to-end chromosome fusions. Telomere dysfunction arising from a failure to maintain telomeric DNA or perturbation of an essential telomere binding protein ultimately activates a DNA damage response. Paradoxically, DNA repair proteins associate with wild type telomeres and are required to prevent telomere fusion. To explore this enigmatic relationship, we took a genetic approach to examine the role of *KU70* and *LIG4*, essential components of the non-homologous end-joining (NHEJ) pathway, and the DNA damage sensing PI-3 kinases, *ATM* and *ATR*, on telomere biology. Our data reveal that *KU70* is a negative regulator of telomere length, and that both *KU70* and *LIG4* act in concert with telomerase to maintain telomere length. When a telomerase deficiency is combined with either *ku70* or *lig4*, telomeres undergo accelerated shortening. Remarkably, triple *ku70 lig4 tert* (telomerase) mutants display a much more severe phenotype and their telomeres are subjected to catastrophic nucleolytic attack. Thus, NHEJ proteins make distinct contributions to chromosome end-protection and telomere maintenance. *ATM* and *ATR* also contribute to chromosome end protection. However, *ATR* plays an additional role in telomere maintenance; telomere shortening is accelerated in *atr tert* mutants at a rate that is similar to *lig4 tert* or *ku70 tert* mutants. Taken together, these data indicate that telomere maintenance and end protection are accomplished by a suite of factors that interface with telomerase and regulate exposure of the chromosome terminus.

Plen3

Unraveling the Meiotic Chromosome Condensation Network using Maize Mutants

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Maize has four rad21/rec8 genes and several of them are expressed in meiotic tissue. afd1 (absence of first division 1), closest in homology to yeast rec8, controls leptotene chromosome assembly and sister chromatid cohesion (SCC). In a deletion allele, equasional instead of reductional segregration of sisters occurs during MI due to a failure of both arm and centromere SCC. Early meiotic prophase chromosome structure is altered; there is little axial element formation, few rad51 foci are loaded onto chromosomes and neither pairing, synapsis nor recombination takes place. In two weak alleles leptotene chromosomes form but their function is abnormal; they do not pair, synapsis or undergo recombination, and SCC is abnormal at MI. We suggest that afd1 regulates leptotene chromosome function in a dose dependent fashion. The mutant mtm99-31, undergoes a normal meiotic prophase and MI, however sister chromatids precociously separate from each other during the second interphase, leading to missegregation of sisters at MII. This phenotype is similar to that described for mutations in yeast Shugoshin (sgo1) and Drosophila, mei-s322. There are at least two sgo genes in maize. mtm99-31is the null allele of Zmsgo1and analysis of weaker alleles of sgo1 are in progress.

Plen4

Function and Specificity of Plant microRNAs: Insights from Whole-Genome Studies

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Small RNAs including microRNAs (miRNAs) are potent agents of gene and genome regulation, but we are only beginning to understand how they discriminate between different potential targets, and how they affect different biological pathways. I will present three topics relating to this question. I will begin by discussing how we discovered two years ago the first plant miRNA mutant. Next, I will describe the use of transcript profiling to identify miRNA targets, including the possibility of off-target effects. In addition, I will present studies on the signaling pathways controlled by one of the miRNAs, miR319. If time permits, I will also briefly discuss insights into plant miRNA evolution.

Talk Abstracts

T1

Functional Specialization of Chloroplasts in Mesophyll and Bundle Sheath Cells in Zea mays: A Comparative Proteome Analysis

(presented by Klaas van Wijk <kv35@cornell.edu>)
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Maize leaves develop from leaf base to leaf tip. The base of the leaf contains undifferentiated chloroplasts, whereas chloroplasts at the tip of the leaves are differentiated in specific bundle sheath (BS) and the mesophyll (M) chloroplasts. M and BS chloroplasts each accumulate distinct sets of photosynthetic enzymes enabling them to cooperate in C4 photosynthesis. This study aims at deciphering potential differentiation of other chloroplast functions through a comparative proteome analysis of fully differentiated M and BS chloroplasts. We present data from 3 complementary approaches: (i) 2-DE gels protein separation of BS and M chloroplast stroma, followed by image analysis and identification of proteins by Mass Spectrometry (MS), (ii) Differential labeling with Stable Isotope Coded Affinity Tags (ICAT), followed by quantification and identification by nano-LC-ESI-MS/MS, and (iii) Comparison of nanoLC-ESI-MS generated ion chromatograms of trypsinized BS and M chloroplast stromal proteomes. Quantified peptide pairs were subsequently identified by MS/MS. After statistical analysis, we found that starch and sulfur metabolism related enzymes are preferentially expressed in the BS fraction, whereas anti-oxidative metabolism was clearly up-regulated in the M chloroplasts. Differential expression of other proteins and pathways will be discussed. We will also comment on search strategies for MS based identification of maize proteins, using available maize and rice EST and genome assemblies. These data will become available via our Plastid Proteome Data Base, PPDB at http://ppdb.tc.cornell.edu/. This is part of a plant genome proposal. The general scope of the genome proposal will be outlined. Funding by NSF-genome - PGRP#0211935

T2

Expression of C4 Photosynthetic Characteristics in Oat-Maize Addition Lines

(presented by Richard Kowles <dkowles@smumn.edu>)
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Oat-maize addition lines have been successfully generated, and they are available for every maize chromosome, 1 through 10. Addition lines are oat plants (C3 photosynthesis) that include one or more chromosomes from maize (C4 photosynthesis). These oat-maize addition lines may be useful to determine the extent to which individual maize chromosomes contribute to C4 photosynthesis and for the identification of the precise chromosomal regions involved in C4 photosynthesis. Investigations have been conducted that focused on the expression of several C4 enzymes and C4 leaf morphological characteristics in these oat-maize addition lines. Immunoblotting with polyclonal antibodies against phosphoenolpyruvate carboxylase (PEPc) and pyruvate orthophosphate dikinase (PPDK) on leaf protein extracts confirmed the location of the PEPc gene to chromosome 9 and the gene for PPDK to chromosome 6. Assays with leaf extracts showed activity of the enzymes in the oat-maize chromosome 9 and 6 lines, respectively. Indirect immunocytological tests with monoclonal antibodies showed widespread gene expression for PEPc in mesophyll cells of the oat-maize chromosome 9 line and PPDK in mesophyll cells of the oat-maize chromosome 6 line. Cytological investigations with cryostat microtomed leaf tissue indicated marked differences in cellular leaf structure, chloroplast size, and leaf attachment in the oat-maize chromosome 3 line. The production of suberin, another C4 characteristic, appeared to occur in the oat-maize chromosome 4 line. These results demonstrate that oat plants containing alien maize chromosomes exhibit both enzymatic and morphological C4 photosynthetic characteristics.

T3

Fourier Transform Mid-range and Near-infrared Spectroscopies in Maize Genomics

(submitted by Tiffany Langewisch <langewit@purdue.edu>)
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Discriminant analysis of Fourier transform infrared (FTIR) and near-infrared (NIR) spectra have been developed as high through-put methods to identify mutations in maize cell wall polysaccharide components and wall architecture. Maize lines carrying segregating sequence-tagged Mu-insertions introgressed into the inbred W22 were generated and used as primary material for mutation screens. Disrupted genes of mutants are identified based on the position of the insertional element. Reverse-genetics DNA grids representing nearly 20,000 lines are being developed to identify those with Mu elements near cell-wall relevant genes of interest. The Type II primary walls of grasses 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 obtained Fourier transform infrared spectra through the entire time course of elongation at one-half-day intervals to establish the spectral features correlated with the dynamics of cellulose, (1,3),(1,4)-beta-D-glucan and and other beta-glycans. Application of Artificial neural and Kohonen network algorithms to the spectral populations showed that walls were classified to their correct one-half-day growth stages with probabilities ranging from 62 to 96%. Using the natural variance we were able to correlate sugar composition for the most abundant monosaccharides by class modeling. We tested the predictive capabilities of the assignments of monosaccharide composition and (1,3),(1,4)-beta-D-glucan content in five maize inbred lines and one hybrid line and found the model to be robust. We now use this model to identify and predict specific defects in cell wall composition of mutants identified in a UniformMu insertional population of maize.
Supported by the NSF Plant Genome Research and REU Programs.

T4

A Modified Shrunken-2 Allele Enhances Maize Yield

(presented by L Curtis Hannah <Hannah@mail.ifas.ufl.edu>)
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A maize transgene containing two changes within the Sh2 locus was transformed into maize, and simple, single copy events were identified. Sh2 encodes the large subunit of the allosterically-regulated, rate-limiting starch biosynthetic enzyme, adenosine diphosphate glucose pyrophosphorylase. Changes were Rev6, a two amino acid insertion in the carboxyl terminus that reduces sensitivity to physiological inhibitor, phosphate, and HS33, a change that enhances heat stability by strengthening interactions between the small and large subunits. Eight transgenic events were crossed twice to an inbred containing the sh2 null allele, sh2-R. This generated plump seed containing (i) only the endogenous gene in heterozygous condition, (ii) only the transgene in hemizygous condition and (iii) the endogenous gene in heterozygous condition and the transgene in hemizygous condition. Seeds of the three classes were planted in the spring 04 in Citra Florida. Following selfing of resulting plants, ear weight, kernel weight and kernel number/ear were determined. Field data detailing the results of this experiment and the impact of expressing transgenic AGP in maize seed will be discussed.

T5
Involvement of an RNAse III Homolog in Group II Intron Metabolism in Chloroplasts

(presented by Amy Cooke <acooke@gladstone.uoregon.edu>)
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Plant mitochondrial and chloroplast genomes each contain ~20 group II introns, a class of ribozyme proposed to resemble the progenitor of the nuclear-spliceosome. Although group II introns are typically referred to as "self-splicing", plant group II introns are degenerate and require proteins for their splicing in vivo. Previously, we used genetic approaches to identify four nuclear-encoded proteins that are necessary for the splicing of different subsets of the group II introns in maize chloroplasts: CRS1, CRS2, CAF1, and CAF2. Genetic and biochemical data suggest, however, that these proteins are not sufficient to promote the splicing of their target introns. To identify additional splicing factors, we immunopurified CRS1, CAF1, and CAF2-containing intron ribonucleoprotein particles from chloroplast extract, and analyzed the copurified proteins by mass spectrometry. An RNAse III homolog, referred to here as RNC, was identified in both CAF1 and CAF2 immunoprecipitate pellets. Bacterial RNAse III functions in rRNA and mRNA processing. Maize RNC has the catalytic "RNAse III" domain but lacks the dsRNA binding domain characteristic of bacterial RNAse III, suggesting it may have evolved distinct functions. To identify RNC substrates, we used RNA coimmunoprecipitation coupled with microarray probing ("RIP-chip") to identify the RNAs to which it is bound in vivo. RNA purified from RNC immunoprecipitations was labeled with fluorescent dyes and hybridized to a full-genome chloroplast microarray. This assay revealed an association between RNC and the unspliced precursors of two intron-containing tRNAs, trnI and trnA. To determine the role of RNC in chloroplast RNA metabolism, we obtained mutant RNC alleles through a reverse-genetic screen of our collection of Mu-induced non-photosynthetic maize mutants. Preliminary analyses suggest that trnI and trnA splicing are disrupted in the RNC mutants. These and other results suggest that the chloroplast RNAse III homolog RNC is a new group II intron splicing factor, and that it may not function in chloroplast rRNA processing.

T6
The Terpene Synthase Family in Zea mays and Its Role in Defense Against Herbivory

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Terpenes constitute the largest group of plant products and display an extreme chemical diversity. Many of these compounds have an essential role in plants and constitute hormones, membrane components and pigments, but most terpenes are not vital for plant growth and development and are involved in plant defense against other organisms. Maize was shown to release complex terpene blends after herbivore attack which attract natural enemies of the attacking herbivores and thereby serve as a plant defense.
In an effort to understand the ecological function of the complex terpene blends as well as the genetic and molecular basis of terpene diversity, we started to study maize terpene biosynthesis. The key step in terpene biosynthesis is catalyzed by the enzyme class of terpene synthases. In maize, we identified a large and diverse gene family encoding terpene synthases. The biochemical characterization of these enzymes revealed that most terpene synthases form multiple terpene products. Comparison of the terpene synthase *in vitro* activities with the terpene blends released by maize plants indicates that five differentially regulated multiproduct terpene synthases are sufficient to produce the complex maize sesquiterpene hydrocarbon blends. To understand the molecular genetic and biochemical mechanisms controlling terpene variation between maize varieties, we studied terpene production in the husks of the varieties B73 and Delprim. The complex stereospecific differences between the terpene blends of both varieties were determined by four amino acid substitutions between the terpene synthases TPS4 and TPS5. Although both varieties contain *tps4* and *tps5* alleles, their differences in terpene composition results fom the fact that B73 has only a single functional allele of *tps4* and no functional allele of *tps5*, whereas Delprim has only a functional allele of *tps5* and no functional alleles of *tps4*.

T7

The Genetic Basis of Increased Maize Silk Maysin Levels Through Recurrent Selection

(presented by Jenelle Meyer <jdfce1@mizzou.edu>)
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Recurrent selection is a standard procedure used by plant breeders to increase favorable alleles for a desired trait in a population, however the nature of the genes underlying the selection gain are generally unknown. Breeders have used recurrent selection to synthesize to populations for high levels of maysin, a C-glycosyl flavone in silks that confers resistance to the corn earworm. The two populations are EPM (exotic populations of maize) and SIM (southern inbreds of maize). Silks from inbred lines derived from cycle 6 of the EPM population (EPMC6S8) and cycle 5 of the SIM population (SIMC5S8) had maysin values of 3.4 and 1.5% fresh silk weight, respectively. To address the question of what genes underwent selection to result in gains to such high levels of maysin, three quantitative trait locus (QTL) populations were analyzed using EPMC6S8, SIMC5S8, and GT119 (a low maysin inbred) as parental lines. The QTL detected have similar locations to the previously reported candidate genes p, whp1, c2, and in1. The p gene encodes the main transcription factor of the flavonoid pathway and chalcone synthase, which synthesizes the first committed step of the flavonoid pathway is encoded by c2 and whp1. The intensifier gene (in1) is believed to regulate whp1 expression. Real time RT-PCR has been utilized to confirm these candidate genes as QTL in the (EPMC6S8 x GT119)F2 population. Our experiments suggest that recurrent selection worked to increase the levels of maysin by accumulating favorable alleles of both regulatory and structural genes in the flavonoid pathway.

T8

The Maize QTL Vgt1 Corresponds to a ca. 2.7-kb Non-Coding Region Upstream of an Ap2-like Gene

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The molecular basis of natural quantitative genetic variation is poorly understood, although it entails the majority of traits of agronomic importance in crop species. The Vegetative to generative transition1 (Vgt1) QTL, controlling most of the variability for flowering time in a nearly isogenic cross, was mapped to a 1.3 cM interval (Salvi et al., Plant Mol Biol. 48:601-613) on chromosome 8 (bin 8.05). Closely associated molecular markers were then utilized for probing a proprietary contigued BAC library. Further mapping allowed us to identify the relevant BAC clone, within which a region of ca. 2.7 kb was found to be completely linked to the QTL. BAC sequencing and annotation revealed that the Vgt1 lies ca. 70 kb upstream of an Ap2-like transcription factor, already known to be involved in controlling flowering time in Arabidopsis. This finding generated the hypothesis that allelic variation at Vgt1 influences flowering time through a modulation of the Ap2-like downstream gene. The involvement of the Ap2-like gene on the control of flowering time in maize has been investigated with different gene validation methods. As an independent proof of the role and position of Vgt1, a significant association was found between haplotype variants at Vgt1 and flowering time on a representative set of maize inbred lines.

T9

Identification and Characterization of DNA Sequences Required for Paramutation of the b1 Gene in Maize

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Paramutation is a natural gene silencing phenomenon whereby interaction between two specific alleles leads to a heritable decrease in gene expression of one of those alleles. At the b1 locus in maize, paramutation occurs when the B-I allele, which confers dark purple pigment, is converted to Bí, an allele that confers light purple pigment. The allele causing paramutation (Bí) is called paramutagenic and the allele sensitive to paramutation (B-I) is called paramutable. Alleles that are insensitive to paramutation (i.e. B-P and b) are called neutral alleles. Sequences required for paramutation and for high expression of the b1 gene were mapped to a 6 kb region, located 100 kb upstream of the b1 transcription start site (Stam et al., Genetics 162:917-930, 2002; Stam et al., Genes Dev 16:1906-1918, 2002). In this region, B-I and Bí have 7 tandem repeats of an 853 bp sequence otherwise unique in the genome; other alleles have one. The 6 kb region is identical in B-I and Bí, demonstrating that epigenetic mechanisms mediate the stable silencing associated with paramutation. We have used a transgenic approach to test whether the repeats are sufficient for paramutation activity. Plants containing the intact tandem repeats and a neutral b1 allele were generated and tested for their ability to paramutate B-I. Plants carrying the repeat transgene (5 independent lines) were able to paramutate the B-I allele, showing that all the necessary sequences required for paramutation are included in the transgene and they can function from an ectopic location. The Bí state induced by the transgene was shown to be heritable and paramutagenic, although not as fully penetrant as the endogenous Bí allele. Trans-acting factors mop1-1 and Mop2-1, which affect establishment and maintenance of paramutation, also affect paramutation activity of the repeat transgene. Plants containing both the paramutagenic transgene and either trans-acting mutant were not able to paramutate the B-I allele.

T10

Heritable Silencing of the *Mutator* Transposon Family is Initiated by the Naturally Occurring Inverted Repeat *Mu killer*

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It is suggested that post-transcriptional (small RNA-based) gene silencing evolved to suppress genomic parasites such as transposable elements. However, these conclusions are based on examination of transposons that are stably silenced - they address maintenance rather than initiation of silencing. Here we describe the cloning and characterization of a naturally occurring maize locus that is competent to heritably silence the otherwise highly active MuDR transposon in maize. This locus, Mu killer (Muk), results from the inverted duplication of a partially deleted autonomous MuDR element located at the breakpoint of a genomic deletion. The resulting locus produces a polyadenylated hybrid transcript that includes a 2.7 kb hairpin that has sequence identity to a portion of the functional MuDR transposase. This hairpin transcript produces small RNAs that are amplified when the target MuDR transcript is present, providing the first direct evidence that initiation of silencing of a transposon family is a small RNA-based process.

T11

Readout Transcripts from the Dasheng Retrotransposon Alter the Expression of Adjacent Rice Genes

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Transposable elements (TEs) are the major component of most plant genomes comprising over 50% of some grass genomes. Retrotransposons with long terminal repeats (LTRs) in direct orientation are the most abundant class of TEs in plants. Retrotransposons resemble retroviruses in their structure and mode of transposition. They move to a new genomic location via an RNA intermediate that is reverse-transcribed into an extrachromosomal cDNA which then integrates into a new location. Transcription of LTR retrotransposons initiates from a promoter in the 5í LTR and terminates in the 3í LTR. Readout transcripts from TEs into adjacent sequences usually initiates from a promoter in the 3í LTR as well as from promoters of solo LTRs. Solo LTRs are those that generated as a result of homologous recombination between the LTRs of full-length elements. While most TEs contain inactivating mutations, others are reversibly silenced by epigenetic mechanisms, mainly cytosine methylation. The presence of TEs in databases of expressed sequence tags (ESTs) suggests that they can escape silencing. Moreover, promoters in LTRs have been shown to be induced by various stresses, such as tissue culture or protoplast isolation. It was previously shown that polyploidization in wheat activates retrotransposon promoters leading, in some cases, to readout transcripts into adjacent genes. In this study we report a more comprehensive analysis of this phenomenon by exploiting the resources of rice, including the precise genomic locations of ~1000 members of a recently amplified retrotransposon family (Dasheng). To this end we performed a whole genome analysis of Dasheng methylation and readout transcription and assessed the impact of LTR methylation on the expression of adjacent rice genes. Here, we show that tissue-specific LTR methylation can lead to tissue-specific readout transcription and to tissue-specific effets on adjacent gene expression. In addition, Dasheng insertion sites that are polymorphic in the two O. sativa subspecies japonica and indica, can also lead to flanking gene expression differences in these two subspecies.

T12

Genetic and Hormonal Regulation of Inflorescence Development

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Axillary meristems produce branches and flowers and therefore play a fundamental role in plant architecture and reproduction. Most dicot inflorescences have two types of axillary meristem - branch and floral meristems. However, maize inflorescences have four types of axillary meristem - branch, spikelet pair, spikelet and floral meristems - leading to the production of a highly branched inflorescence. Therefore, maize is a good model system for axillary meristem development due to the production of these multiple types of axillary meristem. We have shown that auxin transport is required for axillary meristem initiation during maize inflorescence development. Treatment of maize plants with inhibitors of auxin transport, early in inflorescence development, blocks initiation of branch meristems. Moreover, treatment of maize plants with auxin transport inhibitors at later stages of inflorescence development, points to a previously unknown role for auxin in meristem determinacy and/or maintenance. The phenotypes of auxin transport inhibited plants bear striking resemblance to a large class of mutants in maize, called "barren inflorescence". One of these mutants is barren inflorescence2 (bif2) which makes fewer branches, spikelets and florets due to defects in the initiation and maintenance of axillary meristems. The bif2 gene was cloned by transposon tagging and shown to encode a serine/threonine protein kinase homologous to PINOID (PID), which has been proposed to regulate auxin transport in Arabidopsis. bif2 mutants have vasculature defects and reductions in basipetal auxin transport implying that bif2 regulates auxin transport in the maize inflorescence. We are identifying additional genes required for axillary meristem initiation in the maize inflorescence by characterizing other mutants in the barren inflorescence class.

T13

Organ-specific Regulation and Molecular Evolution of the Maize pericarp color1 Gene

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The maize pericarp color1 (p1) gene encodes an R2R3 Myb-like transcription factor that regulates the flavonoid biosynthetic pathway in floral organs, most notably kernel pericarp and cob. Alleles of the p1 gene condition distinct tissue-specific pigmentation patterns; to elucidate the molecular basis of these allele-specific expression patterns, we characterized two novel P1-rw (red pericarp/white cob) alleles, P1-rw1077 and P1-rw751::Ac. Structural analysis of P1-rw1077 indicated that this allele was generated by recombination between p1 and the tightly linked paralogous gene, p2. In the resulting gene, the p1 coding sequence was replaced by the p2 coding sequence, while the flanking p1 regulatory sequences remained largely preserved. The red pericarp color specified by P1-rw1077 suggests that the p1- and p2-encoded proteins are functionally equivalent as regulatory factors in the flavonoid biosynthesis pathway. Sequence analysis shows that the P1-rw1077 allele lacks a 386 bp sequence in a distal enhancer region 5 kb upstream of the transcription start site. An independently-derived P1-rw allele contains an Ac insertion into the same sequence, indicating that this site likely contains cob glume-specific regulatory elements. The striking phenotype of the p1 gene could be the subject of human selection. To elucidate the evolutionary history of p1, 4 new simplex P1-rr and P1-rw alleles were characterized and compared with P1-rr4B2, P1-rw1077 and P1-wr alleles. The results indicated that all p1 alleles examined so far arose from a single ancestral p1 gene, which was generated by a segmental duplication event approximately 2.7 mya. The structure of the distal enhancer region is associated with the expression of each allele in cob glumes. A sequential evolution model is proposed to account for the variations in the distal enhancer. The results suggest that the distal enhancer region diversified rapidly, possibly because the close proximity of the direct repeats flanking the p1 gene increased the frequency of recombination between them.

T14

Identification of Two Maize MADS Box Transcription Factors that Promote Floral Transition

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The transition from vegetative to reproductive development is a critical point in plant development that enables reproductive success. During the vegetative stage of growth, the shoot apical meristem (SAM) produces leaves. At the transition to reproductive growth, leaf initiation is repressed in the SAM and inflorescence initiation commences. Genetic control of the floral transition in maize is poorly understood mostly due to the paucity of flowering time mutants. In order to discover genes involved in the floral transition in maize, we conducted genome-wide RNA expression profiling of the shoot apical meristem at vegetative stages and during the transition to reproductive development using MPSS (Massively Parallel Signature Sequencing), an open-ended expression profiling technology. Computational analysis of MPSS tags revealed a number of candidate genes that were differentially expressed in the meristems before and during the floral transition. We found two MADS box transcription factors that were up-regulated during the floral transition and named them ftm1 and ftm2 (Floral Transition MADS). The pattern of expression, homology and synteny of ftm1 and ftm2 to the wheat vernalization1 gene indicate that the maize ftm genes belong to the subfamily of MADS genes playing a role in the floral transition in monocots. The early flowering phenotype of maize transgenic plants over-expressing ftmís genes confirmed their function as promoters of flowering in maize.

T15

microRNA 172 Downregulates glossy15 to Promote Vegetative Phase Change in Maize

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Shoot development in many higher plant species is characterized by phase change, where meristems and organs transition from one set of identities to another. The transtion from a juvenile to adult leaf identity in maize is regulated by the APETALA2-like gene glossy15 (gl15). We demonstrate here that increasing g115 activity in transgenic maize not only increases the number of leaves expressing juvenile traits, but also delays the onset of reproductive development, indicating that gl15 plays a primary role in the maintenance of the juvenile phase. We also show that the accumulation of a maize microRNA homologous to miR172 progressively increases during shoot development and mediates gl15 mRNA degradation. These data indicate that vegetative phase change in maize is regulated by the opposing actions of gl15 and miR172, with gl15 maintaining the juvenile phase and miR172 promoting the transition to the adult phase by downregulation of gl15. Our results also suggest that the balance of actvities between APETALA2-like genes and miR172 may be a general mechanism for regulating vegetative phase change in higher plants.

T16

RAB GTPases and the Maintenance of Developmental and Cellular Compartments During Leaf Development

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Maize leaves are compartmentalized into relatively distinct developmental zones soon after leaf initiation. Within these zones, cells are aligned and order is maintained by highly oriented cell divisions, directional cell expansion and differentiation. The mutation *warty1-0* causes cells to expand abnormally, resulting in cellular disorder and a typical wart-like leaf phenotype. *WARTY1* encodes ZmRAB2A1, a member of the RAB/YPT family of small GTP binding proteins, known to be involved in vesicle transport and secretion. In yeast and mammalian systems, each member of the large gene family directs vesicle trafficking in a distinct cellular compartment. For example, mammalian HsRAB1 and HsRAB2 function exclusively in the Golgi-ER compartment, as does the solitary YPT1 in yeast. To investigate the role of ZmRAB2A1 as a marker for both whole leaf and cellular compartments, we identified multiple duplicates of RAB1 and RAB2 in maize inbreds B73 and A619. Yeast complementation studies show that only ZmRAB1A and ZmRAB2A1 together fully complement the yeast temperature sensitive *ypt1* mutant. Four related *ZmRAB1* and *ZmRAB2* genes are expressed in unique growing compartments of the leaf and plant, with expression of *ZmRAB2A1(WARTY1)* restricted to the basal blade compartment only. Ectopic expression of *ZmRAB2A1* in upper blade compartments causes uncontrolled expansion and the formation of warts in the blade. We suggest that diverse leaf morphogenetic signals trigger cellular responses of oriented division and expansion, mediated by compartmentalized proteins, such as ZmRAB2A1 and its partners. In this way, order is maintained at both the cellular and whole leaf level during development.

T17

The Maize Domestication Gene, tga1, is Member of a Family of Transcriptional Regulators

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The most critical step in maize domestication was the liberation of the kernel from the hardened, protective casing that envelops the kernel in the maize progenitor, teosinte. This evolutionary step exposed the kernel on the surface of the maize ear such that it could be readily utilized as a food source by humans. Here, we show that this key event in maize domestication was driven by simple changes in a single gene belonging to a family of transcriptional regulatory genes. The functional differences between maize and teosinte map to a 1 kilobase region within which maize and teosinte show only six fixed differences in their DNA sequences. One of these fixed differences, which encodes a non-conservative amino acid substitution, represents the probable causative site. Our results demonstrate that modest genetic changes in single genes can induce dramatics changes in phenotype during domestication and evolution.

T18

Evaluating Sequencing and Assembly Strategies for the Maize Genome

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The repetitive nature of the maize genome poses significant challenges to genome sequencing. Total repeat content has been conservatively estimated in the range of 60-70% and the overwhelming majority of these repeats are class I retroelements. Their large size (up to 14kb in size) and relatively recent activity can cause problems with sequence assembly. In addition to the retroelement sequences themselves, there is also evidence that they have mediated the copying and movement of gene fragments around the genome, adding to the amount of repeated sequence in the genome. Several recent pilot projects funded by NSF and DOE have sampled the maize genome in various ways, including deep and shallow sequencing of BAC clones, sequencing from reduced representational libraries, and whole genome shotgun sequencing. In addition to the inherent strengths and weaknesses of each method for any genome, the specific characteristics of this genome, and the needs of the maize community require that a careful evaluation of these strategies be carried out prior to large-scale sequencing. To that end, we have used the different types of data produced by the pilot studies both alone and in combinations to evaluate which strategies are most likely to produce high quality, cost effective genome assemblies that meet the needs of the maize community. Results of these studies will be presented.

T19

Estimating and Annotating the Maize Gene Space

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Maize genome sequence data have been accumulating from two major sources: genome survey sequencing (GSS) and BAC sequencing. Our PlantGDB project (www.plantgdb.org) aids in the organization and interpretation of these data through the development of integrated database and analytical tools. Roughly 2.7 million maize GSS sequences from the B73 cultivar, generated by gene-enrichment approaches as well as random sequencing, were assembled and annotated with gene models. We have implemented a bottom-up annotation protocol, which seeks to identify contigs containing full-length maize genes based upon high quality spliced-alignment to well-annotated proteins (from Arabidopsis and rice) using the GeneSeqer suite of programs. So far, we have derived 5,735 full-length gene models from our GSS contigs aligning to 6,827 annotated Arabidopsis proteins, which belong to 33 super-families and 301 gene families. These identified maize genes provide a full-length gene collection, a significant addition to our current knowledge of the maize gene space. The full-length maize gene models also have enabled us to evaluate different gene-enrichment sequencing strategies. Our results indicate that the sampling space from High Cot (HC) and Methylation Filtration (MF) approaches are quite different: e.g., only 398 full-length genes are currently completely covered by both HC and MF sequencing methods independently. In addition, we have assembled and annotated about half a million publicly available sorghum GSSs. Complementary to our sites for Arabidopsis (www.plantgdb.org/AtGDB/) and rice (www.plantgdb.org/OsGDB/), a specialized genome browser (ZmGDB) is being set up to present detailed exon-intron gene structures, based on the threading of EST and full-length cDNAs onto the maize BACs, with attached web-based tools to facilitate user contributed annotation.

T20

Characterization of the rice genome based on the complete sequence

(presented by Baltazar Antonio <antonio@nias.affrc.go.jp>)
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As of December 2004, the International Rice Genome Sequencing Project (IRGSP) has completed a map-based and finished quality sequence of the rice genome. The completion of the rice genome sequence will have a great impact in understanding the biology of rice that provides a major food source for almost half of the worldís population. The sequence covers almost 95% of the estimated 390 Mb total length of the entire rice genome. The finished sequence has been characterized in terms of tandem gene families, transposable elements, single nucleotide polymorphisms, simple sequence repeats, insertion of organelle genome sequences and distribution of genes. Analysis of the nucleotide sequence of a centromeric region also provides significant information in understanding the structure of plant centromeres. This high-quality sequence will serve as a gold standard for understanding the function of individual genes and the gene-network involved in expression of complex agricultural traits. Since it is now widely known that rice has syntenic relationships with major grass species including maize, the complete sequence of the rice genome will provide a key informational and experimental tool for cereal genomics.

T21

The Maize TILLING Project: A Progress Report

(presented by Rita Monde <rmonde@purdue.edu>)
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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 in a mutant population. Non-silent, sub-lethal mutations will be valuable for functional genomics, studying a protein's biological function(s), its protein-protein interactions and enzymatic activity. The Maize TILLING Project (http://genome.purdue.edu/maizetilling/) is now open and is a collaborative effort to develop and screen TILLING populations for the maize research community. Populations of the inbreds B73 and W22, have been made by pollen mutagenesis with EMS. Our goal is 10,000 segregating M3 families for each inbred, and we now have ~12,000 M2 ears for each. Preliminary estimations of mutation density indicate the collected populations will segregate for over 100 million lesions. Our current TILLING population contains 3072 mutant lines (~20 million mutations) that are arrayed 2-dimensionally, enabling us to identify individuals harboring mutations in a single TILLING screen. Because maize is an ancient tetraploid we have successfully implemented a pre-screening step that combines amplification and thermal denaturation of the product before doing the TILLING reactions to improve our efficiency at successfully TILLING single target loci. Orders are considered complete when we identify at least one mutation with a 95% confidence level of having a damaging effect on the protein, and users of the service receive sequence information, predictions of mutation effects and stock numbers for seed, available through the Maize Genetic Stock Center. Our primer pre-screening and TILLING protocols will be explained further and a summary of our most recent results presented.

T22

Isolating Maize Kernel Composition Mutants by Near Infrared Spectroscopy

(presented by A. Mark Settles <settles@ufl.edu>)
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There is extensive interest in understanding the molecular control of seed composition in cereal grains. At a genetic level, seed composition is affected by loci that can give rise to visible seed phenotypes or quantitative traits. Quantitative trait loci (QTL) are inherently difficult to identify and track, because QTL have small effects on plant phenotypes and are environmentally sensitive. We tested whether single-kernel near infrared reflectance (NIR) and transmittance (NIT) spectroscopy can be used to observe genetic segregation of seed composition mutants in maize. Single-kernel NIR and NIT data were collected from known visible composition mutants in multiple inbred backgrounds and from visible defective kernel mutants in the UniformMu transposon-tagging population (McCarty et al, 2005). Principal Component Analysis (PCA) of these data indicates that NIR detects known composition mutants more robustly than NIT. To determine if single-kernel NIR is accurately reporting the internal composition of the kernel, we developed calibrations between the NIR spectra and analytically determined kernel composition data using partial least square regression (PLS). The PLS models have a good predictive power based on internal cross-validation and suggest that the NIR spectra report an absolute amount of the kernel components. To determine if single-kernel NIR could be used to identify more subtle seed composition mutants, we screened visibly normal UniformMu M2 families and found 14 putative nir mutants. Heritability tests with the nir isolates suggests that a large fraction of the putative mutants are heritable modifiers of kernel composition. These data indicate that single-kernel NIR can be used as both a qualitative tool to identify novel seed mutants and as a quantitative tool to determine the changes in kernel composition.

T23

A High Resolution Cytogenetic Map of Maize Chromosome 9 Constructed by Single Copy FISH

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High resolution cytogenetic maps provide important biological information on genome organization and function, as they correlate genetic distance with cytological structures, and are an invaluable complement to physical sequence data. The most direct way to generate a cytogenetic map is to hybridize genetically mapped genes directly onto chromosomes, and this has been difficult in maize. We optimized our FISH procedure and successfully detected 6-10 genetically mapped single-copy genes on chromosome 9. All probes were directly labeled with fluorophores, and the smallest probe is 3.4 kb. The FISH results indicate that sh1 and bz1, which are 3 cM apart, still can be distinguished as two distinct signals. The centromere region representing 0.3 cM spans ~30% of short arm of chromosome 9; however, the distal region of 9S that represents almost 30 cM spans only ~ 40% of short arm. In addition, we compared the chromosome positions of these genes to their positions inferred from breakpoint maps and recombination nodules-cM map. Unambiguous identification of each chromosome is of prime importance, and we successfully accomplished this using 6 different repetitive sequences as a probe cocktail to hybridize to 2-D pachytene chromosome spreads. To insure that the 2-D preparations reflected biologically relevant positions, we compared the position of two genes in 3-D preparations, and found that relative distances are preserved. As the cytogenetic FISH map grows, we can address the positions of genes relative to recombination events along the length of pachytene chromosome and even discover which regions of the chromosome are gene-rich. There are many other applications of this technique, including confirmation of contig positions, resolving gene order, and placement of markers which cannot be mapped by recombination-based methods. We hope that this technique can be used by many to generate an excellent cytogenetic map for our community.

T24

The Genomic Extent of Artificial Selection

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Artificial selection promotes rapid phenotypic evolution, but the number and function of loci affected by artificial selection is unknown for any domesticated species. We investigated the history of selection on the maize genome, based on single nucleotide polymorphisms (SNPs) at 774 randomly chosen genes in maize (Zea mays ssp. mays) and its wild progenitor teosinte (Z. mays ssp. parviglumis). Statistical analyses reveal two classes of maize genes: genes that experienced a neutral population bottleneck during domestication and genes that retained the footprint of artificial selection. Two to four-percent of maize genes belong to the selected class. Candidate selected genes, particularly those involved in plant growth, were significantly overrepresented in QTL regions mapped for phenotypic differences between maize and teosinte. Overall, these results suggest that ~1200 genes have been targeted by artificial selection during maize domestication and improvement.

T25

Evolution of DNA Sequence Non-Homologies Among Maize Inbreds

(presented by Stephan Brunner <Stephan.Brunner@cgr.dupont.com>)
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Allelic non-colinearities have recently been reported between maize inbred lines (Fu H. and Dooner H.K., PNAS 2002, 99: 9573-9578; Song R. and Messing J., PNAS 2003,100: 9055-9060). We set out to extend these observations by sequencing and comparing allelic chromosomal regions totaling more than 2.8 Mb, located on maize chromosomes 1L, 2S, 7L and 9S, over distances of 100-350 kb between the two maize inbred lines Mo17 and B73. The alleles contain extended regions of non-homology. On average, more than 50% of the compared sequence is non-colinear, mainly due to the insertion of large numbers of LTR-retrotransposons. Only 27 LTR-retroelements are shared between alleles, while 62 are allele-specific. The insertion of LTR-retrotransposons into the maize genome is statistically more recent for non-shared than shared ones. Most surprisingly, more than one third of the genes (27/72) are absent in one of the inbreds at the loci examined. Such “non-shared” genes usually appear to be truncated and form clusters in which they are oriented in the same direction. However, the non-shared genome segments are gene-poor, relative to regions shared by both inbreds, with up to 12-fold difference in gene density. In contrast, MITEs occur at a similar frequency in the shared and non-shared fraction. Many times, MITES are present in an identical position in both LTRs of a retroelement, indicating their insertion occurred prior to the replication of the retroelement in question. Maize ESTs and/or maize Massively Parallel Signature Sequencing (MPSS) tags were identified for the majority of the non-shared genes or homologs of them. In contrast to shared genes, which are usually conserved in gene order and location relative to rice, non-shared genes violate the maize colinearity with rice. Based on this, insertion rather than deletion events seem to be the origin of the non-shared genes. The intergenic space between conserved genes is enlarged up to 6 fold in maize compared to rice. Frequently, retroelement insertions create a different sequence environment adjacent to conserved genes.

T26

Intraspecific Gene Movement in the Maize Genome

(presented by Jinsheng Lai <jlai@waksman.rutgers.edu>)
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Maize has long been known to be one of the most diverse crops, yet a novel kind of genetic diversity was uncovered recently. The comparative analysis of the 9S bz genomic region in lines McC and B73 demonstrated that the genic content between two different inbreds of maize can be variable. Contrary to the general assumption that individuals within a species have the same gene content in each allelic locus, four or five consecutive genes present in the McC bz region were found to be missing from the same region in B73. To further investigate the possible cause for such a haplotype difference, we report here the isolation of a B73 BAC clone from chromosome 5 that contained some of the genes missing in the bz region. Complete sequencing of the BAC clone indicated that a contiguous 5.8-kb fragment containing two of the adjacent genes missing from the 9S bz region of B73 are present in a different location. Sequencing of a cDNA clone that is >99% identical with its genomic counterpart confirmed that at least one of the two translocated genes is transcriptionally active. The potential implications of the direct gene movement within the maize genome for maize genetic and breeding are discussed

T27

Sequence Comparisons of Seven Mitochondrial Genomes from Maize and Teosinte

(presented by Christiane Fauron <christiane.fauron@genetics.utah.edu>)
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We have completed sequencing of seven mitochondrial genomes within the genus Zea. In addition to the recently published male-fertile maize NB genome (Clifton et al., Plant Physiol. 2004), sequences of the NA and of three male sterile mitochondrial genomes, CMS-T, CMS-C and CMS-S, have been completed. Circular maps have been generated from the mtDNA sequence data for NB, NA, CMS-C and CMS-T, of 569,630, 701,046, 739,719 and 535,825 base pairs, respectively. The map generated from the sequence data for the 569,553-bp CMS-S mitochondrial genome is linear. MtDNA sequences have also been completed for two teosintes from section Luxuriantes. Both Zea luxurians and Zea perennis mtDNAs generate circular maps that are 539,368 and 570,354 bp long, respectively. Sequence comparisons reveal almost no differences in known gene content among all the Zea mitochondrial genomes, but do reveal major rearrangements, large duplications, and insertions of foreign DNA of unknown origin. This is the first detailed analysis of sequences and rearrangements among closely related plant mitochondrial genomes.

This work has been supported by National Science Foundation grant DBI-0110168.

T28

Simplifying the Grass Genome to Evaluate the Maize Genome

(presented by Wade Odland <odla0014@umn.edu>)
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We have developed an improved method for comparative genomics within the grass family that takes into account duplications and gene loss. Current comparative studies use rice as a simple genome for grass comparisons. However, rice is not a simple non-duplicated genome and consequentially masks regions of similarity. Our simplified reference for grass genome evaluations reveals a more complete view of genome duplication and structure. Additionally, we describe a more comprehensive view of candidate synteny regions within and between grass species. An in silico analysis was done using PERL programming, physical and genetic maps, and BLAST analysis with the assumption that regions sharing synteny are related. The model that we constructed can incorporate multiple grass genomes simultaneously so genome structures can be evaluated with a common reference and produces a visual output. An evaluation using this simplified reference in comparison to the maize genome has clarified the duplicate structure of the genome. Our results show that genic blocks within maize have three to four copies in the genome and we identified new regions of commonality. Syntenic blocks were detected across multiple grass genomes. This material is based upon work supported by the National Science Foundation under Grant No. 0110134.

Workshop Abstracts

W1

Ac as a Tool for Forward Genetics in Maize

(presented by Thomas Brutnell <tpb8@cornell.edu>)
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We are currently developing a series of near-isogenic lines each harboring a single active Ac transposon at a precise physical position and a well-resolved genetic location. As Ac tends to transpose to closely linked sites, precisely positioned Ac elements will greatly facilitate regional mutagenesis efforts. To date, we have genetically placed 158 Ac insertions on maize chromosome arms and precisely positioned 60 Ac elements on one of three publicly available recombinant inbred populations. Several of these sequences have also been integrated into annotations of maize genomic assemblies (http://www.plantgdb.org/prj/AcDsTagging/). To explore the potential and reveal the limitations of Ac in gene tagging programs, we have conducted a regional mutagenesis of the Pink scutellum1/Viviparous1 gene using a closely linked Ac as a donor element. We have identified over 21 Ac-induced alleles of ps1 and have created several stable Ac excision or "footprint" alleles. We have also initiated a non-targeted mutagenesis of chromosome 1 to examine the frequency of Ac-induced mutations and to determine the feasibility of such a non-targeted approach in large-scale tagging programs. Approximately 2000 F2 families, each harboring a unique tr-Ac have been screened for mutant kernel, seedling and mature plant phenotypes. DNA blot analysis has been performed on a number of these mutant families and in 15% of the families we have determined that a newly transposed Ac is linked to the mutant phenotype. Based on these findings, I will discuss potential genetic strategies for using Ac in regional mutagenesis programs.

W2

Steady-State Transposon Mutagenesis and Cloning of Tagged Mutants in silico

(presented by Donald McCarty <drm@ufl.edu>)
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We have implemented an integrated strategy for harnessing the power of the high-copy Mutator transposon for efficient molecular analysis of maize insertional mutants identified by forward and/or reverse genetics screens. Steady-state transposon mutagenesis is achieved by continuous backcrossing of active Mutator into an inbred background allowing maintenance of stable high mutation frequencies (7% independent seed mutation rate) with a consistent moderate transposon copy number (~57 total Mu elements; 1-2 MuDR copies per plant). The UniformMu-W22 population consisting of 36000 F2 ears yielded >2000 independent seed mutations. A comparable B73 derived population is under construction. We show that tagged mutants derived from these populations are amenable to conventional as well as bioinformatics based strategies for cloning tagged mutants. Libraries constructed from MuTAIL PCR products recovered flanking sequences from Mu germinal insertions with an estimated efficiency of 72%. Bioinformatics analysis of a dataset derived from 130 diverse seed mutants resolved an estimated 1735 insertions. Importantly, detection of alleles in the MuTAIL dataset facilitates identification and confirmation of mutant genes in silico. We have demonstrated this approach by cloning the widowís peak (wpk) aleurone mutant through MuTAIL analysis of 3 independent wpk alleles isolated from UniformMu. The number of mutants that may be cloned by in silico detection of alleles depends on the number of target loci in the phenotypic class of interest and the total number of mutations included in the MuTAIL database. We estimate that a dataset derived from 1000 mutants representing 300-600 seed loci will yield >200 pairs of allelic MuTAIL insertions identifying the causative insertions in at least 40% of the mutant collection by allele detection alone. These resources will be complemented by a mutant enriched reverse genetics facility will allow rapid PCR screening of the entire (3-6 X) seed mutant collection for confirming alleles for candidate genes.

W3

Chromosome Walking in Maize: Cloning and Expression Analysis of ramosa2

(presented by Esteban Bortiri <ebortiri@berkeley.edu>)

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ramosa2 (ra2) is a maize inflorescence mutant with defects in branching architecture. ra2 tassels produce numerous branches, many of which bear multiple single spikelets. ra2 ears have disorganized rows and often produce branches. Using the rice genome as a ëscaffoldí, we identified ra2 by positional cloning. The candidate gene shows no recombination with ra2 in over 1000 individuals examined. Five mutant alleles support this candidate gene as ra2. Two alleles are Mu transposon insertions in the first intron. The ra2-R allele is an 8 bp insertion near the N-terminus that introduces a stop codon. Another allele has a deletion of the first two thirds of the ORF. The fifth allele consists of a single mutation that causes an aminoacid change in a site conserved among orthologues in Arabidopsis, rice and maize. Northern blotting shows that ra2 is expressed in inflorescences but not in vegetative tissues. The ra2-R allele has very low to null levels of mRNA. In-situ hybridization shows that ra2 is expressed in a group of cells that gives rise to the meristems, but not in the meristem itself. This suggests a role for ra2 in the formation of meristems.

W4

Cloning The Indeterminate Gametophyte1 Gene Of Maize Using Tagging And Comparative Genomics

(presented by Matthew Evans <mmsevans@stanford.edu>)

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Plants have two phases to their life cycle: the diploid sporophyte and the haploid gametophyte separated by meiosis at one end and fertilization at the other. Female gametophyte development in many angiosperms is characterized by a phase of synchronous, free nuclear divisions that produce an eight nucleate syncytium. Mutations in the indeterminate gametophyte1 (ig1) gene of maize cause these free nuclear divisions to be variable in number. Consequently, mature ig1 mutant embryo sacs have a variety of structural defects, including extra egg cells, extra polar nuclei, and extra central cells. These abnormalities in turn lead to a variety of abnormal fertilization events. We have cloned the ig1 gene using a combination of comparative genomics and transposon tagging. The ig1 gene encodes a member of the LOB domain protein family of plant-specific transcriptional regulators. Plants homozygous for a Mu insertion allele of ig1 have abnormal leaf morphology. The identity and phenotype of ig1 in the gametophyte and sporophyte suggest that common logic is used to regulate sporophytic and gametophytic development.

W5

Map-Based Cloning in Maize: What Have We Learned?

(presented by Bailin Li <Bailin.Li@cgr.dupont.com>)
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Map-based cloning is a forward genetics (phenotype-based) gene discovery approach. It enables the discovery of genes or genetic elements responsible for inheritable variations. Map-based cloning requires a large mapping population, a large number of genetic markers for high-resolution mapping, a large-insert genomic library and a physical map (or genome sequence) to cover the gene locus. The first map-based cloned genes in model plant species were reported over a decade ago. However, map-based cloning in maize has not been considered feasible due to its large and complex genome size, and the lack of physical maps/genome sequences. Recently we constructed a whole genome physical map by high information content fingerprinting (HICF) of a maize Mo17 BAC library. Extensive manual curation has reduced the number of BAC contigs to 1836. Moreover, over 10,000 genetically mapped sequences have been placed onto the physical map. This enables us to anchor BAC contigs covering over 80% of the maize genome onto the maize genetic maps. With this integrated Mo17 physical/genetic map as well as the public B73 physical map, and working together with our colleagues and collaborators, seven map-based cloning projects have recently been completed in maize. An example of map-based cloning projects will be presented. General findings/issues (mapping populations size, marker development for fine mapping, comparison of positional cloning between Arabidopsis and maize, implications of co-linearity with rice genome and lack of co-linearity between maize inbred lines, etc) about map-based cloning in maize will be discussed.

W6

Recombinase-Mediated Plant Transformation

(presented by David Ow <ow@pgec.ars.usda.gov>)
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Transgene expression is determined by the molecular configuration of the DNA, and the placement of that DNA into the host genome. The former involves appropriate uses of regulatory elements and codon preferences that affect transcription, translation, localization and stability of the encoded products. Engineering specificities are generally case-specific for the gene, and possibly also for the plant host. In contrast, DNA transformation methods are generally applicable across a broad spectrum of genes and host genomes. Greater precision and efficiency in DNA integration can reduce the currently high rate of rearranged and multiple copy insertions that are more prone to inappropriate and unstable gene expression. The exact placement of DNA into known chromosome locations can also minimize unpredictable affects of neighboring genes. This presentation reviews what can be done, and what might be possible, with the use of site-specific recombination systems for plant transformation and the control of transgene expression.

W7

Position Effect of Targeted Integration at Different Transgene Loci in Higher Plants

(presented by Qiudeng Que <qiudeng.que@syngenta.com>)
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Gene targeting by homologous recombination occurs at low frequency in higher plants. Here we describe targeted insertion in transgenic loci using routine Agrobacterium-mediated transformation in several plants. It was observed that there was position effect of targeted integration at different loci. We also demonstrate that targeted integration into transgenic loci can be achieved in monocot crops rice and maize via homologous recombination by using routine Agrobacterium-mediated transformation. We also tested the effect of cleavage of transgenic loci with a novel mega-endonuclease I-CeuI and observed that co-expression of I-CeuI endonuclease during co-cultivation generally increased gene-targeting efficiency in plants, especially in some loci.

W8

High Frequency Homologous Recombination in Plants Mediated by Zinc Finger Nucleases

(presented by Dan Voytas <voytas@iastate.edu>)
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Homologous recombination offers great promise for studying plant gene function, because it enables specific sequence changes to be introduced into plant genes. This promise has not been realized, however, principally because when DNA enters plant cells, homologous recombination occurs infrequently and random integration predominates. Chromosome breaks, however, enhance localized recombination, and a method to create specific chromosome breaks was developed that uses zinc finger nucleases. Zinc finger nucleases can be designed to recognize any DNA sequence, thereby making all plant genes amenable to cleavage and subsequent modification. In initial experiments, approximately one in ten plant cells that take up DNA sustain a homologous recombination event, suggesting that for the first time, homologous recombination can be reliably employed for plant genome engineering. Current research is focused on developing zinc finger nuclease-assisted recombination for widespread use, including establishing key parameters for high frequency recombination, strategies to introduce sequence changes without incorporating foreign DNA (i.e. marker genes), and robust methods for zinc finger design. The outcome will be a highly facile homologous recombination system for a variety of plant species to study gene function and engineer crop plants with novel traits.

W9

Cre-mediated Site-Specific Gene Integration for Consistent Transgene Expression in Rice

(presented by Vibha Srivastava <vibhas@uark.edu>)
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We studied the utility of Cre-mediated site-specific integration method for streamlining the production of stable transgenic rice. Using this method, we precisely placed a single copy of A-glucuronidase gene (*gusA*) into the designated genomic location. We found that expression variability between transgenic lines produced by this method is greatly reduced. We studied GUS expression in 18 different site-specific integration lines, 11 of which contain precise site-specific integration without illegitimate integration in the background (single-copy lines) and the remaining 7 contain illegitimate integrations in addition to the precise site-specific integration locus (multi-copy lines). We found that *gusA* gene in the single-copy site-specific integration lines is expressed at consistent levels over successive generations, and the gene activity is directly correlated with allelic gene dosage. Segregation analysis of multi-copy lines suggested that in 3 lines illegitimate integrations are genetically linked to the site-specific integration locus. In the remaining 4 multi-copy lines site-specific integration locus was segregated from illegitimate integration locus. Further, the site-specific integration locus derived from silenced multi-copy lines was activated upon segregation from illegitimate integrations. These data suggest that site-specific integration is suitable for streamlining the production of stable transgenic plants.

Poster Abstracts

P1

A 9-lipoxygenase gene functions in maize germination and development

(submitted by Xiquan Gao <xgao@ag.tamu.edu>)
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Lipoxygenases (LOXs) are dioxygenases that catalyze the hydroperoxidation of polyunsaturated fatty acids such as linolenic and linoleic acids at position 9 or 13 of their carbon chains. While 13-LOXs and their derivatives such as jasmonates and C6 volatiles function in defense against pests and pathogens, the physiological role of 9-LOXs and their metabolites are still to be revealed. By using reverse genetics strategy, we have identified mutants in which function of a 9-LOX gene, ZmLOX3, is interrupted by insertions of Mutator transposable elements in its coding sequence. Northern blot analysis of RNA from germinating seeds revealed that lox3-4 mutant allele does not accumulate any detectable levels of ZmLOX3 transcripts and therefore, represents a true null allele of this gene. We generated near isogenic wild type (WT) and lox3-4 mutant lines in B73 genetic background. Phenotypic analysis revealed that the germination of lox3-4 mutants was significantly delayed compared to WT. Moreover, mutant seedlings showed reduced root and shoot elongation. Mature mutant plants also displayed reduced root mass and plant height. To identify LOX substrates and metabolites possibly responsible for these developmental abnormalities, we quantified accumulation of several free fatty acids and LOX-derived oxylipins using HPLC, GC-FID and GC-MS during the early stages of germination in both the mutants and the WT. Although there are several 9-LOX genes expressed during seed germination, lox3-4 mutants are depleted in the accumulation of most of the 9-LOX derived fatty acid hydroperoxides. Surprisingly, the mutants accumulated significantly higher levels of several free fatty acids such as 16:0 and 18:0 compared to the WT. Taken together, these data suggested that ZmLOX3 and the 9-LOX pathway initiated by this isoenzyme have a role in germination and growth of maize plants.

P2

A Functional Genomics Program for the Illinois Protein Selection Strains

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The Illinois Protein Strains (Illinois High Protein (IHP), Illinois Low Protein (ILP), Illinois Reverse High Protein (RHP), and Illinois Reverse Low Protein (RLP)), were derived from the open pollinated cultivar “Burrís White” and are the product of the worldís longest-running breeding and selection experiment. IHP and ILP represent the known genetic extremes for endosperm storage product accumulation and nitrogen metabolism in vegetative tissues. The reverse strains represent 48 years of forward selection for high and low protein followed by reversing the selection for determination of residual variability. The Illinois Protein Strains are thus a unique experimental system to investigate how selection has affected protein deposition. The major endosperm storage proteins in maize are primarily the alpha zeins (19 kD and 22 kD) which are high in prolamine and glutamine but lack tryptophan and lysine. At the DNA level, we have assessed 12 individuals of the 4 strains at two generations (65 and 100) for similarities/differences of the alpha zein allele frequencies during the last 35 cycles of selection. Our Southern blots suggest that there has been limited change at the DNA level. At the protein level, SDS-PAGE analysis of the alcohol soluble proteins (including the alpha zeins) at different developmental stages again suggests limited change. At the RNA level, we have cloned and sequenced transcripts of the 19 and 22 kd zeins. These sequences were compared with the published genomic sequence of B73 and once again show limited polymorphism. Therefore, we believe the differences in protein levels between strains are due to regulation of expression of the major seed storage genes. Microarray analysis has provided clues to this regulation.

P3

An international scientific and cultural experience for undergraduate students

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To expose undergraduate students to scientists working on international efforts to improve maize we have led a group of our undergraduate students on a scientific and cultural experience to New Mexico and Mexico. During this experience six undergraduate students visited the International Maize and Wheat Improvement Center (CIMMYT) located in Texcoco, Mexico. While at CIMMYT the students were exposed to the extraordinary genetic diversity of maize and wheat while learning about some of the challenges that face those who develop genetically improved plants for the developing world. The students not only toured the facility, but also worked for a day in host laboratories in the Applied Biotechnology Center. While in Mexico, the students experienced Mexico outside of CIMMYT on their trips to the nearby pyramids at Teotihuacan, CIMMYTís Tlaltizapan subtropical field station, and the National Museum of Anthropology in Mexico City. En route to CIMMYT the students visited the National Center for Genome Resources in Santa Fe, New Mexico. In addition, while in New Mexico, the students considered the historical and contemporary influences that different human cultures have had on the development of maize while visiting several museums and Tyuonyi and Tsankawi Pueblos in Bandelier National Monument.

P4

An orange endosperm mutation co-segregates with and is associated with mutations in the lycopene epsilon cyclase gene of maize

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Two M2 ears derived from an EMS pollen mutagenesis of the inbred line Qx47 segregated for a distinct change in endosperm color from yellow to orange, with orange recessive to yellow. Two general classes of carotenoids, carotenes and xanthophylls, are primarily responsible for the yellow color of corn grain. The two primary xanthophylls are lutein (yellow in color) and zeaxanthin (orange in color). Based on the carotenoid synthetic pathway and published work in Arabidopsis (Pogson et al., 1996), lycopene epsilon cyclase emerged as a candidate gene. The pathway branches with the cyclization of lycopene to form bicyclic B, B (zeaxanthin) or B,E (lutein) carotenoids. A nonfunctional lycopene epsilon cyclase would be expected to block the lutein branch of the pathway and result in increased levels of B-carotene and zeaxanthin. HPLC analysis of orange endosperm and wildtype kernels confirmed this. Backcrossing of the orange endosperm mutation into the inbred line B73 revealed that lycopene epsilon cyclase co-segregates with orange endosperm. In addition, sequence comparison between the wildtype and orange endosperm mutants indicate changes in lycopene epsilon cyclase. These results show that along with phytoene synthase and B-carotene hydroxylase, lycopene epsilon cyclase is a key component in attempts to genetically engineer maize with higher levels of B-carotene.

P5

Analysis of zeins in mature wild-type and transgenic maize kernels by Matrix-Assisted-Laser-Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS)

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A high-throughput method has been developed to allow rapid analysis of maize seed storage proteins by MALDI-TOF MS. The extraction solution containing an organic solvent, a reducing agent and a volatile base, has been optimized to enable extraction of all classes of zein proteins (a-, b-, g-, and d-). A near-saturating concentration of matrix, 2-(4-Hydroxyphenylazo)-benzoic acid (HABA), was necessary to obtain strong peaks for the most lipophilic zeins, the a-zeins. Zein proteins with small mass differences, difficult to separate by SDS-PAGE, were resolved through this analysis. Mass signals corresponding to the 10-kD d-, 15-kD b-, 16-kD g-, 27-kD g-, and several 19-kD and 22-kD a-zeins were detected. The zein identities were further confirmed by the association of the number of cysteine residues in each zein MS peak, as determined by iodoacetamide derivatization, with the number predicted from its coding sequence. The relative zein abundance in the zein MS peaks was also correlated with the relative zein EST abundance among endosperm EST libraries. This method was utilized to examine the zein composition of a number of corn inbred lines, opaque mutants as well as transgenic lines that displayed zein reduction.

P6

Analyzing the structure and function of maize GBSS and SSI

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Starch synthases (SSs) catalyze the formation of alpha-1,4 linkages that elongate glucosyl chains during starch biosynthesis. There are six known SS isoforms in maize: granule bound SS (GBSS), SSI, SSIIa, SSIIb, SSIII, and SSIV. The molecular mechanisms required for starch synthesis are not fully understood, including the function of each SS isoform. To better understand the roles of GBSS and SSI in the synthesis of endosperm starch, this study generated chimeric and mutant SS proteins and examined their biochemical activities in vitro. The predicted structures of SSs indicate that all contain three highly similar regions: a glycosyl transferase domain (GLYTR), a glucan association domain (GLASS), and the linker region (LINKR) that connects GLYTR and GLASS. The specificity of the GLASS and GLYTR domains was examined by expressing chimeric SS proteins in E. coli that consist of the GBSS GLASS and LINKR regions in combination with the SSI GLYTR domain, and the GBSS GLASS domain in combination with the SSI LINKR and GLYTR domains. In addition, four mutant SSI proteins were generated in which point mutations designed to impact primary sequence rigidity were introduced into the sequence coding for the LINKR to examine the structural importance and/or vulnerability of this region. SDS-PAGE and immunoblot analyses tracked protein size and purification, and glycogen-based native and denaturing zymograms were used to assess protein activity. Gel mobility assays were employed to compare the binding properties of the chimeric and mutant proteins with those of recombinant SSI and GBSS. SS activities were quantified by a radioactive ADPglucose uptake assay.

P7

Assembly state dependent regulation of Rubisco LS translation: Mechanism and physiological relevance

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Rubisco is a key enzyme of the Benson-Calvin cycle promoting CO2 fixation in chloroplasts. The Rubisco complex is constituted by the assembly in the L8S8 form of a large subunit (LS, 55 kDa), encoded by the chloroplast rbcL gene, and a small subunit (SS, 15 kDa) encoded by a nuclear multigene family. Rubisco complex composition reflects a general chloroplast trait; although the chloroplast has its own genome, it is dependent on the nucleus to provide structural subunits as well as proteins required for various steps of chloroplast gene expression. Rubisco subunits accumulate stoichiometrically, a phenomenon which relies both on proteolytic degradation of the unassembled SS subunits, as well as on negative regulation of LS translation in absence of its assembly partner. This latter mechanism has been examined in tobacco and in the green alga Chlamydomonas reinhardtii, and is termed CES (Control by Epistasy of Synthesis). In Chlamydomonas it has been shown to act on at least one chloroplast encoded subunit of each major photosynthetic complex, through a negative autoregulation of translation initiation by the unassembled subunit. Here, we will present work to test the occurrence, mechanism and physiological relevance of CES in Rubisco gene expression in tobacco and maize chloroplasts: RNAi lines directed against rbcS transcripts suggest that the rbcL transcript fails to be translated in the absence of Rubisco assembly. Strategy to unravel the mechanism in place will be presented for tobacco. Finally, the possible involvement of CES in the specific accumulation of Rubisco in maize bundle sheath but not mesophyll cells will be discussed. Our initial evidence shows that untranslated rbcL mRNA is not degraded. This suggests the occurrence of a differential control of rbcL transcript stability in mesophyll and bundle-sheath cells, as proposed for the C4 plant Amaranthus hypochondriacus.
This work is supported by an NSF Plant Genome Award (DBI-0211935).

P8

Bigger endosperm size in maize relative to other cereal grains is due to cumulative action of two cell wall invertase genes in developing seeds

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The dominant Miniature 1 (Mn1) seed locus encodes cell wall invertase-2 (INCW2) that is localized exclusively in the basal endosperm transfer cell layer (BETC) in developing seeds (Cheng et al., 1996, Plant Cell 8: 971). Sucrose hydrolysis by this enzyme and downstream hexose signaling is critical to rapid cell proliferation, total number of cells and cell elongation during the early phases of endosperm development (Vilhar et al. 2002, Plant Physiol 129: 23). Indeed, loss-of-function mutations at the Incw2 (Mn1) locus lead to the mn1 seed phenotype, marked by a loss of > 70% of seed weight at maturity. The mn1 seed mutant is however non-lethal presumably because it retains throughout seed development a residual low level, ~1%, of the Mn1 cell wall invertase activity. Here we show that the residual activity in the mn1 mutant is encoded by the Incw1 gene, and the INCW1 shares 70.1% amino acid sequence identity with the INCW2. Quantitative RT-PCR analyses showed near equal abundance of Incw1 and -2 RNAs in the Mn1 endosperm at 12 days after pollination (DAP); at grain-filling, 20 (DAP) and beyond, however, greater levels of Incw1 was seen relative to Incw2. In sorghum where the normal seed size is nearly the same as the mn1 seed in maize, both genomic database searches and our gene expression analyses show a single gene, an ortholog of Incw1, in developing seeds. In addition, our immunolocalization data reveal a major spatial contrast with maize: the INCW protein was only in the maternal pedicel and not in the filial BETC region of the developing caryopsis. As in sorghum, rice genome harbors only the Incw1 ortholog, and the gene expression pattern in developing seed is similar to sorghum (Hirose et al. 2002, Plant Cell Physiol 43: 452). Thus in maize, an additional locus, Incw2, and the uniqueness of endosperm-specific expression is essential for its bigger seed size as compared to the two closest relatives, sorghum and rice.

P9

Bridging the maize - Arabidopsis divide: Mutants of the latter to a fungal HDAC inhibitor that confers disease in the former

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The leaf spot and ear rot of maize caused by race 1 of Cochliobolus carbonum is the most severe disease of our favorite monocot. The disease is mediated by HC-toxin, a potent inhibitor of histone deacetylases (HDACs) across all species. Arabidopsis thaliana, a dicot dear to many, is among these species, raising the question: why is this plant not susceptible to C. carbonum? Does Arabidopsis have a natural resistance mechanism to inactivate HC-toxin, akin to the natural protection strategy that has evolved in maize against this pathogen, or does it employ a different strategy to contend with HC-toxin? We opted to address this question by generating and identifying EMS-induced Arabidopsis mutants that exhibit altered responses to HC-toxin as well as another HDAC inhibitor, CBHA (m-carboxycinnamic bis-hydroxamic acid).

A genetic screen was devised to identify a number of mutants that exhibited either enhanced sensitivity or tolerance to these HDAC inhibitors compared to wild-type Arabidopsis. The ways these mutations are being analyzed will be described and ideas will be explored to speculate what changes may have occurred to confer sensitive or tolerant phenotypes. It is anticipated that these mutants will not only allow us to understand how HDAC inhibitors interfere with plant defenses, but also impart important tools for gaining insight into the realm of chromatin remodeling in plants.

P10

Candidate Genes for N Utilization Differences in the Illinois Protein Strains

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The Illinois High Protein (IHP) and Illinois Low Protein (ILP) lines of maize, the products of over 100 cycles of selection for grain protein concentration, represent an ideal system for the genetic exploration of nitrogen use efficiency (NUE). A number of studies have demonstrated significant differences between the amino acid compositions and nitrogen-related enzyme activities of IHP and ILP, yet relatively little is known about the genetic basis for these differences. Additionally, little work has been conducted that compares the expression differences between known members of specific nitrogen-related gene families. Using recently compiled gene models, we generated sequence alignments for multiple members of key nitrogen-related gene families. We are employing these alignments for the development of gene-specific assays to monitor expression during development, in response to environmental factors (light/N) and among genotypes. We will report our progress on the development of these assays and our preliminary results for expression differences among the different maize genotypes and tissues. Differences determined at the genetic level should help direct future efforts towards elucidating higher levels of nitrogen utilization processes.

P11

Characterization of a Maize Opaque Mutant mto38

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The investigation of maize (Zea mays) opaque mutants, such as opaque2 (o2), floury2 (fl2) and Defective endosperm* (De*)-B30, has helped us to understand several aspects of the mechanisms that regulate the biosynthesis and accumulation of zein storage proteins in maize endosperm. Here, we characterized a novel opaque mutant, mto38 (Mutator-tagged opaque 38). Homozygous mto38 kernels have a uniformly starchy endosperm and reduced levels of zeins. Although the mutant kernels do not typically germinate, embryo-rescued mutant plants show a reduced degree of apical dominance. Using thermal asymmetric interlaced PCR (TAIL-PCR), we isolated a candidate MTO38 gene with an inserted Mutator tag. The mutant allele is genetically tightly linked to the opaque phenotype. Based on DNA sequence information of the candidate MTO38 gene, we identified additional mto38 alleles by reverse genetics, and we are assessing if these alleles generate any phenotypes similar to the original mto38 allele. Information regarding MTO38 homologs in other organisms, and the nature of the protein-protein interactions with MTO38 suggest the MTO38 gene product may play a role in pre-mRNA splicing and interact with transcriptional machinery and chromatin modifying factors.

P12

Characterization of lycopene-beta-cyclase and manipulation of carotenoids biosynthesis

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Pink Scutellum (Ps1) gene in maize encodes the lycopene b-cyclase, which is required in seed and seedling tissues for the production of both xanthophylls and abscisic acid (ABA). The disruption of this gene results in the accumulation of lycopene in maize embryos and the precocious germination of kernels. We have cloned Ps1 gene by using an Ac mutagenesis strategy, which has the ability to generate multiple unstable insertion alleles. To date, we have identified 24 independent insertions from 1,600 transposition events. Among these insertions, we obtained alleles that condition both strong and weak mutant phenotypes. Excisions of Ac can also generate novel alleles through the creation of ëfootprintí alleles. Duplications or deletions of sequences immediately flanking Ac can give rise to stable new alleles with subtle or dramatic gene function changes. HPLC analysis was used to characterize the accumulation of carotenoid in Ps1 footprint alleles in comparison with near-isogenic parental lines. HPLC analysis was also able to identify that carotenoids accumulate differently between maize embryo and endosperm. We are using HPLC and real-time PCR to characterize the regulatory mechanism of carotenoids biosynthesis pathway in maize kernel. The goal of the project is to identify the functionally important domains of lycopene b-cyclase and to manipulate the accumulation of carotenoids in maize kernels.

P13

Characterization of maize su1, isa2, and isa3 mutants indicates formation of distinct starch debranching enzyme complexes

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Higher plants have two classes of starch debranching enzyme (DBE) that participate in both the synthesis and the hydrolysis of starch, isoamylase-type (ISA) and pullulanase-type. In maize, the sugary1 (su1) gene codes for ISA1. Mutations at the su1 locus cause a dramatic change of the structure of amylopectin and accumulation of the more highly branched polymer phytoglycogen. In this study, two additional maize genes that code for isoamylase-type DBEs, Isa2 and Isa3, were identified and cloned. A TUSC reverse genetics approach identified transposon insertion mutations in both genes, and the effects of these mutations were compared with those of an allelic series of su1 mutants. Mutant isa2 kernels accumulate a small amount of water-soluble polyglucan at 20DAP and have smaller starch granules, although amylopectin structure in endosperm starch of isa2 mutants is unchanged. Zymogram analysis of proteins from developing kernels shows the null isa2 mutant is missing two of three ISA activity bands, and the null su1 mutant is missing all three bands. Purification of ISA activity from wild type and mutant endosperm extracts indicates the existence of distinct DBE complexes. In conjunction with immunoblot analysis, the data show that one complex is comprised of ISA1 homomers, and that the higher molecular mass forms represent complexes containing both ISA1 and ISA2. The latter complex has significant hydrolytic activity toward the slightly branched substrate amylose, whereas the lower molecular mass form has greater activity when amylopectin, beta-limit dextrin, and glycogen are substrates. Temporal analysis of the activities of the two DBE complexes over the course of endosperm development reveals specific activity differences that correlate with changes in the transcript expression patterns of Isa1 and Isa2. These results suggest the two DBE complexes may have developmental- and/or substrate-specific functions that underlie distinct roles in starch biosynthesis.

P14

Characterization of the P450 Carotene Hydroxylase Gene Families in Cereal Crops

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The dihydroxy xanthophylls lutein and zeaxanthin are produced from their respective carotene precursors by the action of carotenoid beta- and epsilon-hydroxylases. The beta-hydroxylase and epsilon-hydroxylase enzymes add hydroxyl groups to the third carbon of carotene beta- and epsilon-rings, respectively. Unlike the well studied non-heme di-iron beta-hydroxylases that have been characterized from a wide variety of organisms, the cytochrome P450 epsilon-hydroxylase has only been defined genetically in Arabidopsis knockout mutants. In addition, recent evidence suggests that there may be an additional beta-hydroxylase of the P450-type present in Arabidopsis. To date, no biochemical demonstration of either epsilon- or beta-ring hydroxylation activity for these P450 enzymes has been reported. Since no P450 epsilon- or beta-hydroxylases have been identified from either maize or rice, we are working on isolating genes encoding all cytochrome P450 beta- and epsilon-hydroxylases, as well as any other homologs that may exist in these cereals. At the molecular level we are using southern blot analysis, expression profiling via RT-PCR, and functional complementation. At the biochemical level we are utilizing HPLC analysis to identify carotenoids processed by these enzymes. We have identified two rice genes and a number of maize ESTs encoding enzymes with homology to the putative Arabidopsis P450 epsilon-carotene hydroxylase and P450 beta-carotene hydroxylase enzymes. Primers designed from rice genomic DNA were used to generate cDNAs from Oryza sativa var. Nipponbare leaves which were expressed in E. coli for enzyme characterization by HPLC.

P15

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 the flanking region using bioinformatics has revealed a candidate zl1 gene that shows a high degree of similarity to a family of genes in Arabidopsis thaliana named KCO1 through KCO6 and to four genes in Samanea saman named SPOCK1, SPICK1, SPICK2 and SPORK1. Published evidence shows that these genes encode a type of novel potassium channel protein in plants. It has also been shown that genes in Samanea saman such as SPOCK1 and SPORK1 are controlled in a circadian fashion. The homology that the zl1 candidate has to these genes combined with the phenotypic analysis allows us to hypothesize that zl1 encodes a potassium channel and that it is controlled in a circadian fashion.

P16

Characterization of the maize gene family encoding beta-carotene hydroxylase (di-iron monoxygenase type)

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Carotenoids and their oxygenated derivatives, xanthophylls, are C40 isoprene compounds. Both have a variety of important biological functions, which include essential health benefits to humans. Significant modulations of the carotenoid and/or xanthophyll content have been bioengineered into crops such as tomato, canola, and rice. However, similar improvements in maize endosperm tissue, a major food staple, have not been accomplished yet. Two classes of beta-carotene hydroxylases are known in plants; the non-heme di-iron beta-carotene hydroxylases (HYD) with the characteristic four iron-binding histidine clusters, and the P450 beta-carotene hydroxylases with a characteristic P450 domain. Collectively beta-carotene hydroxylases convert provitamin-A carotenoids (beta-carotene, alpha-carotene, and beta-cryptoxanthin) to nonprovitamin-A xanthophylls (zeaxanthin, zeinoxanthin, and lutein). In this work, we are assessing the non-heme di-iron beta-carotene hydroxylase gene family, as part of a larger ongoing effort, to lay the foundation for rational metabolic engineering of carotenoid and/or xanthophyll content in maize. A maize cDNA, demonstrated to encode a HYD enzyme, was used to screen a maize B73 BAC genomic library. Combined with southern blot analysis and database mining, we have identified seven members in the maize HYD gene family. On the basis of sequence analysis, two members appeared to share deletions that would likely interfere with expression of a functional enzyme. Initial expression analysis by RT-PCR indicates different tissue-specific and different temporal profiles in the endosperm tissue. Inverse PCR (to clone the promoters and unknown genomic sequence segments), functional complementation of corresponding HYD cDNAs, and HPLC analysis are ongoing.

P17

Codon optimized fluorescent marker genes for maize

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Green fluorescent protein (GFP) and derivatives thereof with shifted excitation/emission variants including blue (BFP), cyan (CFP) 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, YFP and CFP. 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 have been subcloned into the binary plasmid pZP212 and maize transformations have been initiated. Monitoring the fluorescence across varius tissues types in the resultant transgenic lines derived from these experiments will enable us to determine the utility of the maize codon-optimized GFP, and variants thereof, as a means to observe in vivo protein trafficking in maize.

P18

Differential accumulation of maysin and rhamnosylisorientin in leaves of high altitude landraces of maize after UV-B exposure

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Flavonoid induction by UV-B was investigated in five maize landraces from high altitudes and a W23 inbred line lacking the B1 and Pl1 transcription factors required for anthocyanin synthesis. In their natural habitats these landraces receive much higher UV-B fluence than plants at lower altitudes at similar latitudes and would be predicted to have UV-B tolerance by recurrent selection against UV-B stress. We identified two flavones that are induced by UV-B in leaves of high altitude lines: maysin and its biosynthetic precursor rhamnosylisoorientin. Accumulation is controlled by a p-homologous transcription factor expressed in leaves, and this factor is regulated by UV-B. The levels of either maysin or rhamnosylisoorientin are higher in seedling leaves than in subsequent leaves; the highest flavone concentration was detected in silks. Some landraces have only rhamnosylisoorientin; this likely reflects a mutation in salmon silk1 (sm1) or in a duplicate locus, as genetic crosses with W23 restore the production of maysin in heterozygous F1 plants. In conclusion, we demonstrate that maize plants from high altitudes respond to UV-B radiation by accumulating UV-absorbing flavones in leaves; in contrast, these compounds are present at only very low levels in inbred lines such as W23 and are not regulated by UV-B. The project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2003-00745.

P19

Direct and indirect effects of altered Du1 gene expression on starch structure determination

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Starch serves a fundamental role in plants as the primary carbohydrate storage form. Starch consists of two homopolymers of glucose, amylose and amylopectin (Ap). Ap, the more abundant polymer, has a highly organized architectural arrangement of linear and branch chains to efficiently package large amounts of glucose. Starch synthases (SS) catalyze reactions that build a-(1,4)-linked linear glucosyl chains. The five SS classes of plants are highly similar in the catalytic and starch-binding domains of the C-termini but differ at their N-termini, with SSIII having the longest N-terminal arm. In maize, SSIII is the product of the dull1 (dul1) gene. Most SSs affect Ap structure directly via their enzymatic activities, but the SSIII isoform also is likely to regulate other starch metabolizing enzymes via protein-protein interactions. Consequently, the interplay between SSIII and other factors indirectly influences the final molecular architecture of Ap. This study characterized ten allelic du1 mutations with respect to the molecular lesion, changes in SS activity, Ap structural changes, and pleiotropic effects on other starch metabolizing enzymes. Allelic differences in the extent of SSIII activity loss were observed, but all ten du1 mutations affected Ap structure identically, producing more short chains and fewer long chains. The idea that these structural changes are due to altered activities of DU1 binding partners is supported by evidence that isoamylase-type starch debranching enzyme activity is increased in the du1 mutants. To examine potential regulatory effects of the DU1 N-terminus, transgenic maize plants were generated expressing full-length DU1 or two truncated, non-catalytic versions coding only for portions of the N-terminal arm. Starch structures in transgenic kernels were similarly changed in all three lines, indicating that the binding of factors to the DU1 N-terminus is likely to account for the observed alterations. Specific identities of DU1 binding partners are currently being investigated.

P20

Diversity in maize kernel carotenoids content

(submitted by Carlos Harjes <ch20@cornell.edu>)

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In nutrition, carotenoids play an important role as pro-vitamin A and potentially also function as antioxidants. The effect of selection for yellow endosperm color which reflects carotenoid accumulation has been shown to be associated with insertions in the Y1 promoter and a selective sweep flanking this region is evidence of the likely white ancestral endosperm phenotype. We are investigating the genetic and phenotypic diversity using candidate genes for enzymes in the carotenoid biochemical pathway, ultimately with the aim of identifying associated polymorphisms to further characterize the genes that underlie the wide range of carotenoid content among yellow maize lines. The following candidate genes for enzymes in the pathway are under evaluation: DXS, IspD, IspE, IspF, LytB, IPP, Y1, PDS, Ggps, ZDS, LCYB / CRTL-B, LCYE / CRT-E, HYD1, HYD2, ZEP1, VDE1, GGPR, and VINCED. For Y1 diversity estimates using our 281 inbred line panel are contrasted with the results for the regions associated with carotenoids accumulation identified by Palasia et al. SNPs for the remaining candidate genes were selected in an approximately 500bp region of each gene. Phenotypes are based on a panel of 281 diverse inbred lines and their test crosses to B73 grown in three environments; and an additional 3 environments for which we used open pollinated kernel samples from the panel of inbred lines and their hybrids.

P21

Ethylene Signaling Contributes to the Insect-Induced Defense Response in Maize

(submitted by Antoine Harfouche <alh283@ra.msstate.edu>)

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Damage inflicted by herbivore feeding necessitates multiple defense strategies in plants. These defenses are controlled by highly complex regulatory networks which themselves are modulated by interactions with other signaling pathways. In this work, the effects of ethylene signaling on herbivore defense have been investigated in maize. The model developed for the insect-induced activation of the cysteine proteinase (Mir1-CP) protein and the gene encoding it, mir1, in maize establishes the involvement of the plant hormone ethylene as a key component of the insect defense signal transduction pathway. The experimental model involved the inhibition of ethylene biosynthesis and perception in two maize genotypes, resistant and susceptible to insect feeding. Leaf damage and larval growth was higher when resistant plants were treated with the inhibitors prior to insect feeding, which indicated that blocking ethylene signaling resulted in plant that were more susceptible to herbivory. Examination of mir1 and Mir1-CP expression upon insect attack and ethylene inhibitors treatments demonstrated temporal and spatial differences revealing the role of ethylene in the transduction pathway leading from insect attack and acting to regulate the defense response in this plant-insect interaction. We hope to gain greater insight into how plants regulate cellular processes in response to biotic stress, potentially leading to the development of new methods of crop protection based more on the plantís natural defenses rather than chemical pesticides.

P22

Evaluation of water-stress associated changes in root architecture of *viviparous (vp)* maize mutants.

(submitted by Ryan Dierking <rmdq44@mizzou.edu>)

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Throughout the US, millions of bushels are lost due to the damaging effects of drought each year. One of the responses to drought in maize is to produce abscisic acid (ABA), which is involved in reducing water loss by triggering stomatal closure, along with modulating plant growth and other responses. Root architecture is a critical factor in the absorption of water and the overall maintenance of water relations in the plant. In this study, *viviparous* (*vp*) mutant seedlings were evaluated for several root architecture traits under well-watered (ww) and water-stressed (ws) conditions. The characteristics measured included root length, root mass, root branching, seminal root number, shoot mass, and number of leaves present at harvest. A previous study showed that *vp*5-DR3076 and *vp*8 had significantly different root architecture that included branching and root mass changes, respectively. A total of 19 mutants were evaluated here; six *vp*5 mutant alleles, eight *vp*1 mutant alleles, four *vp*9 mutant alleles, and one *vp*2 mutant. Wild-type sibs were also evaluated in comparison to each mutant. In this study *vp*1 displayed a significant difference for root length, root branching, and shoot mass compared to wild-type and root mass and shoot mass in ww vs ws comparisons. *vp*2 had significant differences in seminal root number, root mass, and shoot mass between ww and ws and in comparison to wild-type. Additionally, *vp*5 had significant differences between ww and ws treatments for branching, and seminal root number was significantly different compared to wild-type. No significant differences were observed for *vp*9. The genes identified here along with the mutants identified in a previous study are candidates for use in future studies of root response to water-stress.

P23
High-Throughput Screening for Slowly Digesting Starch in an EMS Mutagenized Maize Population

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Obesity has become a heavy issue in todayís health professions. According to a 1999-2000 NHANES survey, 64 percent of Americans can be classified as overweight or obese. In addition, diabetes and pre-diabetes have become increasingly prevalent. Many of the measures taken to address this problem concern changing the biochemical nature of food itself. Slowly digesting starch is a compound of particular interest because of its potential for prolonging satiety and moderating daily food intake. The goal is a carbohydrate that breaks down slowly in the small intestine, releasing free glucose at a lower rate over a longer time. Rather than current chemical modifications, we have taken the approach that maize varieties can be developed that more reliably modify the starch structure as it is made. To identify such novel starches in a M3 population of EMS mutagenized maize, we have developed a highly miniaturized, high-throughput kernel processing and starch digestion assay. Multiple, steeped and lyophilized endosperms from M3 families are organized individually into microtiter plates and handled on a PCR scale. Retaining their initial plate-level organization, samples in both raw and gelatinized forms are digested using pancreatic alpha-amylase. Changes in digestion rate are determined by measuring amounts of maltose released (a colorimetric DNS-based assay) at three separate time points throughout the digestion and comparison to digestion of different, well-characterized starches. As many as 600 samples can be analyzed per day (several times the efficiencies previously reported). NIR data on intact kernel amylose and protein contents of the same M3 families provide information on possible correlations between unusual digestion patterns and starch compositions. Identification of maize mutants producing slowly digesting starch will provide important new sources of value-added input traits for the development of healthier maize lines.

P24
Identification and genetic analysis of maize male sterile mutant obtained by space flight

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The maize seeds from single hybrid Chuandan No.9 were divided into two sections, one as check, the other were carried into outer space in 1996 by retrievable satellite for 15 days flight. After being returned, the treated seeds along with the check were planted in field. Fortunately, male sterile plants were discovered only in one of the offsrings descended from treaated seeds. The male sterile plants were pollinated with the normal plants of the same ear row. 12 seeds were obtained from the male sterile plants. In order to identify the inheritance and stability of this trait, the same mutant ear and their progeny of male sterile plants were planted in different years, different locations. Test cross, sister cross, back cross, reciprocal cross and self-pollination were conducted with these male sterile plants and their progeny to determine the type of this male sterility and to analysis its heredity law. 10 inbred lines include Zifeng-1 and Hui313 were used as tester lines for the male sterile trait, inbred line ES40 and A318 were used as cytoplasm background donor in the reciprocal crosses.
Based on the fertility survey in field and the observation under microscope in different years, different locations and different generations, we can conclude that it is inheritable and stable, not affected by the temperature or photoperiod, it can be passed on from generation to generation. According to the classification system suggested by Professor Zeng Yong-Lian the male sterile material does not belong to CMS-S, CMS-C and CMS-T. Complete maintainers for this male sterility cannot be found in this experiment. As well known, cytoplasmic male sterility is maternally inherited trait in non-Mendelian fashion, so we conducted another kind of cross experiment named reciprocal, using inbred line ES40 and A318 as cytoplasm donor. These results indicated that this male sterility can express not only in mutant itself cytoplasm background but also in other normal cytoplasm backgrounds. All this evidences suggest that this trait belong to nuclear male sterility. The data from test cross, back cross, sister cross, reciprocal cross combined with self-pollination show that it is controlled by a single recessive nuclear gene. The appearance of male sterile mutant may be the conclusion of gene mutation or gene recombination occurred in nuclear by outer space flight.

P25

Identifying stable Mu-induced knockouts of specific genes for cell wall biosynthesis in the UniformMu maize population using reverse genetics.

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The production of stable gene knockouts through Mu transposon insertions has been exceptionally successful. The goal of our project is to identify roles of specific genes through functional analysis of knockouts on a genomics scale, utilizing the unique "UniformMu" maize population from the University of Florida. These lines have been generated by introgressing Robertsonís Mutator into a W22 background. The resulting UniformMu population has an eight-generation traceable pedigree for each plant, a low mutation load, a high rate of new mutations, and a genetic mechanism for stabilizing desired mutants. Also, because this population is uniformly inbred, an essentially isogenic group of non-mutant, wildtype control plants are available for comparative analysis of mutant plants. Plants from the UniformMu population are now being subjected to a reverse genetics strategy for identifying individual plants where Mu insertions have occurred in specific genes of interest. Two hundred target genes have been selected on the basis of their involvement in cell wall biosynthesis. Gene-specific primers for each have been designed to be utilized along with a Mu-specific primer in a PCR-based screen to identify mutations caused by Mu insertions. Eight grids are being constructed for this screening, each with genomic DNA from 2,304 UniformMu families. Extracts have been pooled into 48 groups of 48 plants each and arranged on x-y axes. An internal control (vp14) has been successfully identified, and a putative new allele of this gene was discovered in the process. Screening of this first grid with 40 unique genes has yielded at least 8 knockouts, indicating a mutation frequency of ~10-4 insertions/ locus/plant. This is consistent with forward mutation rates reported for Mu-active lines of maize. Thus far, putative knockouts have been identified in the following cell wall related genes: CslA9, CslD1, CslF4, CslH1, ExpA10, ExpL3, and Gsl9. Screening is in progress for the remaining 200 candidate genes.

P26

Investigation of ADP-glucose pyrophosphorylase protein-protein interactions and post-translational modification by phosphorylation

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ADP-glucose pyrophosphorylase (AGPase) is a heterotetramer that catalyzes a rate-limiting step in starch synthesis in maize endosperm. It consists of two identical large subunits (~54kDa) encoded by Shrunken2 (Sh2) and two identical small subunits (~51kDa) encoded by Brittle2 (Bt2). In monocot endosperm, the enzyme is cytosolic and its product is presumably transported into the plastids for starch synthesis by BRITTLE1 (BT1), a plastidic adenylate transporter found in the endosperm of monocots. In an effort to investigate whether AGPase interacts with other proteins and particularly with BT1, we utilized formaldehyde as an in vivo protein-protein cross-linking agent. Formaldehyde cross-linking and subsequent protein immunoprecipitation by using a BT2 monoclonal antibody column suggested that AGPase did not interact with BT1 or any other proteins. In separate experiments, AGPase was found not to be phosphorylated.

P27

It's Not Easy Being Green! - Insights into Chlorophyll Detoxification from Lesion Mimic Mutant Studies

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Although the study of chlorophyll has long been the subject of investigation, the process by which photosynthetic organisms degrade chlorophyll has not. It is now becoming recognized that the proper compartmentalization of chlorophyll intermediates during degradation requires a carefully coordinated set of metabolic steps. Valuable insights into this process have come from the study of lesion mimic mutants in plants which exhibit runaway cell death in the absence of pathogen infection. In our lab we found that the Lls1 (Lethal leaf-spot 1) lesion mimic gene encoded an important protection against light-dependent cell death in higher plants. We found that this gene was the ortholog of the Acd1 (accelerated cell death 1) gene from Arabidopsis (Yang et al, Plant Mol. Biol. 54(2):175-191, 2004). A functional genomics approach later revealed that the Lls1 gene encodes pheophorbide a oxygenase (PaO) which catalyzes a key step in chlorophyll degradation (Pruzinsk· et al, PNAS 100:15259-15264, 2003). In the absence of the PAO(LLS1) function, cells fail to compartmentalize pheophorbide a which presumably is photoactivated and initiates cell death. Another lesion mimic gene named Acd2 (Accelerated cell death 2) also exhibits runaway cell death and encodes the next enzyme in the chlorophyll degradation - Red Chlorophyll Catabolite Reductase. The examination of the cell death phenotype of these mutants underscores the proposition that chlorophyll turnover in green plants is more correctly referred to as chlorophyll detoxification. Ongoing studies are aimed at studying the organization and regulation of the PAO(LLS1) enzyme in the inner chloroplast membrane. A bioinformatics survey also revealed that Pao(Lls1) homologs exist in algae and cyanobacteria but not in anoxygenic bacteria (Gray et al.,Plant Mol. Biol. 54(1) 39-54, 2004). The detoxification of chlorophyll in cyanobacteria has never been investigated. A knockout mutagenesis approach is being pursued to examine the possible role of cyanobacterial Pao(Lls1)-homologs in chlorophyll metabolism.

P28

Lepidopteran preference testing of *Glossy* mutants and *Glossy15* alleles for maize resistance.

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Every year fall armyworm and Southwestern corn borer cause severe yield loss in maize. Both fall armyworm and the Southwestern corn borer are known to specifically attack the whorl leaf tissue resulting in major crop losses. Over the past few years several resistant lines have been developed. In our study we have compared the larval feeding habits of both insects on resistant and susceptible genotypes, and on maize mutants that affect epicuticular wax formation. A previous study has shown that the *Glossy15* and *Glossy8* genes have Lepidopteran resistance in maize during the whorl stage. Our objectives in this study are to analyze both fall armyworm and the Southwestern corn borer feeding preferences on various *glossy* mutants, and to further examine their feeding habits on different alleles of the *Glossy15* gene. In both studies we compared feeding preferences on three inbreds (Mp705, Oh28, and Va35) to the *glossy* mutants. Mp705 has resistance to whorl stage Lepidopteran feeding while Oh28 and Va35 are susceptible. An inbred adult leaf was placed next to a *glossy* mutant adult leaf in a Petri dish. We then placed a single larva between the two adult leaves. The larval feeding damage was collected using AlphaEaseFC software. The data indicate that adult leaves of *bm1*, *Gl1*, *gl2-PF*, *Gl3*, *Gl7*, *Gl14*, and *gl15-Sprague* are very susceptible to insect feeding. There was no preference for adult leaves of *bm4*, *Gl4*, *Gl11*, *gl3-N531*, *gl13-U440B*, *Gl18*, *gl18-N166A*, and *Gl21* as compared to wild-type alleles. While *Gl8*, *gl15-KEW*, and *gl15-LAM* exhibit some resistance. We also examined insect preference on different alleles of *Glossy15* gene. We observed allelic differences; *gl15-63* and *gl15-L* are susceptible to insect feeding, while *gl15-S*, *gl15-H*, *gl15-956*, and *gl15-94317* show no preference for insect feeding. These results will allow us to select genes and alleles that can reduce larval feeding damage in maize.

P29

Maize Mitochondrial DNA Binding Proteins

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Plant mitochondrial genomes differ from the well known genomes of animal and fungal mitochondria in terms of size and organization, yet little is known about how or why this genomic complexity is maintained. In order to study the maintenance and organization of maize mitochondrial DNA, we have begun to identify proteins that bind to mitochondrial DNA (mtDNA). After cross-linking mtDNA with maize mitochondrial proteins, the proteins were isolated, separated using SDS-PAGE and silver stained. The two most abundant proteins from the cross-linking were identified by peptide mass fingerprinting and sequencing. The first is the mitochondrial manganese-containing superoxide dismutase (Mn-SOD isoform 3.4). Although Mn-SOD has been shown to bind DNA and provide protection against oxidative damage in prokaryotes (Hopkin et al., 1992; Steinman et al., 1994), there is as yet no report of a role for this protein with respect to mitochondrial DNA in eukaryotes. The second protein identified is Mis1, a member of the aldehyde dehydrogenase family. In maize, Mis1 has homology to Rf2 (restorer of fertility), another aldehyde dehydrogenase involved in restoring cytoplasmic male sterility type T (CMS-T), theoretically by providing a metabolic boost. Interestingly, the rf2 homozygous mutant line WF9 was the background line for generation of several mitochondrial DNA mutants, due to its increased rate of genomic instability. Current work is focused on purification of these proteins by expressing them in E. coli, binding assays of the proteins to mitochondrial DNA and analysis of any potential sequence specificity involved.

P30

Maize brittle stalk 2 (bk2) Gene Determines Mechanical Strength of Tissue by Mediating Cellulose Deposition in Secondary Cell Walls

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A spontaneous mutant from maize, brittle stalk-2 (bk2), which maps to chromosome 9L, exhibits dramatically reduced mechanical strength in the aboveground tissues. Reduction in mechanical strength in the stalk tissue was highly correlated with the amount of cellulose. Lignin concentration was slightly increased in the mutant, which may be explained by the compensation effect resulting from a reduction in cellulose. Scanning electron microscopy revealed that the amount of secondary wall in the sub-epidermal cells as well as perivascular sclerenchyma fiber cells was severely reduced in the mutant, which apparently led to a reduction in dry matter content in a unit length of the stalk. Lignin appeared to be more highly deposited at the cell wall junctions. Brittle culm1 (bc1) gene of rice has recently been isolated by map-based cloning and shown to encode a COBRA-like protein. We demonstrate that a homologous, but non-syntenic maize gene is mutated in bk2. A 1 kb mutator-like element is inserted in the beginning of the second exon, causing a frameshift in the open reading frame of this gene. The Bk2 gene is expressed more highly in the stalk, husk, root, and leaf tissues, which all contain vascular bundles that are rich in cellulose, and not expressed in the tissues like meristematic zone, silk, embryo, endosperm, and pollen. Highest expression was in the isolated vascular bundles, which agrees with its role in secondary wall formation. We have isolated an independent Mu-tagged allele of bk2 referred to as bk2-Mu, which has a phenotype similar to that of the spontaneous mutant. The brittle stalk phenotype co-segregates with bk2-Mu. Our results demonstrate that the Bk2 gene affects stalk strength in maize through interference with secondary wall formation.

P31

Molecular and Genetic Analysis of rgh3 Endosperm Mutant

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The rough endosperm (rgh) class of seed mutants disrupts normal endosperm and embryo development and is characterized by seeds with a pitted or etched surface. We have identified the rgh3 mutant in the UniformMu population by means of phenotype screening as well as SSR and B-A translocation mapping to chromosome arm 5L. The UniformMu population is a Robertsonís Mutator, transposon-active population that it is introgressed into W22 color-converted inbred (McCarty et al, 2005). Phenotype and map-based directed complementation tests identified 3 independent alleles of rgh3 in the UniformMu population. Mature kernel sections of the rgh3 mutants show characteristic endosperm defects such as overproliferation of aleurone cells, and a defective embryo. However, a fraction of the rgh3 mutant kernels are able to produce viable embryos that germinate into small seedlings, turn pale green, and eventually die, indicating that the rgh3 mutant is likely to affect plastid function at some level. Analysis of B-A mosaic kernels of rgh3 suggests that the Rgh3 gene is non-tissue autonomous and is required in the endosperm and the embryo to rescue the embryo developmental defects. Based on these data, we hypothesize that the rgh3 locus will reveal a signaling function in both endosperm and embryo development, and potentially, this signaling may involve the plastid.

P32

Oligopeptide transporters show differential gene expression during germination

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Seed formation and embryogenesis requires the transport and partitioning of specific nutrients, including amino acids which must be transported to areas of elevated protein synthesis during embryogenesis and endosperm formation. Amino acid transport is rapid and amino acids can be transported either freely or as small peptides. Whereas many amino acid transporters have been characterized, few studies have investigated the role that peptide transporters play in seed development. Two peptide transport systems are known to exist in plants, the OPT gene family (Oligopeptide transport) and the PTR gene family (Peptide transport). Phylogenetic analyses have revealed that the OPT family has two distinct clades named the Yellow Stripe (YS) clade and the Peptide Transport (PT) clade based on the first characterized members of each group. Members of the PT clade translocate peptides of 3-5 amino acids and no study has investigated the role that PTs play in loading and unloading amino acids in monocot seeds despite the agricultural importance of seed proteins and the scientific interest in the mechanisms underlying resource acquisition and partitioning in seeds. We are using a genetic, molecular, and biochemical approach to determine if PTs play a significant role in amino acid loading in the developing seed and unloading during germination and seedling growth in rice. This hypothesis makes three predictions: 1) PTs will transport peptides that are rich in amino acids that comprise seed storage proteins, 2) PTs will be expressed in a temporal and spatial manner consistent with translocating peptides to tissues of high protein synthesis, and 3) mutations in PT genes will affect endosperm development, embryogenesis, germination, or seedling development. We present here that OsOPT2, 4, 5 and 7 expression is induced during imbibition.

P33
Pangloss Genes are Required for the Asymmetric Divisions that Give Rise to the Subsidiary Cells of Stomata in Maize.

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The asymmetric cell divisions leading to the formation of stomata are among the most important in the development of terrestrial plants. It is through stomatal pores that plants are able to perform exchange and regulate water loss. In maize, stomata are composed of four cells: a guard cell pair, which forms the stomatal pore, and a flanking pair of triangular subsidiary cells.

We have isolated mutations in two genes responsible for the asymmetric division of subsidiary mother cells (SMCs) to form subsidiary cells. In both pangloss1 and pangloss2 mutants, SMCs fail to polarize correctly, and the resulting subsidiary cells often protrude into neighboring cell files and frequently fail to specify as subsidiary cells. Double mutant analysis suggests Pan1 and Pan2 may act in separate, possibly parallel, pathways required for the formation of subsidiary cells. Cytoskeletal analysis reveals abnormalities in actin organization in both single and pan1;pan2 double mutants.

P34
Photosystem II genes effect Lepidopteran damage in maize.

(submitted by Matthew Meyer <mrmwc2@mizzou.edu>)
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Millions of dollars are spent annually to reduce insect damage via pesticide application. Besides monetary implications, pesticide application has undesirable environmental and potential health consequences. One method of reducing pesticide application is by native resistance. Mp705, a tropically derived maize line, has resistance to multiple Lepidoptera including fall armyworm (FAW) and Southwestern corn borer (SWCB). Several QTL for insect resistance have been identified in progeny where Mp705 was a parent. Proteomic analysis of these materials identified several differentially expressed proteins associated with resistance that are components of photosystem II (PSII). A number of the *high chlorophyll fluorescence (hcf)* mutants in maize have defects in PSI or PSII genes. Insect feeding trials were conducted on these mutants to validate the proteomics findings. A preference test was performed comparing hcf mutants to their wild-type siblings. Mutants included *hcf6-N228B*, *hcf44-N1278B*, *hcf7-N1029D*, *hcf13-N1097B*, *hcf41-N1275C*, *hcf34-N1269C*, *hcf36-N1271B3*, *hcf48-N1282C*, *hcf73*, *hcf23-N1261A*, *hcf49-N1480*, *hcf6*, and *hcf11-N1205A*. Some *hcf* mutants have a lighter green coloration. *Oy1-N1459*, *Oy1-Anderson*, *pg2*, *yg1*, and *pg15-N340B* mutants were also compared to their wild-type siblings to determine if pigmentation was not a factor in insect resistance. *yg1* and *pg2* showed no significant difference in feeding damage compared to wild-type, while *pg15-N340B* showed less feeding damage compared to wild-type. These results indicate that color *per se* is not a determining factor in insect feeding behavior. *hcf44-N1278B* showed increased damage from fall armyworm feeding. *hcf49-N1480* showed increased feeding resistance to both Lepidoptera, *hcf11-N1205A* showed increased feeding resistance to SWCB, and *hcf7-N1029D* showed increased feeding resistance to FAW. The differential feeding response of *hcf11* and *hcf7* indicate that some genes may be specific to a particular Lepidoptera. These results support the proteomic analysis and indicate that PSII genes may play a role in native plant resistance.

P35

Role of Opaque2 in Regulation of Zein Production in the Illinois Protein Strains

(submitted by Lisa Haney <lhaney@uiuc.edu>)

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The Illinois High Protein (IHP) and Illinois Low Protein (ILP) lines that have been selected for over a century vary greatly in their seed protein concentration (IHP = 35%, ILP = 4%). Much of this difference can be attributed to the accumulation of zein storage proteins. Opaque2 (o2) is a gene known to regulate zeins, but the effects of o2 in the Illinois protein selection lines is unknown. The o2 null mutation greatly reduces the zein protein accumulation in IHP while still maintaining an increased grain protein concentration. There are also changes in o2 allele frequencies that appear to be associated with protein concentration. These studies of the o2 gene in the Illinois Protein Strains serve as a model system to investigate the functional role of candidate genes that mediate phenotypic differences in the selection experiment. The o2 studies also serve to provide insights to the mechanisms for continued genetic variability in these populations.

P36

Semi-dominant alleles of Oil yellow1 contain single amino acid substitutions in a magnesium chelatase subunit

(submitted by Ruairidh Sawers <rjs47@cornell.edu>)

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Tetrapyrrole metabolism in photosynthetic organisms results in the synthesis of two major classes of products, the heme-related compounds and the chlorophylls. The allocation of the common precursor protoporphyrin IX to the synthesis of heme or chlorophyll is determined by chelation of either ferrous iron or magnesium respectively. The insertion of magnesium to form Mg-protoporphyrin is catalyzed by the enzyme magnesium chelatase. In higher plants, magnesium chelatase consists of three subunits CHLI, CHLD and CHLH. CHLI and CHLD subunits contain a P-loop (Walker A) NTPase domain and are members of the AAA+ protein family.

Maize genetic collections contain many chlorophyll deficient mutants. In order to exploit this rich resource, we have used a candidate gene approach to link a maize CHLI ortholog to the locus oil yellow1 (oy1). In addition to recessive alleles, a number of semi-dominant Oy1 mutants have been characterized, each of which is potentially informative as to mechanism. We have sequenced two semi-dominant Oy1 alleles and identified single amino acid substitutions representing potential mutant lesions. We have confirmed candidate lesions by transient expression of mutant forms of maize CHLI in Nicotiana. This system offers a powerful approach for in planta analysis of Oy1 mutant alleles and for further targeted mutagenesis of a CHLI protein.

P37

Sexual transfer of B-chromosomes from maize, Zea mays L., into oat, Avena sativa L.

(submitted by Howard Rines <rines001@umn.edu>)

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B-chromosomes (B's) are supernumerary chromosomes described in several animal, fungal, and plant species. B's likely evolve from complements of autosomes (A-chromosomes, A's) and heterosomes (sex-chromosomes), and inherit species-specific structure and behavior. Although B's appear to be gene poor, relatively high numbers of B's can affect the fertility and fitness of the host genotype as reported in the intensely studied species Zea mays L. B's have not been reported in oat, Avena sativa L. From crosses of oat 'Starter' with a maize 'B73' derivative that carries B's from 'Black Mexican Sweet', out of 31 recovered F1-plants 14 tested positive for the maize repetitive element Grande-1. Two of the F1 plants had maize B's and no maize A's retained along with the haploid oat chromosome complement, based on positive assays for a B-specific marker that was obtained from J. Birchler, University of Missouri-Columbia, and absence of SSR-markers specific to each arm of the ten maize A's. In situ hybridization with labeled maize genomic DNA revealed two F1-plants that had one and three added maize B's, respectively. Twenty F2-offspring of each F1-plant were analyzed by cytological and molecular means. All F2-offspring of the F1-plant with one B lacked B's. The F1-plant with three B's produced three F2-plants with one B, six F2-plants with two B's, and eleven F2-plants with chimeric root meristems showing cells with one to five B's. All of the F2-plants with B's showed a regular 'Starter' oat phenotype with no distinct morphological characters. These oat plants with added maize B's separated from all maize A's provide an opportunity to investigate sequence uniqueness of B's and their possible evolutionary relationship to specific A's as well as transmission behavior of alien maize B's in an oat host.

P38

The 5íUTR region of maize Hsp101 mRNA contains an IRES.

(submitted by Jorge Nieto-Sotelo <jorge@ibt.unam.mx>)

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Cap-dependent translation is severely inhibited by heat stress in plants. To understand the mechanisms by which heat-shock protein mRNAs are preferentially translated during heat shock, we focused our analysis on Hsp101 mRNA that encodes HSP101, a heat-shock inducible disaggregase. In maize Hsp101 gene is essential for induced thermotolerance, its function negatively influences the growth of the primary root, and it is expressed in the embryo during its development where its mRNAs remain at maturity. We observed that the translation of Hsp101 in vitro was very efficient via a cap-independent mechanism. These results were achieved both in rabbit reticulocyte-lysate and wheat germ in vitro translation-systems where the initiation factor eIF4G was proteolytically cleaved or the eIF4E and eIFiso4E initiation factors were depleted, respectively. Deletion of the 5í untranslated region (5í UTR) from the Hsp101 mRNA showed that it was required for its cap-independent translation. We then asked if the translation of Hsp101 mRNA during heat stress could be mediated by cap-independent mechanisms such as an IRES (internal ribosome entry site). Similar to the foot-and-mouth disease virus (FMDV) IRES region, the 5í UTR of maize Hsp101 mRNA translated a downstream reporter gene when placed in the middle of a bi-cistronic construct in the sense but not in the anti-sense orientation. We conclude that maize Hsp101 mRNA contains an IRES or IRES-like element that is responsible for its highly efficient cap-independent translation during heat shock. The potential applications of the Hsp101 IRES in plant biotechnology will be discussed.

P39

The Etched 1 gene of Zea mays encodes a plastid protein with a zinc ribbon-like domain and similarity to eucaryotic transcription elongation factors.

(submitted by Udo Wienand <udo.wienand@uni-hamburg.de>)

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Etched 1 (et1) is a pleiotropic, recessive mutation of maize that conditions fissured and cracked mature kernels and virescent seedlings. Microscopic examinations of the et1 phenotype revealed an aberrant plastid development in mutant kernels and leaves. The et1 gene was cloned from several Mutator-induced et1 mutant and Et1 wild type alleles and analyzed at the molecular level. The data proved that a defect of the cloned gene is responsible for the observed mutant phenotype in maize.

The Et1 gene encodes a novel protein of about 19 kDa with an N-terminal plastid localization signal. Transient expression experiments with a GFP-fusion protein as well as in vitro import studies confirmed that ET1 is translocated into chloroplasts. The mature 12 kDa ET1 protein shows similarity to the zinc ribbon domain of several eucaryotic transcription elongation factors. Database search revealed that ET1 is a member of a small gene family in maize and Et1 homologous genes are also present in other monocotyledonous and dicotyledonous plants.

A polyclonal antibody raised against a peptide derived from the mature ET1 protein was used for Western-experiments and detected a protein of about 12 kD in a highly enriched fraction of the transcriptionally active chromosome (TAC) prepared from maize chloroplasts. These preliminary data suggest that ET1 may play a role in the transcription apparatus of plastids.

P40

Two Flavonones and Two Flavonols effects on *Aspergillus flavus* Growth

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A. flavus affects a broad range of plants and animals and produces aflatoxin which is the most potent natural carcinogen. A 7-fold increase in toxin levels was observed when there is a defect in the chalcone synthase gene in maize, the rate limiting step in the flavonoid biosynthesis. Flavonoids have been recognized as anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic compounds. They are act as potent metal chelators and free radical scavengers. Some flavonoids restrict the growth of *A. flavus*. Previous studies have examined the effect of flavonoids on fungi but the majority of the upper levels tested are beyond those observed in planta. In this study biologically relevant levels of compounds were tested for their effect on *A. flavus* growth. Two flavonones and two flavonols were tested in this experiment. Hesperetin, naringenin, quercetin, and rutin at concentrations of 0 *micro*M, 50 *micro*M, 100 *micro*M, 150 *micro*M, 200 *micro*M, and 250 *micro*M were added to Czapek-Dox agar supplemented with 10 g/L NaCl. Solvent controls were also tested by adding DMSO or ethanol depending on the compound solvent. Plates were inoculated with a 1 cm square of *A. flavus* NRRL3357 and grown in at 37 C. Fungal growth was measured at 2, 4, 6, 8, 10, 12, and 14 days after inoculation. Fungal diameter was averaged over all days for each compound and hesperetin was found to have the greatest effect on restricting growth. Rutin at 200 *micro*M inhibited fungal growth for 6 days as compared to the controls and hesperetin on a level basis. All other compounds tested inhibited fungal growth for 4 days. This information will contribute to our understanding of how these classes of compounds restrict fungal growth.

P41

Use of abundant retroelements to cytologically distinguish Tripsacum and Zea genomes

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Repetitive DNA elements, especially LTR retroelements make up a large portion of Tripsacum and Zea genomes. The genomic distribution of seven of the most common elements was examined on maize somatic chromosome spreads revealing three distinct patterns of hybridization: Grande was evenly distributed along the length of the chromosomes; Opie, Huck and Prem2 were abundant near the ends of chromosomes; and Tekay, Prem1, and Cinful were enriched near, but not at, the centromere. These retroelements were applied to chromosome spreads of Tripsacum andersonii, which is thought to contain the genomes of Zea luxurians and Tripsacum dactyloides, a hybrid between Zea mays ssp. mays and T. dactyloides, and to a "triple hybrid" which is an allopolyploid (Mangelsdorf and Reeves, 1935, provided by Dr. Mary Eubanks) that contains a haploid contribution from Zea mays ssp. mays, Tripsacum dactyloides and a Teosinte species. Only Tekay, the oldest element, was abundant in both Tripsacum and Zea and the other elements could distinguish the chromosomes from the two genera. Two retroelements, Huck and Opie, hybridized more intensely to Z. mays ssp. mays chromosomes than to the Teosinte ones and allowed all three genomes to be distinguished in the "triple hybrid." Additionally, screening genomic libraries by successive hybridization of whole genomic DNA from different species allowed clones containing elements expanded only in Tripsacum to be isolated.

P42

mRNA Differential Display of Drought Tolerant Inbred Lines Under Water Stress in Maize

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The method of mRNA differential display was used to research the differential mRNA expression of a drought tolerant maize inbred line under drought stress and irrigated control. 3 specific fragments (MD1, MD2 and MD3) were found under drought treatment and sequenced. The expression of MD1 and MD2 was regulated downward and the expression of MD3 was regulated upward under drought stress. Sequence alignment showed that MD1 had 97% homology with matK, a gene in maize chloroplast genome encoding RNA maturase; MD2 had 88% homology with encoding sequence of serine / threonine phosphorylase type 2C, concerned with stress signal transduction; and MD3 had 81% homology with encoding sequence of metacaspase (endopeptidase) in rice.

P43

tie-dyed2 is impaired in sugar movement out of leaves

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Successful growth of the plant requires movement of sugar from photosynthetically active leaves to other regions of the plant with little or no photosynthetic capacity. Loading of sugars from photosynthetic cells into the vascular system is crucial to this process. The recessive tie-dyed2 (tdy2) mutant of maize is characterized by a variegated yellow/green sectoring pattern in the leaves. Iodine staining of leaves revealed that starch accumulates to excessive levels in tdy2 yellow sectors while tdy2 green sectors and wild types leaves show the same low starch levels. Maize is a C4 plant and exhibits two distinct photosynthetic cell types, mesophyll and bundle sheath (BS) cells. During the day, starch accumulates in the chloroplasts of BS cells but is rarely seen in those of mesophyll cells. Histological and transmission electron microscopy (TEM) studies of tdy2 yellow sectors determined that starch is found in much higher levels in the BS chloroplasts and also accumulates uncharacteristically in the mesophyll cell chloroplasts. One explanation for the excess starch is that plasmodesmata (PD), the cytoplasmic strands connecting cells within the plant, are blocked preventing sucrose movement toward the vein. This mechanism has been proposed to explain the phenotype of sucrose export defective1 (sxd1) mutants, which hyperaccumulate starch in the leaves as a result of callose blockage of PD between BS and vascular parenchyma cells. Aniline blue staining of callose in tdy2 yellow sectors revealed no blockage at the interface between BS and vascular parenchyma cells. Likewise, no callose blockage of PD was detected in yellow sectors of tie-dyed1 (tdy1), a mutant phenotypically similar to tdy2. Further TEM investigations of tdy2 and tdy1 yellow sectors found no altered PD structures. These results suggest starch hyperaccumulates in tdy2 and tdy1 by a different mechanism than in sxd1. These results are concordant with genetic studies which show that plants doubly heterozygous for tdy1 and tdy2 display a mild tdy phenotype, suggesting the two genes function in the same pathway.

P44

A pattern matching approach to identify microRNAs in plant genomes

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The ability to control gene expression during development in plants could be used for improving crop yields, resistance to disease, and environmental adaptability. It has been suggested that microRNAs, or miRNAs, control developmental processes such as meristem cell identity, organ polarity, and developmental timing by interfering with the expression of mRNAs.
Since many noncoding RNAs display sequence conservation amongst eukaryotic genomes, we expect the microRNA precursors to be conserved in multiple plant genomes. Through sequence alignment we demonstrate that both the microRNA and opposing stem is conserved in more closely related cereal genomes, while the mature microRNA product tends to be conserved in more distantly related species.
To expand this analysis, we retrieved Arabidopsis thaliana microRNA precursors from 19 different gene families submitted to the microRNA Registry, a database of published microRNAs. Using a pattern matching approach to exploit multiple hairpin characteristics, while requiring microRNA sequence conservation, we identified 94 putative precursors within Oryza sativa indica, Zea mays, and Sorghum bicolor. In addition to phylogenetic analysis, stable secondary structures were found with MFold, therefore meeting both the biogenesis and phylogenetic criteria for submission to the microRNA Registry.
By expanding the precursor sequences and then aligning them together we have identified recently duplicated precursors, which is supported by their physical location near syntenic blocks between chromosomes. Given the temporal- and spatial-specific expression patterns within microRNA gene families, further understanding of how the families are evolving in conjunction with large-scale expression data should elucidate their individual roles in developmental regulation.

P45

Analysis of the origin of a small inverted repeat sequence found in a tissue culture induced allele of C2

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DNA sequence analysis revealed a 51bp of insertion sequence in the exon of a tissue culture-induced C2 allele. The insertion was composed of a nearly perfect 21bp of palindromic sequence with a 1bp "loop" and had an 8bp of direct duplication for the insertion site. The c2 insertion sequence was not homologous to any previously characterized transposon. An allele of the maize A2 gene [Genebank accession # X55314] had a larger palindromic sequence with homology to our c2 insertion sequence. In silico analysis of maize genome sequence identified 50 contigs with homology to the c2 and A2 insertion. In eleven cases, the sequence was present as an inverted repeat. In each case, the end of the homology was consistent with the end of the c2 insertion, and there was a 7 to 9 bp target site duplication. Additional sequences had only one of the repeat present. This analysis indicates that the small insertion found in our tissue culture-induced c2 allele may have originated from a previously uncharacterized family of transposons.

P46

Bioinformatic, phylogenetic and gene expression analyses of maize microRNA172 genes and their APETELA2- like gene targets

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One of the emergent topics of interest in biology is "what regulates the regulators?". Part of an answer to this question now includes the findings that many eukaryotic transcriptional regulatory proteins are regulated by genes encoding RNAs capable of directing gene-specific mRNA degradation and/or translational repression. In order to understand the functions of these genes and to characterize the rules that govern their interactions, we are examining the family of APETALA2-like transcription factors and the miR172 family of microRNA genes that regulate them in maize, rice and Arabidopsis. Overexpression of an miR172 gene in Arabidopsis has been shown to cause precocious flowering through downregulation of APETALA2-like genes. We have recently shown that overexpression of glossy15, a maize APETALA2-like gene, leads to delayed flowering as well as delayed vegetative phase change. The fact that these gene families show conservation of function across 135+ million years of plant evolution is indicative of their functional importance as well as their experimental utility. Members of the microRNA class of regulatory RNAs form a stem-loop structure that is processed into a 21-25 nucleotide RNA oligo that has ~95% complementarity to a binding site in a target gene. We have used these parameters to identify five miR172 genes and nine AP2-like target genes in maize, including gl15 and indeterminate spikelet1. We have elucidated the gene structure of two and five members of these families respectively and are using them to build phylogenies based on gene structure and amino acid homology. We have also mapped more than ten of these genes and are using these data to predict orthology with rice based on microsynteny. Finally, we report our progress toward developing gene-specific qPCR assays for these genes as well as RACE strategies that will characterize the microRNA intermediates.

P47

Examine the synteny between maize and rice

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Establishing a synteny relationship between two species allows us to leverage information from the genome of a well-studied organism (e.g. rice) to examine a less understood genome (e.g. maize). In this study, we use a densely marked, high-resolution maize physical map to calculate the pseudo-coordinates of more than 100,000 markers and BAC clones that have known sequences. Based on the alignment of these sequences to the rice pseudo-molecules by BLAST search, and using a novel algorithm that uses a fifth-order Markov chain, we established a high resolution synteny map between maize and rice with 5054 potential orthologous relationships. Our results suggest extensive genome collinearrity between these two species across all chromosomes.

P48

Gramene Diversity Module: Sharing the Data Behind Germplasm, QTL, and Breeding Studies

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Over the last decade, thousands of quantitative trait locus studies, germplasm surveys, and molecular maps have been published using a wide variety of germplasm resources. While there has been substantial progress in understanding the population structure, QTL, and the genetic architecture of plants, it has been very difficult to synthesize, share, and/or reanalyze the basic data behind these studies. Currently with the support of NSF and USDA-ARS, the Gramene, Maize, Wheat, and Rice diversity projects are collaborating to build the infrastructure to start sharing this basic molecular and phenotypic diversity data. We have developed a data model and schema (GDPDM; www.maizegenetics.net/gdpdm/), a Java XML-SOAP middleware component (GPDC; www.maizegenetics.net/gdpc/), a sequence alignment-SNP viewer (www.panzea.org), and an association diversity analysis tool (TASSEL; www.maizegenetics.net). All the software and schemas are open source. Species specific portals and basic query tools to these data sets are currently available for maize (www.panzea.org) and rice (rice-evolution.plbr.cornell.edu). Over the next year, we will be working to (1) incorporate more of the data from the various diversity projects, (2) facilitate sharing of data with species specific databases, (3) develop community upload tools for smaller datasets, (4) implement mechanisms to share data with germplasm databases (eg. GRIN and IRRI), and (5) create enhanced query, display, and analysis tools. We encourage community input and collaboration on this effort, so that the largest possible community can access and productively use diversity data.

P49

MaizeGDB Community Curation Tools

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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 (1) made their data available by contributing to MaizeGDB and (2) helped to guide the efforts of the MaizeGDB Team by giving lots of needed input. In an effort to enable researchers to contribute their data directly via the web, we have developed and tested community curation tools which are now available for general use. Stop by this poster to learn how you can become a community curator for MaizeGDB!

P50

MaizeGDB In A Nutshell: A Tour Of The Maize Community Database

(submitted by Trent Seigfried <devolver@iastate.edu>)

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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 PlantGDB's EST and GSS 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.

P51

Multi-K means Clustering - Capturing the Natural Shape of Data

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Microarray technology permits the analysis of the expression patterns of thousands of genes at one time. Clustering groups genes with similar expression patterns and is therefore a useful analytical approach. However, the clustering results from all available clustering algorithms are strongly influenced by the choice of distance/similarity measure used to find the distance between or similarity of the data points being clustered. The Multi-K means clustering algorithm compensates for the intrinsic shape of a distance/similarity measure. During Multi-K means clustering a function of the data (the cut plot) is computed that can be used to suggest the natural numbers of clusters for the microarray data. Multi-K means clustering was tested on four synthetic data sets that had been designed to defeat K-means clustering. Our simulations show that Multi-K means clustering successfully discovered the designed cluster structure of all four synthetic data sets. We also present the clustering of actual microarray data from maize.

P52

PathBinderH - a tool for Linnaean Taxonomy-Aware Literature Searches

(submitted by Karthik Viswanathan <vkaru@iastate.edu>)

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As the amount of published literature increases it becomes increasingly difficult to identify relevant publications. PathBinderH is a new tool that allows users to more easily retrieve relevant abstracts from PubMed. The most innovative feature of PathBinderH is that users can limit searches to abstracts mentioning either a user-specified taxon or any subordinate taxa. For example, specifying “Poaceae” (the grass family) as the taxon will cause PathBinderH to search among those abstracts that explicitly mention Poaceae or any of its synonyms, as well as those that mention wheat, rice, maize or corn, or any other taxon subordinate to Poaceae. Within that taxonomy-qualified set of abstracts, PathBinderH retrieves individual sentences matching the userís query. In addition to taxonomy-based retrieval, PathBinderH displays sentences with the query terms highlighted to enhance its user friendliness by further increasing the ease with which users can identify material of interest. PathBinderH is a web-served tool, available at www.plantgenomics.iastate.edu/PathBinderH/.

P53

Plant RNA Binding Proteins: Tools for functional genomics and application to chloroplast biogenesis

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Interactions between proteins and RNAs underlie every aspect of plant development and function. Plant genomes encode many hundreds of proteins that harbor predicted RNA binding motifs, few of these proteins have been studied in detail. To dissect RNA-protein interaction networks in plants, it is necessary to catalog plant RNA-binding proteins, to identify the RNAs with which they interact, and to determine how those interactions influence the fate of the RNA and downstream processes. We have developed the following tools to address each of these issues, and we are applying them to a set of 40 plant-specific RNA binding proteins that function in the chloroplast. The 40-protein set emphasizes the CRM and PPR protein families, which are largely specific to plants and together contain ~500 members, most of which are predicted to be targeted to chloroplasts or mitochondria.
1) A relational database of plant RNA binding proteins (RBP) that will integrate data from rice, maize, and Arabidopsis. The database will feature cross-referenced orthologs and paralogs, annotated with gene models, targeting predictions, and experimental data.
2) RIP-CHIP analysis to identify the RNAs associated *in vivo* with chloroplast-localized RNA binding proteins. This approach couples coimmunoprecipitation with microarray technology.
3) Reverse genetics in maize and rice to pinpoint aspects of RNA metabolism that are influenced by each of these 40 proteins. Mutants will be sought in maize through reverse genetic screening of our PML collection (pml.uoregon.edu). RNAi or Tos17 insertions will be used in rice when informative mutants are not recovered in maize.
4) We are also working to develop a microarray based approach to facilitate the cloning of Mu-tagged mutations. The approach is based on the fact that most Mu-insertions that cause strong phenotypes also cause a severe loss of mRNA from the disrupted gene, and takes advantage of the recently released maize 70-mer microarrays (Chandler et.al).

P54

Study of the unorthodox breeding behavior of shrunken 1 Bombay

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The Shrunken (Sh) locus on chromosome 9 of Zea mays encodes the enzyme sucrose synthase (EC 2.4.1.13) that catalyzes the cleavage reaction of sucrose to UDP-glucose and fructose in both directions. The enzyme is involved in starch metabolism of the developing endosperm. In maize strains homozygous for recessive sh mutations, sucrose synthase activity is decreased to 2-6% and starch content in the mature kernels is decreased to 60%. This causes the shrunken phenotype.
A new allele of sh1 is reported. Along with kernels having somatic instability for aleurone pigmentation, metastable shrunken kernels have been seen in the progeny. These kernels on crossing to sh1 tester yielded all shrunken kernels indicating it to be a sh1 allele (designated sh1 Bombay) and is located on the short arm of chromosome 9.
The behavior of sh1B is puzzling. Somehow these phenotypes appear to be mere reminiscent of activation and inactivation of an allele by methylation. The DNA extracted from sh1B seedlings, when cut with HpaII and HhaI; both enzymes are sensitive to CG methylation. Southern blots probed with sucrose synthase I, showed that methylation is quite stable upon selfing but subjected to change upon crossing back to the sh1 tester. Speculatively, there is a possibility that when the sh1B enters the dent background of the American tester, it is restricted in its expression which results in a phenomenon known as allelic “cross-talk.”
The nucleotide sequence of sh1 B has been elucidated. Further computational sequence analysis performed with an objective of identifying the determinants of its functional features will also be reported.

P55

Technical Aspects of the MaizeGDB Project

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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. Datatypes stored at MaizeGDB include (but are not limited to) sequence, locus, variation, probe, map, metabolic pathway, phenotype, quantitative trait locus (QTL) experiment, stock, and contact information for hundreds of maize researchers worldwide. Technical aspects of the MaizeGDB project are largely unknown to many maize geneticists. Here we aim to convey how data is stored and maintained, and outline the various methods by which data are made accessible. We outline how the database infrastructure was built and is currently maintained, and the machine architecture is explained in detail. How each copy of the database is utilized is illustrated, and standard operating procedures employed at MaizeGDB are described.

P56

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

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~850,000 methyl-filtered (MF) and high Cot (HC) GSSs generated by the Consortium for Maize Genomics from the "gene space" of maize were assembled into MAGIs (Maize Assembled Genomic Islands, Emrich et al., 2004). Similarly ~500,000 gene-enriched sorghum GSSs produced by Orion Genomics were assembled into SAMIs (Sorghum Assembled genoMic Islands). A web-resource is available at http://www.plantgenomics.iastate.edu/maize/ 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. A new genetic map containing ~3,500 markers (produced by the ISU project and the MMP) has been generated using MultiMap. This genetic map, including linkages to the physical map produced by the AGI, can be viewed via CMap. Detailed annotation regarding all ISU markers is available on-line. 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.

P57

The Plant Ontology Consortium: Comparing phenotypes and gene expression among angiosperms.

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A primary goal of the Plant Ontology Consortium (POC; http://www.plantontology.org) is to develop simple, yet robust and extensible controlled vocabularies that accurately reflect the biology of plant structures and developmental stages. These provide a semantic framework for meaningful cross-species queries across diverse databases. The current ontology and gene annotation release integrates the diverse vocabularies in use to describe Arabidopsis, maize and rice anatomy and morphology. This integration spans two major taxonomic divisions: monocots and dicots, and two grasses with very distinct anatomy. Using our ontology browser, the Plant Ontology AmiGO, over 3000 gene annotations from three species-specific databases: TAIR, Gramene and MaizeGDB, can now be queried and retrieved. This year, POC will extend this controlled vocabulary to include terms for growth and developmental stages, and to integrate other species groups: legumes, Solanaceae, poplar, and the Triticeae. We will present the organizing principles and rules followed in developing the plant ontology. We will address progress towards defining standards and methods for using the Plant Ontologies to annotate gene expression and phenotypes. We will provide examples of queries using annotations from member databases where the PO supports gene discovery, predicting phenotypes, determining functions of gene products and accessing mutant stocks and germplasm. The project is supported by National Science Foundation grant No. 0321666.

P58

Two Novel Arginine/Serine (SR) Proteins in Maize are Differentially Spliced and Utilize Non-Canonical Splice Sites

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The serine-arginine (SR)-rich splicing proteins are highly conserved RNA binding nuclear phosphor-proteins that play important roles in both regular and alternative splicing. Here we describe two novel putative SR genes from maize, designated zmRSp31A and zmRSp31B. Both genes contain characteristic RNA binding motifs RNP-1 and RNP-2, a serine/arginine-rich (RS) domain and share significant sequence similarity to Arabidopsis atRSp31 family of SR proteins. Both zmRSp31A and zmRSp31B produce multiple transcripts by alternative splicing, of which majority of the alternatively spliced transcripts utilize non-canonical splice sites. zmRSp31A and zmRSp31B produce at least six and four transcripts, respectively, of which only one corresponds to the wild type proteins for each gene. All the alternatively spliced transcripts of both the genes with one exception are predicted to encode small truncated proteins containing only RNP-2 domain of their first RNA recognition motif and completely lack the carboxyl terminal RS domain. We provide evidence that some of the alternatively spliced transcripts of both genes are associated with polysomes and interact with the translational machinery.

P59

Use PGROP to locate educational activities, programs, and resources about plant genomics

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PGROP, an educational site ancillary to PlantGDB, provides a centralized repository for various Plant Genome Research "Outreach" Program materials and related activities (http://www.plantgdb.org/outreach). PGROP's intended audience includes (but is not limited to) high-school students and teachers, undergraduates (including special target groups like minorities), journalists, and the general public. It is the goal of the PGROP project to broaden participation of these various groups in plant genome research by helping them find materials, information, and answers to questions that are related to plant genomic research.

P60

Agrobacterium-mediated stable transformation of multiple maize inbred lines using a standard binary vector system

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This study was undertaken to identify a tissue culture media regime for the efficient stable transformation of one or more maize inbred lines using a standard binary Agrobacterium-mediated gene delivery system established at Iowa State University Plant Transformation Facility. In 2003, ten public maize inbred lines were tested on two N6 based divergent medium backgrounds using field embryo materials. In 2004, the same media backgrounds, but with MS or N6 salts and vitamins, were compared with a subset of these inbreds (B114, B104 and Ky21). While stable transformants were generated from both medium backgrounds MS-based media appeared to enhance stable recovery of transgenic inbred maize compared to N6 salts for these three inbred lines. Molecular evidence and progeny analysis data will be presented.

P61

Characterization of Cyclins and Cyclin-Dependant Kinases in Developing Maize Endosperm Cells

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In developing maize endosperm, three types of cell cycle occur successively. Syncitial development takes place during the first three days after pollination (DAP). A mitotic stage follows and persists until 8-10 DAP. Finally, endoreduplication takes place, resulting in polyploid cells. The mitotic stage comprises the four classic cell cycle phases (G1, S, G2, M), while the endoreduplication stage consists of alternating G and S phases. Progression through cell cycle phases is controlled by cyclin-dependent kinases (CDKs), which are regulated by association with particular cyclin subunits. In plants, D-type cyclins control the G1/S transition; A-type cyclins control S-phase progression and the G2/M transition; and B-type cyclins control G2/M and mitotic transitions. The nature and role of cyclins during the mitotic and endoreduplication periods of endosperm development are unknown. To investigate these questions, we characterized cyclins A1;3, B1;3, D5;1, and D2;1. Polyclonal antibodies were generated to determine the pattern of cyclin accumulation, associated CDK activity, and spatial localization of cyclins in developing endosperm. Cyclin A1;3, and B1;3 RNA levels decreased sharply as the endosperm transitioned from the mitotic to the endoreduplication stage, whereas cyclin D2;1 and D5;1 RNA levels decreased only slightly. Cyclin protein levels remained nearly constant throughout development, except cyclin A1;3, which declined more abruptly. CDK activity associated with cyclin A1;3 was highest at 7 DAP, while that associated with the other cyclins peaked at 11 DAP. Cyclins A1;3, B1;3 and D2;1 were found throughout the endosperm while D5;1 was mostly in aleurone and subaleurone cells. At the cellular level, cyclins A1;3 and B1;3 localized predominantly to the cytoplasm with some diffuse staining in the nuclei of interphase cells. Cyclin D2;1 localized to nuclei, whereas D5;1 formed aggregates in the cytoplasm of aleurone cells throughout development.

P62

Drought Tolerant and Male Sterile Material Screening from Maize Callus Mutated by Gamma Ray and Sodium Azide

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Maize callus was mutated by 60Co gamma ray and sodium azide (NaN3), and screened on high osmotic medium with 1.0% NaCl. The lines of the regenerated plants were identified for their drought tolerance. The result indicated that gamma ray absorption dosage of 20 Gy and NaN3 concentration of 1 mmol/L were suitable treatment combination to mutate maize callus. From 22 lines of regenerated plants, 5 lines were identified to be more tolerant to drought than non-mutated control, and one of them had almost the same tolerance with drought tolerant inbred line 81565. Moreover, 1 M1 plant was found to be male sterile and identified as spore male sterility controlled by one karyon recessive gene.

P63

Functional loss of a COBRA family protein in the maize brittle stalk2 (bk2) mutation

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First described in 1940, bk2 is characterized by a developmentally-programmed recessive mutant phenotype in which all parts of the maize plant turn suddenly brittle following the 4-leaf stage. Consequently, bk2 plants snap easily, making it hard to grow them unassisted. There appears to be a link between the bk2 phenotype and cellulose deficiency, as the mutants typically have 20 to 30% less cellulose compared to their wild-type siblings. To clone the gene underlying bk2, a directed transposon tagging approach employing Mutator (Mu) was used that resulted in the identification of two mutants. However, before we had an opportunity to identify Mu elements responsible for these mutations, a brittle culm (bc1) gene was cloned from rice that mapped to a location in the rice genome having synteny with the maize bk2 region. This raised the possibility that bk2 was same as bc1. Confirmation of this kinship came from a reverse genetic approach that employed sequence information from a maize homolog of bc1 and template DNA from Mu-tagged mutants. The bc1/bk2 gene encodes a protein of the COBRA family. Since all COBRA proteins are predicted to be anchored to the outer surface of the cell membrane through a glycosylphosphatidylinositol linkage, it is thought that they serve important coordinating roles at the plasma membrane-cell wall interface. A bizarre feature of the maize bk2 gene was revealed: its transcriptional activity appears opposite to what would be expected from its mutant phenotype.

P64

Maize Shugoshin is required for Centromeric Cohesion during Meiosis

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During meiosis, the formation of four haploid gametes requires two rounds of chromosome segregation, first, a reductional division segregating homologous chromosomes, and, second an equational division segregating chromatids. To control this two step mechanism, sister chromatid cohesion is released along the chromosome arms during meiosis I but is retained at the centromeres at anaphase I to allow the segregagtion of homologs. Centromeric cohesion is then required for the formation of the metaphase II plate and is released to allow the segregation of chromatids at anaphase II (reviewed in Nasmyth 2001 and Petronczki et al. 2003). Drosophila MEI-S332 and yeast SGO1 proteins are essential for centromere cohesion in meiosis (Kerrebrock et al. 1995; Moore et al. 1998; Kitajima et al. 2004; Marston et al. 2004; Rabitsch et al. 2004 and Katis et al. 2004). We have isolated two maize mutants in the ZmSGO1 gene and demonstrated that the absence of the ZmSGO1 protein induces premature loss of centromeric cohesion at prophase II. We also showed that, although ZmSGO1 is loaded at the centromeres as early as leptotene in the wild-type, chromosome structure, bouquet formation, pairing, synapsis, and mono-polar sister chromatid kinetochores orientation were not impaired in the zmsgo1 mutants during the first meiotic division. In the afd1 (rec8) mutant background, no ZmSGO1 immunostaining was observed, suggesting that AFD1/ZmREC8 is required for the recruitment of ZmSGO1. Based on these results, we propose that ZmSGO1 is responsible for the maintenance of centromeric cohesion during meiosis. We plan to investigate further the phenotypic variation among the zmsgo1 alleles, the relationship between ZmSGO1 and the spindle, the extent and control of loading of the ZmSGO1 protein onto the chromosomes as well as the role ZmSGO2, a close ZmSGO1 homolog, during meiosis in maize.

P65

Mitochondrial biogenesis and function in developing pollen of normal and S male-sterile maize

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In the S cytoplasmic male sterility (CMS-S) system of maize, expression of mitochondrial open reading frames (orf355-orf17) conditions collapse of developing haploid pollen following the microspore mitosis. The orf17 gene predicts a protein that is 65% identical to the mitochondrial ATP synthase subunit 9 (ATP9) C-terminal membrane domain. Antibodies raised against the ORF17 N-terminus recognized a 6 kDa protein that accumulated predominantly in CMS-S microspores. Two approaches were used to assess whether ATP synthase function is compromised during the development of CMS-S maize pollen. Western blot analysis showed decreased accumulation of ATP synthase subunits 4, 6, and 9 in CMS-S microspores as compared to normal microspores. Measurements of ATP demonstrated similar ATP levels in the CMS-S microspores and pollen compared to normal microspores and pollen. We propose that ATP synthesis in microspores and pollen relies upon glycolysis and fermentation. Consistent with this hypothesis, mitochondria of late-stage normal pollen failed to accumulate ATP9. Aberrant ATP synthase assembly in CMS-S microspores does not, therefore, alter bioenergetics, but may condition the release of cell death signaling molecules from the mitochondria. Mitochondrial release of the intermembrane space protein cytochrome c is a hallmark of programmed cell death in plants and animals, and mitochondria of collapsed CMS-S pollen were depleted of the intermembrane space protein cytochrome c.

P66

Phosphoserines on Maize CENTROMERIC HISTONE H3 and Histone H3 Demarcate the Centromere and Pericentromere during Chromosome Segregation

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We have identified and characterized a 17- to 18-kD Ser50-phosphorylated form of maize (Zea mays) CENTROMERIC HISTONE H3 (phCENH3-Ser50). Immunostaining in both mitosis and meiosis indicates that CENH3-Ser50 phosphorylation begins in prophase/diplotene, increases to a maximum at prometaphase-metaphase, and drops during anaphase. Dephosphorylation is precipitous (approximately sixfold) at the metaphase-anaphase transition, suggesting a role in the spindle checkpoint. Although phCENH3-Ser50 lies within a region that lacks homology to any other known histone, its closest counterpart is the phospho-Ser28 residue of histone H3 (phH3-Ser28). CENH3-Ser50 and H3-Ser28 are phosphorylated with nearly identical kinetics, but the former is restricted to centromeres and the latter to pericentromeres. Opposing centromeres separate in prometaphase, whereas the phH3-Ser28-marked pericentromeres remain attached and coalesce into a well-defined tether that binds the centromeres together. We propose that a centromere-initiated wave of histone phosphorylation is an early step in defining the two major structural domains required for chromosome segregation: centromere (alignment, motility) and pericentromere (cohesion).

P67

AFD1, a maize REC8 homolog, displays dose-dependent functions during meiosis

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Isolated in 1975, absence of first division (afd1-1) is a well characterized maize mutant impaired in sister chromatid cohesion, synapsis and establishment of leptotene chromosome structure (Golubovskaya and Mashnenkov 1975; Golubovskaya et al., 1993; Chan and Cande, 1998; Kaszas and Cande, 2000; Yu and Dawe, 2000; Pawlowski et al., 2003). Furthermore, afd1-1 is impaired in the establishment of leptotene chromosome structure, a process that is not at all understood. REC8, a key component of the meiotic cohesin complex, is required for establishment of chromosome structure and sister chromatid cohesion (Uhlmann, 2003 ; Lee and Orr-Weaver, 2001 ; Nasmyth, 1999). Here we show that AFD1 encodes a REC8 homolog. Using four afd1 alleles, we demonstrate that AFD1/ZmREC8 is absolutely required for sister chromatid cohesion maintenance and pairing. Surprisingly, in weak afd1 alleles, we observed wild-type leptotene chromosome structure as well as bouquet formation at zygotene. Maintenance of these processes was correlated with the level of AFD1/ZmREC8 expression, suggesting for the first time that the establishment of early prophase chromosome structure does not require a full level of REC8. Based on these results, we propose a model in which distinct REC8 functions are dose-dependent during meiosis.

P68

Behavior of minichromosomes derived from the B chromosome of maize

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Twenty-one 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. A specific repetitive sequence of the B chromosome centromere (called B repeat) was used to determine the transmission frequency of different minichromosomes. In two cases, the minichromosomes contained two B chromosome centromere sequences. Three minichromosomes contained two B repeat clusters. One minichromosome is extremely small. Eleven minichromosomes can pair with each other with a frequency of 100% to 25% at the pachytene stage. Ten other minichromosomes could not pair with themselves and in these cases sister chromatids divide equationally at meiosis I. In the plants containing one minichromosome, the sister chromatids of minichromosomes separated precociously at meiosis I. In anaphase II, the minichromosomes progressed to one pole or the other. Thus, most tetrads had two B specific signals. Some minichromosomes were probed with CentC and CRM sequences and CenH3 antibodies to examine the nature of the remaining centromere. Mature pollen FISH results indicated that there is no nondisjunction of any the 21 minichromosomes. The transmission rate of univalent minichromosomes is below the theoretical 50% frequency as determined by FISH of the B repeat signal in mature pollen.

P69

Breakage-fusion-bridge cycles, chromosome healing and unequal recombination events in maize callus cultures

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Cytological analysis of callus cultures derived from tropical inbreds has revealed the occurrence of breakage-fusion-bridge (BFB) cycles initiated after chromosome breakage in anaphase bridges resulting from delayed separation of chromatids at knob sites (Ann. Bot.78: 73, 1996). Chromosome 7 possessing knobs on the short (K7S) and long (K7L) arms was the most affected. Here, we examined aberrations of chromosomes 7 and 9 in C-banded metaphases from samples taken from 2 to 3-year-old calli. In one culture (3-57), a chromosome 7 bearing reverse tandem duplications (RTDs) in the short arm and a deficiency at K7S showed a stable broken arm in most samples. FISH using a probe of the telomeric repeat TTTAGGG gave evidence of healing of this arm. Culture 3-57 was homozygous for K9S, which was stable in some subcultures, but deleted in other ones. A stable minichromosome appeared in metaphases showing chromosome 9 aberrations induced by BFB cycle. Plants homozygous for normal chromosome 7 and heterozygous for the RTD chromosome were observed in R1 progenies derived from culture 3-57, but RTD homozygotes were not detected. In other callus (12F), an amplification of K7L was observed and FISH showed telomere signals in both arms of chromosome 7. Plants homozygous and heterozygous for the K7L amplification were found in R1 progenies. The presence of homozygotes suggests that the K7L amplification may be derived from unequal recombination, resulting in amplification and partial deficiency at K7L, and maintaining intact the distal segment between K7L and the chromosome end, whereas a BFB cycle would delete this segment. Metaphases showing sister chromatids of the chromosome 7 with C-bands (K7L) with different sizes were detected in 2-month-old cultures, thus providing evidence of unequal recombination at knob sites in vitro. These results are interesting in that they show the response of knobbed chromosomes and how knob size may be altered in vitro.

P70

Characterization of a Maize Isochromosome 8S:8S

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An isochromosome was found in the HiIIB line during karotyping with a multi-probe FISH system developed by Kato et al. (2004). Cytological analyses show that it pairs with the short arm of chromosome 8 during the pachytene stage of meiosis, which suggests its origin from chromosome 8. The 8S origin was confirmed by FISH. Metaphase chromosomes from root-tips were probed with a knob probe, and weak signals were present at the short arms of chromosome 8, and also observed at both ends of the isochromosome. This isochromosome can form a univalent through pairing of its two homologous arms as well as a trivalent by pairing with the normal chromosome 8 at diakinesis and metaphase I. At anaphase, this isochromosome lags behind other chromosomes. It has a low transmission rate of about 17% from both male and female parents. The heterozygotes that contain one isochromosome are phenotypically normal as compared with the original HiIIB plants. However, the homozygotes that contain a pair of this isochromosome (six total copies of 8S) grow slower and are shorter than the normal HiIIB plants, and have a reduced fertility.

P71

Construction of a High-Density Cytogenetic Map of Maize Chromosome 9

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The goal of this research is to develop a detailed pachytene FISH map of maize chromosome 9 using 40 different loci. This project will establish an optimized procedure for the production of a cytogenetic map of the entire maize genome. The maize chromosome 9 is visualized by chromosome painting in an alien addition line of oat. Maize marker-selected sorghum BACs were identified by one of several methods and used as FISH probes. We have developed a multi-wavelength direct-labeled FISH method in which the paint, sorghum BAC, and the centromere are directly visualized after hybridization without the need for secondary detection. The 3-D FISH image data were collected using the DAPI (total DNA), FITC (maize 9), Rhodamine (sorghum BAC probes), and Cy5 (maize Cent-C) channels. Following 3D image deconvolution, the chromosome fibers were straightened, and the arm ratio and the average cytogenetic location of each locus were determined. The 40 loci fall into four categories: core bin markers (CBM), framework markers (FWM), centromere-linked markers (CLM), and off-frame markers. We have recently FISH-mapped the following BACs: wx1 / CBM9.3 (9S.13), cdo17 (9L.03), rgpr3235a (9L.04), and sbb16685 (9L.54). FISH-mapping of centromere-linked markers provides a new method for indirect assignment of the centromere to a defined genetic interval on the linkage map (e.g., currently between wx1 and cdo17). This cytogenetic map will also enhance future research on structural genomics, positional cloning, and comparative genome analysis among the grasses. The cytogenetic FISH map data and chromosome images are available online through MaizeGDB.

P72

Development of a Pachytene Cytogenetic FISH Map of the 90 Core Bin Marker Loci

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We are in the process of developing a pachytene cytogenetic FISH map of the maize genome using sorghum BACs corresponding to the 90 maize Core Bin Marker (CBM) loci. The CBM loci were chosen because they are uniformly distributed and they delineate the widely used genetic bins derived from the UMC98 linkage map. This project will provide insights into the structure of the maize genome, while creating new technologies and reagents for downstream chromosome research. We are using the new single-locus cytogenetic FISH mapping system for maize that was first described by Koumbaris and Bass (2003) and later revised by Ferdinand Amarillo (see nearby poster). Maize marker-selected sorghum BACs are used as FISH probes which are hybridized to maize pachytene chromosomes from addition lines of oat. In addition, we have initiated an RFLP full-length insert sequencing project to enable in silico screening for suitable sorghum BACs. So far, at least 20 RFLPs have been annotated and deposited into GenBank. Current cytogenetic mapping of the CBM loci is focused on chromosomes 1, 3, 4, and 6. The specific procedure for selecting sorghum BACs will be illustrated using CBM 1.11 (umc161) as an example. The completion of a cytogenetic map will provide a means for integrating the physical, genetic, and cytological maps of the maize genome.

P73

Endoreduplicated Chromosome Structure

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The chromosomes of the maize endosperm, in later stages of development, proceed through an endoreduplication phase. Endoreduplication is a process is which the cell cycle changes from a normal type to one that skip the M phase, thus allowing multiple rounds of chromosome synthesis. When this occurs, the normal triploid endosperm can reach ploidy levels greater than 200x in some lines of maize. There have been many studies on endoreduplication that have investigated the genes involved, influence of hormones, timing, epigenetic control, and environmental influences. Nevertheless, the molecular mechanism has not been elucidated. In this study, we examined the structure of the endoreduplicated chromosomes. Previous cytological work has indicated that, although the DNA content per cell increases, the number of nucleoli and knobs remain the same. It has been suggested that only regions with transcribed genes are amplified. With the use of fluorescence in situ hybridization and slot blot techniques, we have shown that the highly repetitive heterochromatic areas both on the A and B chromosomes, as well as several actively transcribed genes, are replicated. This result suggests that the entire genome follows the same trend. Further evidence shows that the multiple copies, after they have been replicated, stay associated throughout the length of the chromosomes, and that the DNA at the centromeric and knob regions are more tightly associated than the other regions of the chromosomes.

P74

Inactivation of the B chromosome centromere in an A-B translocation

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A plant carrying an A-B translocation chromosome of maize was found among the progeny of a hemizygous B-9-Dp9 chromosome together with two 9-B chromosomes. Multicolor FISH results indicate that this translocation chromosome was derived from chromosome 9 of maize and the B chromosome centromeric region. Fluorescence in situ hybridization (FISH) revealed that this chromosome carries sequences typical of the centromeres of both chromosome 9 and the B chromosome. Both of the centromeric regions contained CentC and CRM signals. Pairing between this dicentric chromosome and a normal chromosome 9 was observed at meiosis. CENH3 detection revealed that the centromere from chromosome 9 is functional, whereas the B centromere sequences are inactive. Homozygous plants of this chromosome were albino and seedling lethal because the translocation chromosome does not contain the very distal part of 9S. Because all of the sequences surrounding the centromere of the normal B chromosome are present in this translocation but do not attract CENH3, the translocated B centromere is apparently inactivated.

P75

Mitochondrial DNA Insertion into Nuclear Chromosomes of Maize

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Over evolutionary time, the transfer of genes from the mitochondrion to the nucleus has proceeded to a degree that mitochondria now require nuclear genes to function. Our study suggests that this DNA transfer is an ongoing process in maize. Using fluorescence in situ hybridization we have identified the locations of mitochondrial DNA within the nuclear chromosomes of the B73, B37, Mo17 and Black Mexican Sweet (BMS) lines. Twelve cosmids, representing over 70% of the 570 kb NB mitochondrial genome, were fluorescently labeled and hybridized to metaphase root tip chromosomes. In B73, we identified 8 different insertion sites on chromosomes 2, 3, 4, 7 and 9, predominantly near centromeres and telomeres. Interestingly, the detectable nuclear insertion sites were not consistent among the lines studied. Although each of the lines showed 6 to 8 sites of mitochondrial DNA insertions, only chromosome 2 was labeled in all 4 lines. This result raises the possibility that mitochondrial DNA sequences have been recently and independently transferred into the nuclear DNA of different maize lines.

P76

Progress from the Maize Centromere Consortium

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This poster will describe prior results and future goals of the NSF Plant Genome project "Functional Genomics of Maize Centromeres." Centromeres are large chromosome domains that organize and regulate chromosome movement. They contain thousands of simple repeated sequences and hundreds of RNA-based transposable elements, all of which are difficult to sequence and manipulate in the laboratory. Despite these challenges, mammalian centromeric DNA has been exploited to create 'artificial chromosomes' that can carry long segments of engineered DNA. Artificial chromosomes could also have widespread applications in agriculture. For instance, entire biochemical pathways or multiple disease resistances could be introduced simultaneously. The experiments carried out under this award are designed to fill major gaps in our understanding of plant centromeres and build the foundation for creating artificial chromosomes in maize and rice.

During the last five years we have: 1) Sequenced over 200 kb of centromeric DNA and shown that centromeres are composed of satellite repeats and retrotransposons, many of which are centromere-specific; 2) Generated a high-resolution fiber-FISH map of the maize B centromere; 3) Used ChIP to demonstrate that CentC and the centromeric retrotransposon CRM interact strongly with Centromeric Histone H3 (CENH3); 4) Identified a phosphorylated form of CENH3; and 5) Transformed rice with centromeric BACs and shown that large centromere-like inserts can be recovered at single sites. In the next five years, we hope to: 6) Identify and map centromeric contigs using the public BAC library and database; 7) Fully sequence Centromere 4; 8) Map at least three kinetochore proteins along centromeres at high resolution; 9) Test the idea the maize centromeres evolve by meiotic drive; and 10) Make functional artificial centromeres in maize and rice.

P77

The Effects of Monosomy on the Rate of Transmission of Abnormal Chromosome 10

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When a diploid maize genome contains at least one copy of Abnormal Chromosome 10 (Ab10; also referred to as K10 elsewhere), three phenomena are observed: an increased rate of recombination, neocentromeric activity, and preferential segregation (meiotic drive). Whether Ab10 requires a pairing partner for preferential segregation to occur is unknown. The r-X1 deficiency is an X-ray induced deficiency that includes the R locus on chromosome 10. A high rate of non-disjunction occurs in embryo sacs that contain the r-X1 deficiency, producing monosomics at a rate of ~10%. To find out how Ab10 behaves in the unpaired condition, crosses are being carried out to produce plants that are monosomic for Ab10, N10 (the normal variant of chromosome 10), and smd3-Ab10 (Ab10 carrying the suppressor of meiotic drive 3 mutation). Each will be crossed to N10/N10 plants, and the rates of seed set will be documented. If rates of seed set are the same among all three types, we will conclude that Ab10 requires a pairing partner to cause preferential segregation. However, if seed set is higher in monosomics for Ab10 than in monosomics for N10 or smd3-Ab10, we will conclude that Ab10 does not require a pairing partner for preferential segregation to occur. Crosses have been developed using various alleles of R (which is tightly linked to the Ab10 differential segment) enabling transmission rates for each chromosome variant to be determined directly by kernel phenotype. Molecular markers are in development for confirming the chromosome constitution of plants via PCR amplification of sequence variations associated with each chromosome 10 variant involved in the experiments. Preliminary data suggest that r-x1/Ab10 heterozygotes show the expected rates of transmission for each chromosome variant. Preliminary transmission rate data also have been collected for r-X1/smd3-Ab10 heterozygotes. Thus far, no data have been gathered for the monosomics.

P78

The Maize-10-Maze project, a public field replica the maize pachytene karyotype, decorated with mutants.

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The *Maize-10-Maze* project is a public outreach effort of the *Cytogenetic Map of Maize* project (www.cytomaize.org). The pachytene karyotype will be used to develop a field-scale version, as a serpentine maze, of the maize genome for public exploration and education. Each row will represent a single chromosome. Selected mutants will be grown within the rows, located at their known or predicted cytogenetic position (e.g. bz1 at 9S.65). K-12 science classes are making field placards that will describe the individual mutants. Our goal is to produce a fun and educational self-guided public tour of the maize genome. The criteria for choosing which mutants to include were that the mutant (1) should exhibit a visually striking or cool plant or seed phenotype - such as *Knotted1*, or (2) should be of agronomic importance - such as *shrunken2*, or (3) should be of major scientific or historic importance - such as *pericarp color1*. We have selected, grown, and photographed over 120 mutant stocks so far and now seek additional feedback in advance of the display, scheduled for summer 2006 in Quincy, FL and 2007 in Quincy or Tallahassee, FL. A photographic model will be presented. The project is expected to help raise public awareness about plant genome research, while also highlighting the remarkable and historic genetic diversity within maize. If successful, the project can be scaled up or down and replicated anywhere corn can be grown.

P79

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* (*n*on-*r*eduction *m*utant) 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. 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.

P80

A Maize Chimeric Promoter Drives High Level GFP Expression in Endosperm and Embryo

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Seed storage proteins accumulate in a tissue specific manner due to promoter elements driving transcription during seed development. The embryo seed storage protein globulin-1 accumulates to high levels in embryo tissue and to a lesser extent in aleurone seed tissue. An endosperm seed storage protein, the 27 kDa gamma zein, is present in one or two copies in the genome, yet is one of the most abundant seed proteins representing up to 15% of total endosperm protein content. In our previous studies, promoters of these genes have been used to produce high-levels of GFP in transient expression assays and in transgenic maize plants. In this study we have created a chimeric promoter consisting of regulatory cis-elements originating from both the globulin-1 and 27 kDa gamma zein promoters. Specifically, a gamma zein promoter region containing the powerful bifactorial endosperm box was inserted upstream of a globulin-1 promoter region containing the Em, Em1a, and Em1b cis-elements. This chimeric promoter was then fused to GFP for transient transcription analysis. Transient expression in immature maize endosperm and embryo tissue results in GFP accumulation in both tissues at a level not significantly different from the level produced by the native promoters. This unique combination of regulatory elements creates a seed specific transgene expression pattern that can be used to produce high levels of desirable proteins in maize grain.

P81

A PCR-based forward genetics screen to identify mutants in endosperm transfer cell development.

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The identification of new mutants impaired in the development of a specific domain within the endosperm is made difficult by the unpredictability of the phenotype in mature kernels. The seeds have to be examined during development, which limits the number of candidate lines that can be examined by techniques involving microscopy or in situ hybridization. We have developed an efficient screening procedure that allowed to monitor the expression of 7 domain-marker genes and a constitutively expressed one in 100 Mu-induced mutant lines, segregating for miniature-like phenotypes.

Our marker set included 4 transfer cell specific markers, one embryo-surrounding-region marker, one aleurone marker and one inner-endosperm marker. RNA was isolated from sibling wild type and mutant kernels extracted from the same cob, at the earliest time in which a phenotypic difference was noted. 12 lines were selected after the real-time PCR analyses of the marker genes. Northern-blot analyses confirmed that at least 6 of the lines show a molecular phenotype consisting in the absence/reduction of the expression of transfer cell specific genes.

This work was supported by the Spanish grant BIO2003-03721 and internal funds at Biogemma SAS

P82

A reverse genetic approach to find new members of the ERF family of transcription factors involved in maize inflorescence development.

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The ERF family is a large family of plant transcription regulators. They are involved in key developmental steps and in stress response. BD1, a putative ERF transcription factor, plays a fundamental role in the maize spikelet development controlling meristem identity. A reverse genetic approach was taken in order to clone new ERF factors involved in the development of the maize inflorescence architecture. A maize cDNA library from immature female inflorescence meristems was screened using the BD1 ERF DNA binding domain as a probe. The screening allowed us to isolate 15 clones coding for putative ERF transcription factors. Northern blot and RT-PCR analysis narrowed down the pool of clones to four ones that showed specific expression in ear and tassel. Interestingly, all these four genes show a high similarity among each other either at the DNA and protein level. In addition, all of them have an EAR repression domain at the C-terminus. To investigate their possible action in the BD1 pathway their expression was tested in different bd1 mutants. All of them are down-regulated in the bd1 mutant alleles. The level of expression matches the strength of the bd1 allele: almost no expression in a bd1 null allele and partial expression in a weak bd1 allele. Moreover, the promoter analysis of these four ERFs shows the presence of at least one BD1 binding site in each of them. The binding of BD1 to these promoter sequences was confirmed by a gel shift essay. A better understanding of the role of these transcription factors will come from the analysis of the Mu insertion mutant alleles that have been found for all the four genes.

P83

A search for upstream signals controlling the promoter of ZmMRP-1, an endosperm transfer cell specific transcriptional activator.

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The base of the maize endosperm differentiates into a specialized tissue, the transfer cell layer, to facilitate uptake of nutrients from the phloem terminals at the maternal side (the pedicel) and to protect the developing seed from the entrance of infectious agents. Very likely, the transfer cell layer develops in response to signals released from the vascular tissues, since there is an exact correspondence between transfer cell-specific markers expression and phloem terminals distribution. The transcriptional activator ZmMRP-1 is a myb-related gene specifically expressed in the transfer cell layer of the maize endosperm. The expression pattern of the gene is consistent with its presumable implication in the development of this specialized tissue. The gene is expressed at the base of the endosperm even before the cellularization phase takes place, and seems to control the expression of other transfer cell specific genes. In this work we have studied the signals controlling the expression of ZmMRP-1. A ZmMRP-1promoter-GUS construct was introduced in tobacco and Arabidopsis, were we found that the gene is expressed in phloem unloading zones, in addition, we have shown that the construct responds to the sink strength and that can be induced by metabolites produced from phloem components.

This work was supported by the Spanish grants BIO2000-0848 and BIO2003-03721

P84

An Investigation of the Branching Pathways of Maize Inflorescences: A Screening for Enhancers/Suppressors of the ramosa1 Mutant

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Maize inflorescences, tassel and ear, are mainly formed by the activity of lateral meristems. These meristems, called branch meristems, spikelet-pair meristems, spikelet meristems and floral meristems, are responsible for the ordered series of branching events that characterize inflorescence formation. Several mutations are known that perturb the development of these meristems, causing distinct, aberrant inflorescence phenotypes. In the ramosa1 mutant (ra1), both tassel and ear are highly branched, as branch meristems are specified in place of spikelet-pair meristems. To identify new genes affecting the branching program in both inflorescences, we initiated a search for modifiers of the ra1 mutant phenotype by mutagenesis of a weak allele of ra1 (ra1-RS) with ethylmethane-sulphonate. More than a thousand M2 families have been screened for enhancement of ear branching, and for either suppression or enhancement of tassel branching. Sixteen families segregating putative recessive mutations have been identified over a period of four years. We identified three classes of mutations: enhanced branching of the ear alone, enhanced branching of both ear and tassel, and suppressed tassel branching alone. We sequenced the ra1 gene in all mutants to identify those mutants representing new ra1 alleles. One of 16, showing an increased branching of the ear, is indeed caused by a point mutation that produces an amino acid substitution in a repressor domain of the Ra1 protein. We are focusing in particular on five mutants that differentially affect the development of both inflorescences. Their phenotypes are being carefully characterized through light and Scanning Electron Microscopy. In addition, crosses are being made to test for complementation among these putative new mutants and for allelism with known inflorescence mutants. Segregating populations are being generated for mapping the mutations by mean of SSR markers. This study should provide new insights into the developmental pathway regulating inflorescence branching in maize.

P85

Analysis of the Dominant Maize Mutant Corngrass1 (Cg1)

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Corngrass1 (Cg1) is a dominant mutation that affects juvenile to adult phase transition. Cg1 mutants mature and flower later than their wild type siblings, and retain juvenile phase characteristics such as leaf waxes, tillering, and prop root production well into the adult phase. SEMs of Cg1 inflorescences show that lateral meristem initiation and phyllotaxy is also affected. Although the mutant phenotype is variable, we hypothesize that the gene plays a role in activating adult phase identity.

Previous researchers have genetically mapped the Cg1 gene to the short arm of chromosome 3. We are using a combination of SSR and RFLP mapping to positionally clone the Cg1 gene. From a population of 427 plants, we have identified a single probe that shows zero recombination with the Cg1 locus. Recently, we have obtained the complete sequence for the BAC that contains this probe. We will continue our mapping on larger populations to better define the Cg1 genomic interval. Our goal is to clone the Cg1 gene and determine its molecular function in phase change.

P86

Axis formation in the maize leaf - inside, out, upside, down.

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The Wavy auricle in blade1 (Wab1) locus is defined by three fully penetrant, dominant alleles that disrupt the proximal-distal pattern of maize leaves. Both narrow sheath1 and Wab1 mutations cause reduced leaf width and map to the same bin on chromosome 2. Our results, however, indicate that they are different genes. We are following a map-based approach to clone Wab1. Using available molecular markers, we have located the gene into a 1.8-cM interval. One contig of the most recent physical map encompasses several of these markers, allowing orientation of the clones relative to our genetic map. The use of additional probes taken from the physical map and from the sequencing of selected BACs will allow us to further restrict the position of the gene.
The milkweed pod (mwp) phenotype was originally described by Oliver Nelson and has been maintained by the Maize Genetics Stock Center. It is inherited as a monogenic recessive trait affecting the abaxial-adaxial polarity of leaves. Originally described as specific to husk leaves, we have been able to detect signs of adaxialization in the sheath of every leaf after introgression into the appropriate inbred backgrounds. Sectors of adaxialized sheath extend down from the sheath-auricle boundary in mwp plants. An ectopic, abaxial ligule develops when these sectors meet the auricle. The sectors have supernumerary minor veins that lack the abaxial hypodermal schlerenchyma and that are not attached to the abaxial epidermis, when compared to the wild type. Our progress in the genetic characterization of mwp function will be presented.

P87

Basal Endosperm Development In Maize Depends On Maternal Baseless1 Activity

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Plants have two phases to their life cycle: the diploid sporophyte and the haploid gametophyte separated by meiosis at one end and fertilization at the other. Two cells of the female gametophyte, the egg and central cell, are fertilized by two sperm cells of the pollen grain to produce the embryo and endosperm, respectively, of the seed. Proper development of the seed depends not only on the action of genes in the resulting embryo and endosperm but also on maternal gene expression. Among other functions the embryo sac (the female gametophyte) also lays down patterning information for subsequent endosperm development. Mutant baseless1 embryo sacs produce defective seeds with abnormal endosperm and embryo development. The phenotype of baseless1 mutants suggests that basal fate determinants need to be properly localized within the central cell of the embryo sac for subsequent basal endosperm cell development and that basal fate localization requires a normal 2 maternal : 1 paternal genome ratio for its maintenance in developing endosperms.

P88

Characterization of members of a zinc finger 'ID domain' protein family in Rice (Oryza sativa L).

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In a rice genome, around 15 genes are present that have a highly conserved zinc finger domain called "ID domain". OsPER1 is a member of the plant-specific zinc finger protein family. A mutant, named Oryza sativa Perennial1 (OsPER1), was identified via Ac/Ds transposable element mediated mutagenesis system in rice. Phenotypically, the mutant showed severely reduced gravitropism in stem pulvinus and nodes, and increased the number of tillers during plant reproductive stage. GUS-stains revealed the expression of OsPER1 at vascular tissues near shoot meristems and young immature leaves. Also, the expression was detected in the tips and vascular tissues of roots. Further study revealed that the expression of OsPER1 responded to gravitropism. Upon gravistimulated, rice plants changed the GUS pattern of OsPER1. Gravi-stimulated plants not only expressed OsPER1 in the bending region of leaf sheaths but also showed asymmetric expression of OsPER1 in roots. Moreover, exogenous treatment of developing root with an auxin transport inhibitor (NPA) eliminated asymmetric GUS expression pattern. Phenotypes such as lateral shoot branching (tillering) and graviresponse, and expression pattern suggested that OsPER1 could be involved in developmental processes determined by auxin. Besides, other homologous mutants (OsPER6 and OsPER9) were suspected to show auxin related phenotypes. Our current working model is that at least one of functional actions evolutionally conserved among the ID domain-zinc finger protein family, would be active participation in auxin-related developmental processes.

P89

Characterization of the bif1 mutant in maize

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The “barren inflorescence” (bif) class of mutants in maize are generally characterized by reduced branching in the male inflorescence, reduced production of spikelets and production of single spikelets, as well as floral defects. These mutations are thought to be due to defects in auxin signaling that cause the failure of axillary meristem maintenance and initiation. We have characterized one of the bif mutants, bif1, discovering additional defects and phenotypes unique to this mutant and producing evidence of a role for bif1 in axillary meristem development.
The original bif1-ref allele is semi-dominant. We have characterized vegetative tassel (vt)1, vt2 and 19746, three recessive alleles that have a more severe phenotype. The morphology of mature bif1 plants was studied, with significant phenotypes discovered in both vegetative and inflorescence structures. bif1 has a reduced stature compared to normal plants, and also displays leaf defects. In the male inflorescence, some dichotomous branching of the tassel is observed, as well as the appearance of kernels and leaves in the tassel. In some cases the tassel is completely barren, and takes on a twisted, zigzag morphology, with unidentified wispy bract-like structures growing from the tassel.
Scanning electron microscopy, histology and in situ RNA in situ hybridization using the meristem marker knotted1, showed that branch meristem initiation was deficient in bif1, leading to the production of fewer branches. Spikelet and floral meristem production was also deficient, resulting in barren patches on the inflorescence and single spikelets and deformed flowers. Cloning of the Bif1 gene is currently in progress and this work will also be presented.

P90

Chromosome walking to the tasselseed4 locus of maize

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The tasselseed4 (ts4) mutant of maize affects inflorescence branching as well as sex identity. Early ts4 spikelet pair primordia appear to be ideterminate and branch-like, while the male florets display a lack of pistil abortion. Interestingly, the basal portion of the inflorescence does not seem to be affected in any of the four alleles of ts4 we have isolated, indicating that the gene may be active only in the upper part of inflorescence.

Since numerous tagging crosses have failed to yield tagged ts4 alleles, we are attempting to clone the gene by chromosome walking. From an F2 backcross population of 1800 plants, we have defined the ts4 gene to an interval contained on three BACs. All three BACs have been sequenced, and candidate genes are being amplified and sequenced from each ts4 allele to identify mutations. In rice, these three BACs cover a 100kb region and show a high degree of synteny with maize.

P91

Cloning and tissue-expression studies of a KANADI4-like gene from Zea mays

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As part of a collaborative effort to identify genes involved in meristem function and early aspects of leaf development in maize, we are cloning maize homologs of genes required for these processes in Arabidopsis. In Arabidopsis, the four KANADI gene family members are GARP domain-containing putative transcription factors. They are expressed in meristematic tissues of the plant, including vegetative and floral meristems. KANADI1-4 perform redundant functions and are involved in pattern formation in primordial leaves, specifically by designating the abaxial (lower) side of the leaf. Using the Arabidopsis KANADI protein sequences as queries of various maize sequence databases we have identified an EST which is likely to encode a maize KANADI4-like protein. Using primers designed from this EST, RT-PCR was performed on RNA isolated from shoot apical meristem-enriched tissue to demonstrate that this sequence is expressed in this tissue in maize. RT-PCR experiments on a variety of tissues demonstrates that the maize Kanadi4-like gene is expressed in shoot and root apical meristem-enriched tissues, 7-day-germinated seedlings and unfertilized developing ears. This data is consistent with expression of Aradidopsis KANADI4.

P92

Cloning of narrow sheath/Pressed Flower homologue in tomato and its evolutionary implication in lateral organ development

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During the development of simple leaves in maize and Arabidopsis, the WUSCHEL-like homeodomain protein NS/PRS recruits meristematic cells to become founder cells in a specific lateral domain of the SAM. Founder cells recruited by NS/PRS function form the lateral domain of the lower leaf. Disparate ns/prs mutant phenotypes in maize and Arabidopsis provide new insight into the evolution of leaf morphology, and support a model whereby monocot and eudicot leaf primordia undergo differential elaboration of upper versus lower leaf zones. Compound leaved species form a single primordium as well, however organogenesis continues with the formation of leaflets from primordial margins. Current models suggest that compound leaf primordia have retained meristematic potential, which is supported from the expression patterns of indeterminacy-promoting °Æmeristem genes°Ø in leaf primordia of tomato and other compound leaved species (Kessler & Sinha 2004). We are interested in determining the contribution of NS/PRS in the development of compound leaf morphology of tomato. Based on sequence homology, we have cloned a tomato candidate designated LeNS that is homologous to NS/PRS; DNA gel blot analysis indicates that this clone is from a single copy gene in tomato. Analysis of the expression pattern and mutant phenotype of LeNS in compound leaves of tomato will reveal whether LeNS function is conserved within the SAM as in simple leaves, expanded into the leaf primordia in keeping with a current model for compound leaves, or has evolved novel functions. Genetic mapping of the clone is under way to help identify any candidate tomato leaf mutants that map in its vicinity. Recessive leaf mutations such as solanifolia (sf), potato leaf(c) and trifolia2 (tf2) mutants show reduced margin complexity, whereas wiry displays reduced laminar expansion and entire generates nearly simple leaves (Kessler et al., 2001). These mutant phenotypes may constitute the best-predicted loss of function mutations in the LeNS gene. These comparative studies will expand our understanding of the role of NS/PRS during the evolution of diverse angiosperm leaf morphology.

P93

DNA sequence diversity at an ANGUSTIFOLIA-like gene among inbred lines and open-pollinated landraces of maize

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The Arabidopsis ANGUSTIFOLIA (AN) gene, a homolog of the human C-terminal binding protein, encodes a protein that regulates the arrangement of cortical microtubules in epidermal and mesophyll cells, thereby influencing leaf morphology. AN also regulates MERI5, a gene involved in cell-wall formation in plants. Overlapping maize ESTs were identified that account for all but the first twenty amino acids in the Arabidopsis AN protein. Oligonucleotide primers were designed which during the polymerase chain reaction amplify a ~700bp genomic sequence that contained both intronic and exonic sequence. RT-PCR was performed on RNA isolated from 1 week-old seedling leaf tissue in order to demonstrate that this sequence is expressed in maize; sequencing this amplification product confirmed the intron/exon boundaries which had been predicted by sequence database analysis. The genomic sequence was cloned and sequenced from six North American inbred lines, five open-pollinated Mexican land races, and four open-pollinated land races from Native American Pueblos in New Mexico. The calculated values for silent-pi indicate that the Mexican land races show the most diversity at this locus. Interestingly, the open-pollinated New Mexican Pueblo lines exhibited slightly less sequence diversity than the North American inbred lines.

P94

Defining the modules of epidermal phase transition and identifying their genetic regulators

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The transition from juvenile to adult leaf identity in maize is qualitatively evident for several epidermal traits, including transitions from dull blue alcohol-rich to glossy green alkane-rich waxes, from totally glabrous to pubescent with three types of epidermal hairs, from unexpanded to expanded bulliform cells, from brick-like to crenulated (puzzle piece-like) pavement cells, and from pink staining to blue staining with toluidine blue-O, reflecting a difference in cell wall biochemistry. We recently demonstrated that the balance of microRNA172 and glossy15 (gl15) acts pleiotropically to affect all epidermal aspects of vegetative phase transition. For this study, these traits were examined in a population segregating for glossy15 and dwarf1 (d1), whose plants have transition boundaries that are precociously adult and incessantly juvenile based on the respective effects of these mutations. We have observed that the traitsí transitions can be grouped into either three or four co-regulated modules, with the wax and macrohair traits each comprising their own modules. We also show that microRNA172 is downregulated in d1 mutants relative to WT siblings, explaining the uniform pleiotropic effects on these transitions. Our recent QTL studies of these traits using the IBMRILs have demonstrated that Wax modulator1 (Wam1) regulates the timing of the wax transition without affecting the other epidermal aspects of vegetative phase change. Here we report on the development and utility of a set of 170 RILs derived from inbred parents NC61 and W23, which represent late and early extremes of vegetative phase change, respectively. This population will be used to identify additional module-specific regulatory loci. Finally, we introduce a recessive mutant recovered from an EMS screen that delays transition of the cell wall biochemistry trait without impacting macrohairs or wax. Further characterization of this mutant will better define the remaining modules and offers an opportunity to clone another regulatory gene.

P95

Diversification of SEPALLATA genes in grasses

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Members of the SEPALLATA (SEP) family of MIKC-type MADS-box genes exhibit distinct patterns of expression in rice and maize and have been linked to both the origin and diversification of the grass spikelet. To better understand the evolution of SEP genes in grasses we have isolated orthologues of the five rice SEP genes LEAFY HULL STERILE1 (LHS1), Oryza sativa MADS5 (OsMADS5), OsMADS7, OsMADS8 and OsMADS34 from 12 phylogenetically disparate grass species. Phylogenetic analysis of 120 SEP genes from diverse flowering plants indicates that only OsMADS7 and OsMADS8 are demonstrably orthologous to an Arabidopsis SEP gene; the remaining three grass SEP genes are restricted to monocots. Using a combination of RT-PCR and in situ hybridization, we document diverse patterns of gene expression. All grass SEP genes are detected in inflorescence tissue, but at least one SEP gene is also detected in root, culm and leaf tissue in each of the sampled species. The identity of the widely expressed SEP gene differs from species to species. Using in situ hybridization, we show that LHS1 is expressed in several of the upper florets of Eleusine indica (yard grass) spikelets, but only in the upper floret of Panicum maximum (guinea grass) spikelets. These data confirm our earlier observation that LHS1 may act as a “selector gene” (specifying the terminal floret of the spikelet) only in species with basipetal maturation of the florets. OsMADS5 is expressed in the palea and lemma of several florets of the spikelet in oats, barley, Chasmanthium (sea oats) and sorghum, but is expressed only in the stamens and pistil in rice. These heterogeneous patterns of expression support the hypothesis that SEP genes have diversified extensively in grasses and may have different roles in different species.

P96

Double Mutants at the thick tassel dwarf1 and fasciated ear2 loci reveal complex interactions affecting vegetative and inflorescence meristems

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In Arabidopsis, the CLAVATA pathway acts to maintain the stem cell population of the SAM, IM and FM. Currently, it is hypothesized that clv3 is an extracellular ligand that binds to the clv1/clv2 receptor protein kinase complex leading to transcriptional repression of the homeodomain transcription factor, wuschel. To investigate a possible genetic interaction between the maize clv1 orthologue, thick tassel dwarf1, and the maize clv2 orthologue, fasciated ear2, segregating double mutant families were created and analyzed. Leaf number and the number of spikelets at the widest point of the ear were counted to explore interactions in the vegetative and inflorescence meristems, respectively. Double mutants had an increase in spikelet number and a decrease in leaf number relative to plants homozygous for either single mutation. Therefore, these proteins do not act in an exclusive co-receptor complex and they probably interact with other LRR proteins to control both vegetative and inflorescence meristem development and organ initiation. Double mutants also showed aberrant phyllotaxy and irregular internode lengths when grown in the greenhouse, suggesting that environmental conditions affect the severity of the defects. To more fully investigate the vegetative defects of td1 single mutants, we compared the phenotypes of the td1-glf mutants in the B73 genetic background to that of td1-R mutants in the W22 background. By t-test, td1-glf (B73) mutants were significantly shorter and made significantly fewer leaves. In contrast, td1-R (W22) mutants had significantly fewer leaves but did not differ in height from their normal siblings. Thus, expressivity of the td1 phenotype is background and or allele-dependent. No notable phyllotaxy defects were observed in td1 single mutants. The respective roles of td1 in maize development, and of clv1 in Arabidopsis development, are discussed.

P97

Fate and Consequence of the Zag1/Zmm2 Gene Duplication Across Grasses

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Gene duplications are prevalent feature of eukaryotic genomes. Understanding the evolution of gene duplications is important to our understanding of genetic redundancy and the evolution of new gene function. We present data on two developmentally important duplicate genes, Zag1 and Zmm2, that appear to be key determinants of stamens and carpel specification in grasses. Zag1/Zmm2 are maize orthologues of C-function organ identity genes as defined in the ABC model of floral development. In maize, Zag1/Zmm2 have partitioned C-function activity through the evolution of sub-functionalized 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 grass lineages, 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. Post-duplication 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 initial work on screens of coding region subfunctionalization using Arabidopsis as a heterologous system. We are continuing to examine the evolution of Zag1/Zmm2 across grasses and are also beginning to examine a more ancient duplication event in the D-Class genes Zmm25/Zmm1.

P98

Functional analyses of genes involved in meristem organization and leaf initiation

(submitted by Kazuhiro Ohtsu <kazohtsu@iastate.edu>)

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All above ground organs of higher plants are ultimately derived from specialized organogenic structures called shoot apical meristems (SAMs). The SAM exhibits distinctive structural organization, marked by tissue zonation and cell layering. The structures of plant SAMs are correlated with their functions, such that new leaves are initiated from the peripheral zone of the SAM and the central zone replenishes new meristematic cells that are lost during organogenesis. The goal of our project is to identify genes required for meristem function and early stages of leaf development in maize. Laser micro-dissection is a powerful technique that permits the isolation of RNA from specific cell types within fixed plant tissues. RNA collected from 1,000-10,000 cells is sufficient for use in microarray analyses of gene expression, which permit the simultaneous examination of expression profiles of thousands of genes. Intact SAMs were fixed, embedded in paraffin and sectioned. SAM cells were isolated from these sections via laser micro-dissection. RNA was isolated from these cells and whole seedlings, amplified and hybridized to microarrays spotted with ~20,000 maize cDNA clones. This experiment identified many genes that are preferentially expressed in the SAM. A summary of our current experiments will be shown and the functions of the genes that are preferentially expressed in maize SAMs discussed.

P99

Fungal Induced Sex Change in Male Buffalograss Mimics Tasselseed-2 Mutant

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The feminizing effect of Tilletia buchloeana Kell. & Swing. infection on the sex expression of dioecious buffalograss (Bouteloua dactyloides (Englm.) Columbus; syn. Buchloì dactyloides (Nutt.) Englm.) is similar to mutants of maize Tasselseed-2 (Ts-2) gene. Ts-2 induces programmed cell death in sub-epidermal gynoecium cells of florets destined to be male. However, upon infection, the fungus suppresses expression of Ts-2 homologue in buffalograss, thereby, inducing the development of pistils in an otherwise staminate floret. This observed phenotype is analogous to maize ts-2 mutant. Detailed morphological analysis supplemented with SEM revealed other secondary effects of fungal infection, ex. induction of an additional pistillate floret per spikelet in each sex form. Ts-2 homologue in buffalograss was cloned and sequenced showing approximately 89% homology with maize. Real time RT-PCR analysis performed over different stages of inflorescence development quantitatively estimated 5 to 8 fold reduction of Ts-2 expression in infected vs. non-infected male buffalograss. Study of this unique host-pathogen interaction, which leads to induced hermaphrodism, will potentially enable us to elucidate the developmental pathway involved in the separation of sexes in dioecious buffalograss and to produce seeds in male florets thus facilitating seed germination and mechanical seed harvest.

P100

Gene expression profile analysis of maize (Zea mays L.) pericycle cells

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The pericycle is a single layer of cells that represents the outermost cell file of the central cylinder in the primary roots of maize. Primordia of lateral roots, which constitute a major part of the maize branch root system, are initiated in the pericycle. Our understanding of how lateral roots are initiated from pericycle cells is limited due to the difficulty of analyzing gene expression in specific internal cell types. To explore the global gene expression profiles of pericycle cells and to thereby identify genes involved in lateral root initiation, Laser Capture Microdissection (LCM) was used to separately collect pericycle cells and the remaining non-pericycle cells of maize primary roots 3 days after germination. RNA from both cell-types was isolated and amplified prior to microarray experiments on ISUís 12k maize cDNA microarrays. Genes that are preferentially expressed in pericycle cells were identified.

P101

Invertase-Mediated Sucrose Utilization during Early Reproductive Development in Maize and Arabidopsis

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The high C-cost of reproductive development necessitates early adjustment of total sink load through optimal integration of diverse signals. These include environmental stimuli, hormonal cues, and global sugar status. Invertases catalyze the irreversible cleavage of sucrose into glucose and fructose and have been implicated in sink tissue establishment and expansion. As pivotal generators of hexoses for turgor adjustment, respiration, and signaling, invertase gene family members are carefully regulated. We have investigated the quantitative expression and localization of four invertase gene family members (two vacuolar and two cell wall forms) in developing Arabidopsis and maize flowers. Temporal and spatial transcript accumulation of AtBfruct3, a soluble invertase of Arabidopsis, suggests an analogous relationship with the maize Ivr2A gene for vacuolar invertase. Both are expressed at key points during the early phases of reproductive development. In addition, previous work showed that changes in invertase expression under drought stress contribute to adjustment of reproductive load in maize. Therefore, in the present work we also tested whether invertase expression during the pollination process is influenced through disruption of the ABA-sensing system by using a vp1 maize mutant (germinates precociously due to the lack of a functional activator/repressor of ABA-dependent gene expression). Developmental comparisons between wild-type and vp1 mutant ovaries, silks, and surrounding floral structures were shown using a sensitive Q-PCR technique. Mutants tended to show a greater amplitude of developmental change for vacuolar Ivr2A in the ovaries as well as altered localization patterns (ie. decreased Ivr2A in floral structures). These data suggest a possible role for VP1 in maternal tissues where it may, directly or indirectly, mediate invertase gene expression through endogenous ABA perception.

P102

Isolation and expression studies on a Fasciata1-like gene in maize

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Several Arabidopsis genes that are known to be involved in meristem maintenance and development have not yet been isolated and characterized in maize. The Arabidopsis FASCIATA1 protein is a subunit of a chromatin assembly factor that is known to be involved in both shoot and root apical meristem function. Degenerate PCR primers were designed from the Arabidopsis and rice FAS1 proteins and were used in RT-PCR to amplify a Fas1-like mRNA sequence from shoot apical meristem-enriched tissue. This sequence was cloned, sequenced and used to design a primer pair that was used in subsequent expression studies. RT-PCR using a variety of tissues demonstrates that the maize Fas1-like gene is expressed in shoot and root apical meristem-enriched tissues, as well as other tissues that are mitotically active. We have used 3íRACE to amplify, clone and sequence the 3í end of this Fas1-like mRNA. In addition, a genomic sequence is present in the maize database which accounts for most of the N-terminal coding region of the Fas1-like gene which is not present in our cDNA clones.

P103

Isolation and mapping of defective endosperm mutants by using AFLP markers

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A collection was made of viable defective endosperm (de) mutants developed by selfing plants of open pollinated maize varieties or from mutagenized and random tagging materials. Genetic characterization was begun by transferring all mutants to the B37 and A69Y inbred backgrounds, through 5-6 backcrosses. Allelism tests were carried out. The vast majority of the mutants were not linked, and in most cases not additive, indicating that the phenotype can be caused by mutations in many unrelated genes. We are currently starting to identify AFLP markers linked to individual de alleles with the aim of mapping the mutants. For this purpose, it was necessary to obtain F3 families in which the mutation segregated in crosses with the mapping parents; to place a sufficient number of AFLP markers on a map constructed from the cross between the mutant and the mapping parents; to integrated the new mutant linked AFLP markers into the intermated B73/Mo17 genetic map. The maize map will be enriched of AFLP markers and mutant loci for which developmental mutants are described.

P104

Maize C-class homeotic genes control floral meristem and reproductive organ identity

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In the model species Arabidopsis and in other core eudicots, floral organs are patterned by the A, B, and C classes of MADS-box genes. C-class gene activity is necessary to specify stamen and carpel identity, in addition to a floral meristem determinacy in the center of the flower. Consequently loss of function for the sole C-class gene in Arabidopsis AGAMOUS (AG) results in a mutant flower where stamens replace petals, and a new flower arises in the place of the carpel reiterating the sequence of sepal, petal, petalÖ. Little is known about C-class gene function in plants outside the core eudicots, and thus it is not clear that C-class genes are playing a conserved role across the angiosperms. Since maize is phylogenetically distant from Arabidopsis and has highly derived flowers, it makes a good model to test the conservation of C-class function. Unlike Arabidopsis, three maize C-class orthologs exist: Zag1, Zmm2, and Zmm23. zag1 mutant flowers show a partial loss of determinacy, but have little or no effect on organ identity. We have recently identified mutant alleles for both Zmm2 and Zmm23. Alone or in a zmm2 zmm23 double mutant these mutations do not result in a striking phenotype. However, a zag1 zmm2 zmm23 triple mutant has a significant effect on both stamen and carpel identity. Furthermore, floral meristem identity is lost in the triple mutant indicating that, unlike Arabidopsis, the maize C-class activity is necessary to specify floral meristem identity.

P105

Map based cloning of RTCS, a gene controlling crown and seminal root formation in maize

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Our research focus is to elucidate the genetic basis of root architecture in maize. Our goal is to understand genetic variability and to improve root structures for increased yield. The genetic analysis of root development and function is still at its early stages in maize, partly because of the difficulty in phenotyping underground organs, and because of the plasticity of root development, influenced by the environment. During the course of plant growth, maize plants develop various types of roots, including crown roots derived from above-ground nodes. Recent genetic studies indicate that there are distinct pathways for the development of each root type, having embryonic as well as post-embryonic origins. In order to shed light on the pathways, we are taking a forward genetic approach: map-based cloning of mutant genes significantly altering root development and function. Here we describe our first successful cloning and molecular characterization of the RTCS gene, which is essential for the development of crown and seminal lateral roots in maize.

P106

Microarray analysis of vegetative phase change in maize

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Young plants cannot flower, even in the presence of flower-inducing environmental cues, whereas once they reach a certain developmental stage they can flower. This gain in competence to flower or "phase change" is often associated with a suite of morphological changes. In maize, a number of characters specific to the nonflowering juvenile or the flowering adult phase have been recognized. Experimentally, phase can be "reset" to the juvenile phase by culture of isolated shoot apices. Mutations that hasten or delay the transition to the adult phase have identified genes involved in phase change; however, the signals that drive this process and the genes that respond by bestowing phase-specific patterns of differentiation are unknown. The nature of phase change, in which a large number of morphological traits are affected simultaneously, renders this developmental process particularly amendable to a genome-scale gene expression experiment. We used RNA isolated from a juvenile leaf (leaf 4), an adult leaf (leaf 9), and culture-derived plantlet leaves 3-4 to explore the magnitude of differences in gene expression between phases. RNA samples were analyzed using spotted cDNA microarrays. Three types of comparisons were made: adult vs. juvenile, juvenile vs. culture-rejuvenated and adult vs. culture-rejuvenated. Analyses of these experiments continue; however, we have found that thousands of genes show statistically significant ($p < 0.001$) differences in expression among the three sample types. Among all test groups, 770 genes show a greater than twofold difference in expression. One hundred twenty of these genes show similar expression profiles when comparing between adult vs. juvenile and adult vs. culture-rejuvenated test groups. These represent bona fide juvenile-phase-expressed genes. Similarly, of the 770 genes, 74 genes shared between juvenile vs. culture-rejuvenated and adult vs. culture-rejuvenated test groups show similar expression profiles. These likely represent differences resulting from growth from a seed vs. from meristem culture.

P107

Millets, maize, and the control of morphological diversity

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Maize is the pre-eminent model system in grasses for the dissection of morphological development. Genes found to affect development in maize may be candidates for similar developmental changes in other grasses. However, there is not necessarily a one-to-one genetic correspondence between mutant phenotypes in maize and similar phenotypes in other grasses. One approach to discovering which maize genes are responsible for evolutionary changes in related grasses is to combine quantitative genetics and detailed comparative developmental morphological analysis within a phylogenetic context. We have done this in the millet grasses using QTL analyses of foxtail millet, followed by comparative mapping and genomic analysis between maize, rice and foxtail millet. This approach identifies candidate genes from maize that are most likely to be involved in vegetative and reproductive morphological change in the millets, including barren stalk1 (ba1), zea floricaula leafy1 (zfl1) and tasselseed4 (ts4). Interestingly, we find that not all genes that affect development in maize are found in millets, and that there are QTL in millets that are not accounted for by known developmental genes in maize. This approach is one way of leveraging genetic information from maize to other grass species.

P108

Molecular analysis of the ubiquitin system in the maize egg cell

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Post-translational modification of proteins by small polypeptides, such as ubiquitin (Ub), has emerged as a common and important mechanism for regulating protein function in plants, fungi and animals. We have analyzed 988 ESTs from a maize egg cell-cDNA libary for the existence of transcripts encoding Ub and Ub-like proteins. Three novel genes (ZmUbl15, ZmUbl26a/b) encoding Ub-like proteins have been identified and compared with Ub-like protein sequences from other organisms. We have classified Ub-like proteins into four different groups including the SMT3 (Similar to Ubiquitin3)/SUMO (Small Ubiquitin Modifier), RUB (Related to Ubiquitin), and two novel groups encoding hypothetical proteins of rice and a class of Ub-like proteins, which might encode an egg cell-specific group. Based on the phylogenetic tree, ZmUbl26a/b are closely related to the SMT3/SUMO subgroup, while ZmUbl15 forms an own group together with an egg cell-specific Ub-like protein from wheat and a predicted hypothetical protein of rice. Other plants Ub-like proteins group into the SMT3/SUMO and RUB group. RT-PCR was applied to study the expression of the three novel maize Ub-like genes. While ZmUbl26b is ubiquitously expressed and ZmUbl26a was detectable in most of the tissues, ZmUbl15 is exclusively expressed in the egg cell. This specific expression of ZmUbl15 suggests that it might play an important role in egg function where it might target proteins for degradation. RNAi has been applied to down-regulate ZmUbl15 gene activity. Phenotype(s) of the first transgenic lines will be shown. Future work aims to localize ZmUbl15 in vivo using reporter gene constructs and to identify target protein (s).

P110

Pollen beta-Expansins in Maize

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Group-1 grass pollen allergens from maize make up a subfamily of beta-expansins, which are cell wall proteins that loosen plant cell walls in characteristic ways. Group-1 pollen allergens are specifically expressed in grass pollen and are proposed to loosen the cell walls of the silks to aid in the penetration and growth of the pollen tube through the silk. There are two divergent classes of the pollen beta-expansins (A and B), each containing multiple genes.

Two approaches are being used to assess the function of the pollen beta-expansins. We have utilized a maize line, obtained from Pioneer Hi-Bred, with mu transposon insertion in one class B gene, EXPB1. Several lines show an unexpected phenotype (delay in pollen penetration into silk, continued silk growth post-pollination) that appears to be associated with the disruption of EXPB1. Pollen competition studies indicate transmission of mu is significantly decreased when heterozygous pollen is placed in excess on wild type silks. Further studies revealed that silk collapse was delayed by 9-12 hours when the silks were pollinated with expb1 pollen in comparison with EXPB1 pollen. Genotype of the female was not relevant.

Also, we transformed maize plants with an RNAi construct, driven by a pollen specific promoter, to silence the class B genes. Many lines in the first generation were infertile. Those that survived have the following unusual traits: low pollen production, shriveled anthers, continued silk growth post pollination and low seed set. These phenotypes are similar but more extensive than those found previously for the maize line carrying a mu insertion. These results indicate an additional role of pollen beta-expansins in pollen development. Segregation analysis from crossing transgenic plants to wild type plants suggests problems in the transmission of the transgene through the pollen and ovule. This may indicate additional roles of the pollen beta-expansins in the female gametophyte and/or embryo.

P111

Positional Cloning of the Maize Sex Determination Genes tasselseed1 and silkless1

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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. The focus of our research is to study the role of the tasselseed1 (ts1)gene in pistil cell death and the function of the silkless1 (sk1)gene in protecting pistil cells from tasselseed-mediated cell death. Currently, we are working on the molecular identification of ts1 and sk1 using a positional cloning strategy. This takes advantage of the multiple resources available for the maize community, as the sequence databases, the genetic and physical maps and the annotation of the rice genome which emphasizes the extensive synteny between the genomes of maize and rice. The physical region containing ts1 has been delimited by two flanking markers spanning about 500 kb. Further characterization of this physical interval will refine the location of ts1. This should lead to the identification of ts1 candidate genes, which will be used to perform sequence analysis in the multiple ts1 mutantl alleles currently available in our lab. As to sk1, a closely linked (1.5 cM) proximal marker has been identified. We are working in the detection of a distal marker since the only one found so far is about 9.5 cM away from sk1.

P112

RNAi-mediated transcriptional silencing of anther-expressed genes results in male sterile maize

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In maize, a large number of genes have been identified as tassel-preferred in their expression pattern both by traditional methods and by recent high-throughput expression platforms. RNA suppression approaches may provide a rapid alternative means to identify genes that are directly related to pollen development in maize. The maize male fertility gene Ms45 and several anther-preferred genes of unknown function were used to evaluate the efficacy of generating male sterile plants by transcriptional gene silencing (TGS) approaches. Inverted repeats of promoter sequences expressed by the maize Ubiquitin promoter and targeting Ms45 conferred a high-frequency of male sterile plants that lacked Ms45 mRNA due to transcriptional inactivity of the target promoter. Moreover, fertility could be restored to these promoter IR-containing plants by expressing the MS45 coding region from non-target promoters. Transcriptional silencing of other anther-expressed genes also resulted in altered fertility phenotypes and methylation of target promoter DNA sequences. These studies provide evidence that gene activity can be disrupted in monocots by RNAi constructs directed against either native or transformed promoter regions. This approach not only enables the correlation of monocot anther-expressed genes with functions important to maize reproduction, but also has broad applications for studying gene function.

P113

Specific features of the RB/E2F pathway in cereals: RBR1 regulates RBR3 and controls endoreduplication during maize endosperm development

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Although cereal grains are the most important source of food calories worldwide, little is known about the molecular mechanism(s) controlling the development of the cereal seed and its storage tissue - the endosperm. In maize, the endosperm undergoes dramatic growth and differentiation in mid-development, coincident with a transition from a mitotic to an endoreduplication cell cycle. The retinoblastoma (RB)/E2F pathway plays a major role in cell cycle regulation in higher eukaryotes, including plants. We are investigating how the RB/E2F pathway controls cell cycle progression during endosperm development, and how it influences the overall growth of this tissue. To relieve the G1-specific, RB-mediated block on the E2F pathway, we down-regulated retinoblastoma-related 1 (RBR1) in the endosperm by RNAi. In one RNAi line, RBR1 RNA was decreased by 50% and the encoded protein virtually undetectable. A general up-regulation of E2F target genes was observed, which was associated with a 30% increase in mean ploidy. Experiments are in progress to determine the physiological consequences of this stimulation on endosperm development and the endoreduplication cell cycle.
The Arabidopsis genome has a single RBR gene, as is currently believed for many other plants. However, cereals like rice, maize and wheat possess at least two RBR genes suggesting specific regulatory mechanisms. We have isolated a novel member of the maize RB family, RBR3, which shows distinct features and regulation. RBR3 expression, in contrast to that of RBR1, declines during endosperm development raising the possibility that RBR1 and RBR3 play distinct roles in cell cycle regulation and development. Indeed, in vitro and in vivo experiments in transgenic calli and endosperms indicate that RBR3, far from being a redundant RB-related gene, is regulated by RBR1 through the activity of E2F. The results suggest that the RB/E2F pathway in cereals involves additional regulatory mechanisms from those identified in Arabidopsis.

P114

Striga KNO1 (KNOTTED1-like homeobox) RNAi as a Resistance Mechanism in Maize

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RNAi (RNA interference) is the process by which production of double stranded RNA leads to the targeted degradation of mRNA with sequence similarity and the 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 and proper leaf patterning of organs will be targets for RNAi. A dsRNA inverted repeat construct was made by cloning 1900bps of the PCR pasted coding sequence of STM1 and KNAT1 orthologs from Striga asiatica in both sense and antisensence direction and separated by 1100bps of the rice WAXY intron under the control of 35S promoter in pMCG161. The resulting construct, pMCG161-SAKNOXi will be transformed into maize via Agrobacterium tumefaciens. Transcription of the inserted construct will produce a hairpin structure then a dsRNA necessary to mediate silencing of targeted STM and KNAT1 genes. It is now known that a specific mobile silencing signal exists that can travel between cells via plasmodesmata and long distances via the phloem. Striga infects maize by sending an infectious peg (haustorium) that connects into the hosts vascular system. This physical contact between host and parasite provides a conduit for transfer of macromolecules in both directions. Evidence that gene transfer between host and parasite occurs as a result of direct physical contact between the two is provided by phylogenetic data in which plants that penetrate host plants intracellularly have been shown to pass their genes to their hosts.

P115

Sucrose affects ID1 protein expression in developing maize leaves

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The indeterminate1 gene (id1) controls the transition to reproductive growth in maize. Mutant id1 plants remain in a prolonged vegetative state and are unable to undergo a normal transition to flowering. Both id1 mRNA and ID1 protein are detected only in developing leaves, suggesting that id1 may control the production of a leaf-derived floral inductive signal that is transmitted to the shoot apex. Levels of ID1 in developing immature leaves decrease and become undetectable as leaves emerge from the whorl and become green. We used lemon white 1 (lw1) albino mutant seedlings to determine if ID1 expression follows a developmental pattern or if it is directly correlated with the sink to source transition. Albino lw1 seedlings form 3 to 4 leaves before dying and all tissues remain as carbon sinks. Western analysis with anti-ID1 specific antibody detected ID1 protein only in immature leaves but not in leaf blades of albino or green plants. This suggests that ID1 expression is controlled developmentally and is not correlated with the sink to source transition in maize leaves. However, we also found that immature leaves of albino plants grown in the absence of added sucrose had very low or undetectable levels of ID1 protein, whereas albino plants grown in the presence of sucrose had similar amounts of ID1 protein as green siblings. No difference in ID1 levels were observed in green plants grown with or without sucrose, suggesting that endogenous sucrose produced by photosynthesis in green plants is sufficient to induce ID1 expression to its maximum level. A possible connection between carbon partitioning in maize and the transition to flowering will be discussed.

P116

Sugar and Starch Accumulation Patterns Are Altered in tie-dyed1 Leaves

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Our lab focuses characterizing genes responsible for coordinating leaf development. We used a genetic approach to identify a recessive maize gene, tie-dyed1 (tdy1), which develops yellow and green sectors in leaves. Yellow sectors hyperaccumulate sugars and starch while green tissues have carbohydrate levels similar to wild type (WT). We determined these data from iodine staining starch accumulation in leaves and quantifying leaf sugars and starch levels. Starch staining was carried out at two time points, one at the end of the light period and the other at the end of the dark period. The results showed that tdy1 yellow sectors had significantly more starch than green sectors and WT leaves at the end of the day. At the end of night, tdy1 yellow sectors stained slightly for starch accumulation while tdy1 green sectors and WT leaves did not. This suggests that tdy1 yellow sectors may accumulate more starch during the light period than can be broken down during the night. We hypothesize that Tdy1 functions in a sugar or starch metabolic or export pathway.

sucrose export defective1(sxd1) is another maize mutant displaying sectors in leaves, containing increased sugars and starch concentrations and defective in exporting sugars. To determine if tdy1 and sxd1 function in the same genetic pathway, we created F2 families by self-crossing sxd1/+ and tdy1/+ double heterozygous plants. Surprisingly, we observed a mild sectored phenotype in some double heterozygous plants indicating they may function in the same pathway. We determined that sectors in double heterozygotes accumulate a large amount of sugars and starch. We measured carbohydrate levels in all genotypes in the F2 family to assess if Tdy1 and Sxd1 are dosage sensitive and act in the same pathway. Our findings support Tdy1 and Sxd1 acting in separate pathways to promote sugar export from leaves.

P117

The Terminal acidic SANT 1 (Tacs1) gene of maize is expressed in meristem-enriched tissues and encodes an acidic SANT domain similar to some chromatin-remodeling complex proteins

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Screening for maize homologs of single Myb telomeric-complex proteins, we isolated a full-length cDNA for a gene that we named Terminal acidic SANT 1 (Tacs1). The ORF predicts a 45-kDa protein with a C-terminal Myb/SANT-like domain. The SANT motif was first described as a putative DNA-binding domain (DBD), and later found to be present in subunits of several chromatin-remodeling complexes. The SANT domain is characterized by an acidic isoelectric point and a negative electrostatic surface potential. Protein homology modeling was performed on several SANT, Myb, and Myb-like domains and we determined that TACS1 and other plant TACS-type models have a negative electrostatic surface potential. Surprisingly, these acidic patches are not compatible with direct DNA binding and may reflect areas for the binding of basic moieties, such as histone tails or basic regions of other proteins. We mapped Tacs1-specific PCR products using the IBM Recombinant Inbred Lines DNA mapping kit to the genetic bin 2.08 between the markers umc1604 and mmc0381. The Tacs1 gene appears to be homologous to the recently identified ANTHER INDEHISCENCE1 (AID1) gene of rice. To learn more about the possible function of the Tacs1 gene, we examined its expression using maize EST data. BLASTn searches with the full-length Tacs1 cDNA as a query revealed that the Tacs1 gene is expressed at relatively low levels, mostly limited to very young reproductive organs, such as the primordia of ear and tassel shoots. The gene-expression and protein-modeling data indicate that the TACS1 protein may function in chromatin remodeling within shoot primordia or meristematic tissues.

P118

The maize aberrant pollen transmission 1(apt1) gene is homologous to the Arabidopsis SABRE gene and encodes a Golgi protein required for pollen tube growth

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The Ac-linked apt1 mutation was detected by the aberrant pollen transmission of Ac. Reciprocal cross demonstrates that Ac transmission is normal in the female germline, but aberrant in the pollen. The DNA blot and revertant analysis show that Ac insertion causes the apt1 mutation. The apt1(Ac) is rarely male-transmitted, and the transmission ratio is 1000:4. apt1 homozygous mutant reveals twisted and short pollen tubes. The apt1 gene is 26 kb at length. The transcript is 8.3 kb with 23 exons, and encodes 2595 amino acids. The APT1 protein is homologous to SABRE and KIP (SABRE-LIKE), unknown proteins involved in root cortex cell and pollen tube elongation in Arabidopsis. Northern blots show that apt1 is expressed in pollen. Subcellular localization analysis demonstrates that a region in APT1 C-terminus is responsible for the localization, and APT1 protein localizes to Golgi in growing pollen tube via cobombarment shooting. We hypothesize that the APT1 protein may be involved in membrane trafficking in pollen tubes and may be specialized to optimally support the high secretory demands in this tip growth cells.

In addition, DNA blot analysis of Ac-carrying progeny from the cross between apt1(Ac)/+ males and wild-type females shows that not only apt1(Ac) is rarely male- transmitted, but also most of the spotted seed progeny from the cross carry a transposed Ac (trAc). Because of this gametophytic screen, trAcs are recovered at a frequency of 94%, which represents a considerable enrichment over the usual transposition frequency of 2-3%. Our genetic data shows that trAcs are preferentially linked to apt1(Ac) donor site. Thus, apt1(Ac) is an excellent tool for selecting Ac transpositions.

P119

The role of polar auxin transport in inflorescence development in maize

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Polar auxin transport (PAT) has been shown to play a very important role in plant developmental pattern formation. PAT is regulated by asymmetrically localized proteins which form the auxin efflux complex. The chemical N-naphthylphthalamic acid (NPA) blocks part of the auxin efflux complex, inhibits PAT and causes abnormal phenotypes in plant development and growth.
We found that NPA treatment inhibits axillary meristem initiation in maize inflorescences. Maize has four types of axillary meristem: branch, spikelet pair, spikelet and floral meristems. SEM analysis and RNA in situ hybridization using kn1 as a marker for meristems shows that NPA inhibits branch, spikelet pair and spikelet meristem initiation. Treatment with other auxin transport inhibitors such as Tri-idobenzoic acid (TIBA) and 9-Hydroxy-9-fluorene-carboxylic acid (HFCA) causes similar abnormalities as NPA. These results indicates that PAT is required for axillary meristem initiation in maize.Surprisingly, although bif2 mutants have a similar phenotype as NPA treated plants, bif2 is still expressed in these NPA treated meristems.
Although polar auxin transport function in floral meristem initiation in dicots is well known, this is the first time its function in inflorescence development in maize has been shown.

P120

Toward in silico transposon tagging of genes controlling aleurone development

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Identification of multiple independent alleles is typically the limiting step in cloning of maize genes by transposon tagging. We have developed a bioinformatics protocol that automates detection of allelic insertions among MuTAIL sequences derived from the UniformMu seed mutant collection. Putative alleles detected by bioinformatics are then confirmed by complementation testing. The efficiency of this approach can be improved by enriching the MuTAIL dataset for mutants that are probable alleles. We have taken two systematic approaches to enrich coverage of genes of interest; phenotypic classification and genetic map location. Mutants with aleurone phenotypes are a relatively rare class of mutants (small number of expected loci) that are readily detected in the pigmented seed background of the UniformMu population. A preliminary screen based on aleurone pigmentation has yielded at least nine seed mutants classified representing four complementation groups. One distinctive class designated "widow peak" included three alleles that were subsequently confirmed complementation tests. The wpk mutant seeds produce aborted embryos with a nearly normal sized endosperm. Intriguingly, aleurone differentiation is partially blocked on the germinal face of the kernel. MuTAIL libraries derived from Mu-off derivatives of each wpk allele were included in the MuTAIL dataset. Blast cluster analysis detected a unique cluster containing MuTAIL products derived from independent Mu insertions in two of the alleles. Subsequent PCR analysis of the third allele confirmed presence of a third Mu insertion located in the same gene. As a test of map based enrichment strategies, the bz1-mum9 marker in the UniformMu provided a convenient means of enriching for mutants linked to the bz1 locus on chromosome 9. A screen of the mutant collection identified five bz1 linked embryo mutants some of which have altered aleurone pigmentation. Because of the tight linkage to the locus, some of these mutants are likely to be allelic. MuTAIL analysis and complementation testing of these mutants is in progress.

P121

Transcriptional regulation of the maize vp1 gene

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The maize viviparous1 (vp1) gene is a transcription factor that serves as a key mediator between the seed maturation and germination programs. VP1 inhibits transcription of genes associated with seed germination and activates transcription of genes associated with seed maturation, desiccation and dormancy. vp1 is also the most upstream known regulator of the well-studied aleurone anthocyanin pathway, activating transcription of the c1 gene. Loss of function vp1 mutant kernels germinate precociously on the ear instead of entering maturation and quiescence, and are yellow in genotypes that would normally pigment the aleurone with anthocyanin. The function of VP1 in regulating these developmental programs has received considerable attention, but little is known about how the expression of the vp1 gene itself is regulated. We have identified a fragment of 5í genomic DNA that confers the proper expression pattern to a GUS reporter, Vp1::GUS, in maturing embryos and aleurone. A comparative analysis of the 958 base promoter region revealed four sequence blocks that are highly conserved among maize, sorghum and rice vp1 genes. Deletion of the distal two blocks showed no significant effect on Vp1::GUS expression in transient assays. We have identified two oligonucleotides that specifically bind embryo and aleurone nuclear proteins in electrophoretic mobility shift assays.

P122

camouflage1 patterning results from a defect in the chlorophyll biosynthetic pathway

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Our labs are studying genes involved in signal transduction pathways controlling leaf differentiation and development. We are characterizing a large collection of mutants that develop abnormally pigmented sectors or necrotic lesions late in leaf development. 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. Portions of the yellow-green sectors occasionally progress to necrotic tissue. 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. Examination of the photosynthetic pigments in yellow-green and green sectors showed that the levels of carotenoids and chlorophylls are reduced in yellow-green cf1 leaf sectors. Immunoblot analyses of wild-type and cf1 protein extracts using antibodies against photosynthetic proteins showed reduced amounts of bundle sheath cell proteins in yellow cf1 sectors compared to the green sectors and wild-type. To understand the function of the cf1 gene, we cloned it via Mutator (Mu) transposon tagging. Cosegregation analysis found a Mu8 element tightly linked to the cf1 mutant phenotype. This fragment was cloned and sequenced. The transposon inserted into the 5’ portion of a gene involved in chlorophyll biosynthesis. Analyzing two additional cf1 alleles derived from Mu active populations revealed independent Mu1 insertions in the 5’ end of the gene demonstrating we cloned the correct gene. Expression analyses are underway to determine if any of the alleles produce transcripts downstream of the insertions or are molecular null alleles. A model discussing the enzymatic function and phenotype will be presented.

P123

empty pericarp (emp) Mutants As A Tool To Study Embryo-Endosperm Interaction In Maize.

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empty pericarp (emp) mutants represent the class of defective kernel (dek) mutants with the most severe reduction in endosperm development, and are easily recognizable in segregating mature ears because they are devoid of endo-sperm material and flattened by compression from surrounding normal seeds. We present the characterization of a group of three emp mutants, defining three different genes, in term of defects in embryo and endosperm develop-ment. Histological analysis reveals an endosperm reduced to a variable extent but still able to differentiate the basal endosperm transfer layer, the embryo surrounding region, and the aleurone. The placento-chalazal layer is significantly reduced and a loss of adhesion between pedicel tissues and the basal transfer layer is evident. In all three mutants, the endoreduplication process as well as endosperm programmed cell death are delayed. Mutant embryos appear retarded in their growth but not impaired in morphogenesis. Both scutellum and coleoptile are present, the embryonic axis is properly established and root and shoot primordia are well formed. Two mutants differentiate leaf primordia, while the third stops at the coleoptilar stage. Even tough mature dry seeds do not germinate, a successful germination is obtained by culturing immature mutant embryos on MS medium. Their seedlings, although not significantly altered in architecture, appear retarded in their growth. These observations provide a picture of a general delay in processes related to growth. Maize seed formation implies the co-ordinated development of embryo and endosperm and the establishment of a flow of signals and metabolites between these two compartments. Mutation affecting seed development can be instrumental to gain more insight into such processes. In particular, analysis of seeds with discordant embryo-endosperm phenotype (mutant embryo, normal endosperm and viceversa), obtainable with the use of B-A translocations, can provide indications about the effect of the mutation on a specific seed compartment and illustrate the influence exerted by one of the two compartments on the other. The histological analysis of seeds with discordant embryo-endosperm phenotype obtained by crossing +/emp females with the appropriate B-A translocation suggest that emp expression in the embryo is necessary but not sufficient for proper seeds development, implying an effect of the endosperm on embryo development.

P124

ragged seedling2 Leaves Fail to Expand Despite Retention of Adaxial/Abaxial Polarity

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ragged seedling2 (rgd2) is recessive mutation affecting lateral organ development in maize. The mutant phenotype of homozygous rgd2 leaves is variable. Mild leaf phenotypes have a reduced midrib and may be moderately narrow and furcated; severe rgd2 leaves are filamentous or even radial. Despite their radial morphology severe rgd2 mutant leaves develop distinct adaxial and abaxial anatomical features. Previous work revealed that RGD2 functions during recruitment of leaf founder cells and during expansive growth of leaf primordia. To examine rgd2 function in relation to other genes involved in mediolateral expansion or adaxial/abaxial polarity, ns rgd2, rgd1 rgd2, and Rld1 rgd2 double mutants were generated. Red auricle and red sheath backgrounds were used to gauge proximo-distal leaf defects of rgd2. Overall, these observations suggest that development is uncoordinated in rgd2 mutant leaves, so that leaf components and tissues may develop quasi -independently. Currently, the cloning of rgd2 employs a map-based strategy exploiting the ever-increasing map and sequence data available in silico.

P125

A putative histone methyltransferase is a candidate gene for rmr2, a modifier of pl1 paramutation

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Paramutation, a process resulting in heritable epigenetic changes in gene regulation, has been well studied at several maize loci involved in pigment production. At the purple plant 1 (pl1) locus, the Pl1-Rhoades allele stably exists in two heritable regulatory states, a highly expressed Pl-Rh or a repressed Plí state. Several loci have been identified by mutation that are required to maintain repressed gene expression of the Plí state. In vitro transcription assays show that one factor, required to maintain repression 2 (rmr2), is required to maintain transcriptional repression of Plí. SSR-based mapping has refined the genetic position of rmr2-1 and allowed identification of a likely candidate gene encoding a SET domain protein SDG104. Arabidopsis orthologs of these proteins are involved in maintaining proper CNG methylation patterns through their ability to methylate histone H3 Lys-9. Similarly, Southern blot analysis shows that maintenance of CNG methylation in the 3í region of Plí is dependent on rmr2 function. This result prompted sequencing of the Sdg104 allele in an rmr2-1 mutant, which revealed a G to C transversion resulting in a non-conservative tryptophan to serine change at amino acid 174. Since this mutation occurs in a region of unknown protein function we have undertaken molecular, genetic, and reverse genetic approaches to test this candidate gene assignment. dCAPs primers targeting the W174S polymorphism have been designed to screen through recombinants from the rmr2-1 mapping population to test cosegregation of the polymorphism and the rmr2-1 phenotype. Additional alleles are currently being analyzed from Mutator lines, EMS treatment, the Maize Gene Discovery Project and TILLING. The hypothesized role of chromatin structure changes in maize paramutation along with the presumed histone modification function of Sdg104 provides mechanistic support for its current assignment as a candidate for rmr2.

P126

Differential CG and CNG methylation modifications of maize pericarp color1 epialleles in the presence of an epigenetic modifier Ufo1

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The pericarp color1 (p1) gene encodes a protein with Myb DNA binding domain that regulates transcription of structural genes of phlobaphene biosynthesis. Alleles of p1 are classified based on pigmentation accumulation patterns of pericarp and cob glumes: P1-rr plants have red pericarp and red cob, where as P1-wr conditions white pericarp and red cob glumes. The phenotypic differences between p1 alleles have been attributed to their gene structures, copy numbers and epigenetic modifications observed as changes in DNA methylation. The Unstable factor for orange1 (Ufo1; Chopra et al., 2003, Genetics 163: 1135) is a dominant epigenetic modifier that upregulates the expression of P1-wr leading to enhanced accumulation of phlobaphenes in various tissues. Southern blot data from P1-wr and P1-wr Ufo1 plants showed that increased phlobaphene pigmentation correlates with increased transcription and decrease in DNA methylation of P1-wr. Further studies of interaction of Ufo1 with P1-wr and other p1 epialleles are currently under way. These additional epialleles are: P1-pr (a hypermethylated epiallele of P1-rr) and P1-wrSM (a supermethylated epiallele of P1-wr). Genetic data together with DNA methylation analysis indicated that Ufo1 modifies the expression of these epialleles as well. To define the molecular nature of interaction of Ufo1 with multi- and single copy alleles and epialleles of p1, we have now performed genomic bisulfite sequencing. DNA methylation changes representing allele specific signatures may provide a better understanding of mechanism through which Ufo1 interacts with p1 alleles and epialleles.

P127

Elucidating the transcriptional regulation of the class I knox gene 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 but it is absent in differentiated cells such as leaves. Transposon insertions throughout the kn1 locus cause its transcript to be ectopically expressed in leaves. The normal and ectopically expressed transcripts are the same size.
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 (L. Bartko, and E. Kellogg, unpublished data). It has been predicted that these CNSs are key regulatory elements that maintain these genes in a meristem-specific manner by an unknown regulatory mechanism. We propose that the transposon insertions in intragenic sequences disrupt the chromatin environment that maintains the transcription of kn1 silenced in leaves. Therefore, we are investigating the possibility that changes in the chromatin environment in this CNS-rich region in a mutant background are responsible for the improper expression of kn1. The DNA methylation of knotted and normal individuals has been explored in leaves and ear meristem but so far there are no major differences detected by McrBC-PCR. Moreover, we are currently using the H3mK9 and H3mK4 antibodies to investigate the possibility that histone modifications in the CNS-rich region can be used to analyze the chromatin environment in normal and mutant individuals. To further investigate the function of these CNSs in the regulation of kn1, we propose to use the high forward mutation rate of the Mu system to find deletions of kn1 sequences near the Mu insertions by using pool PCR.

P128

Functional genomics of maize chromatin

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In plants, chromatin-level regulation is responsible for many different types of non-Mendelian inheritance, such as paramutation, transgene silencing, transposon activity, gametic imprinting and nucleolar dominance. Chromatin is also critically important for many processes during growth and development of the plant, including cellular differentiation, phase changes, flowering, and development of embryo and endosperm. Changes in chromatin can be mitotically, and sometimes meiotically, heritable, serving as a mechanism for the plant to remember environmental or cellular cues inducing gene expression changes. In some cases, the memories are passed - as altered chromatin states - to future generations. Such heritable changes in gene expression, that occur in the absence of changes in nucleotide sequence, are termed epigenetic.
Our research is aimed at identifying and functionally characterizing chromatin genes in maize. To understand how these genes work, we are analyzing the phenotypes of maize lines in which chromatin-gene function has been knocked out. The knockout lines are being produced either transgenically via RNA-interference based strategies or by screening for recessive mutants using TILLING. To characterize chromatin genes and assess their functions, we are taking three basic approaches. First, to dissect gene function genetically, we are performing genetic assays that include looking for effects of chromatin gene knockdowns on: 1) epigenetic reporters, such as paramutation at the b and r genes and epigenetic variegation at pl and p; 2) transgene silencing; and 3) imprinting. Second, to investigate the molecular outcomes of altering chromatin gene function, we are conducting biochemical assays to assess effects of chromatin gene knockdowns on: 1) DNA methylation; 2) histone modification of repetitive elements; 3) changes in expression profiles of genes and repetitive elements, including transposons. Third, we are building high throughput tools to assay nuclear sub-localization and interactions among proteins encoded by chromatin genes. Project status can be viewed at www.chromdb.org.

P129

Maize Sdg118 is the Functional Ortholog of Arabidopsis thaliana Kryptonite

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The Arabadopsis thaliana SET domain protein Kryptonite (KYP), a histone H3K9 methyltransferase, has been shown to play a role in the maintenance of CpNpG DNA methylation and epigenetic silencing at SUP and PAI. The putative orthologous gene in maize (Sdg118) was identified, sequenced and targeted for RNAi-mediated silencing (see www.chromdb.org). Two transgenic cell lines expressing an inverted-repeat construct for the silencing of Sdg118 were recovered. Real-time PCR was used to verify that the endogenous Sdg118 expression was reduced in these two lines. DNA gel blot analysis using methylation sensitive restriction endonucleases shows that CpNpG methylation levels are reduced. Microarray results from Sdg118 mutant cell lines will be presented. The reduction in DNA methylation is consistent with that observed in kyp mutants in Arabidopsis, suggesting Sdg118 plays a similar role in maize.

P130

Mutations in Required to maintain repression6 suggest evolutionary processes mechanistically related to paramutation

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Paramutation occurring at the maize purple plant1 (pl1) locus provides a convenient model system for studying epigenetic mechanisms of gene regulation. pl1 encodes an R2R3 Myb-like transcription factor regulating expression of anthocyanin biosynthetic genes. Opposed to most pl1 alleles, Pl1-Rhoades can exist within a continuum of epigenetically controlled expression states. Typical of paramutation interactions, repressed states, referred to as Plí, are meiotically stable and invariably direct repression of highly expressed states, referred to as Pl-Rh, in heterozygous combination. Somatic maintenance of the repressed Plí state requires the trans-acting factor encoded by the Required to maintain repression6 (rmr6) locus. In homozygous condition, recessive rmr6 mutations cause Pl-Rh-like anthocyanin pigmentation in genotypes predicted to be Plí / Plí. Furthermore, pedigree analyses show that Rmr6 helps maintain an epigenetic mark characterizing the Plí state and thereby assures its meiotic heritability. Additionally, Rmr6 appears to restrict Pl1-Rhoades expression from aleurone tissues thereby maintaining an apparent paralogous pl1 and colorless1 (c1) subfunctionalization of expression patterns. Phenotypic abnormalities occurring in rmr6 mutant plants indicate that Rmr6 helps define developmental patterns of gene expression at other loci. Although developmental phenotypes appear only after inbreeding or intercrossing sibling plants for several generations, they have arisen in divergent families derived from two independent rmr6 mutations. Epistasis analysis indicates that an apical inflorescence feminization phenotype is caused by ectopic action of silkless1. Thus stable maintenance of the Zea mays monoecious architecture requires Rmr6 function. Additional phenotypes suggest Rmr6 roles in apical internode elongation, polar restriction of adaxializing factors, and repression of lateral meristem activity. Once established, these phenotypes reliably cosegregate with homozygous rmr6 mutant genotypes. The fact that developmental abnormalities are only manifest in rmr6 mutant F2 individuals, indicates they are not caused by stable, unlinked, epialleles. The action of Rmr6 in pl1 paramutation and its apparent role in stabilizing otherwise labile developmental processes supports the hypothesis that the nuclear system responsible for paramutation is related to normal mechanisms of developmental gene regulation and is a potential node of evolutionary change.

P131

Mutations that Affect Maintenance of Paramutation at b1 and pl1 Have Little or No Effect on Maintenance of Silencing in p1 Paramutation

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Paramutation involves both establishment (transfer of epigenetic silencing from a paramutagenic allele to a paramutable allele) and maintenance (heritability of the silenced state). To identify trans-acting factors that may affect maintenance of p1 paramutation, we tested several candidate mutants that are known to affect b1 and pl1 paramutation: mediator of paramutation 1, 2, 3, and required to maintain repression 1, 2. Surprisingly, none of these mutations significantly disrupted the silenced P1-rrí state. This is in sharp contrast with paramutation at the b1 and pl1 loci, where the same five mutations can restore the expression of paramutated epialleles (Dorweiler et al., 2000. Plant Cell 11:2101-18; Chandler, unpublished data). These results suggest that distinct regulatory mechanisms may govern maintenance of paramutation at different maize loci. Although these mutations failed to interrupt maintenance, they may affect the establishment of p1 paramutation, as has been observed for r1 paramutation (Chandler and Kermicle, personal communication). Further genetic tests are being conducted to address this possibility. We also tested the effect of Ufo1 (Unstable factor for orange1) on maintenance of P1-rr paramutation. Ufo1 is a dominant factor that relieves the epigenetic suppression of the P1-wr (white pericarp and red cob) allele and induces demethylation of P1-wr sequences (Chopra et al., 2003.Genetics 163:1135-1146). Interestingly, our results showed that Ufo1 also can reactivate expression of a silenced P1-rrí allele, leading to recovery of dark red pigmentation characteristic of P1-rr. Thus far Ufo1 is the only mutation known to affect maintenance of p1 paramutation. In further experiments, we will test whether Ufo1 affects the establishment of p1 paramutation, and determine whether Ufo1 induces demethylation of a paramutated P1-rrí epiallele.

P132

Paramutation: long-range epigenetic interactions in maize

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We investigate the molecular basis of paramutation. Paramutation is a mitotically and meiotically heritable change in the expression of one allele caused by a trans interaction with another allele. Our paramutation model system, the b1 locus in maize, involves trans communication between alleles, long-range regulatory interactions in cis and the establishment of heritable epigenetic states. These processes are important elements in gene control in many higher eukaryotes and are associated with DNA methylation and specific changes in chromatin structure.
Recombination experiments showed that seven 853 bp tandem repeats, located about 100 kb upstream of the b1 coding region, are essential for both b1 paramutation and the activity of a b1 enhancer activating the b1 promoter 100 kb downstream (Stam et al., 2002, Genes & Dev. 16:1906; Genetics 162:197). Recombination experiments also indicated that sequences between 8.5 and 49 kb upstream of the b1 coding region contain regulatory sequences required for the basal b1 expression level. We hypothesize that the upstream enhancer sequences interact in cis with the b1 promoter proximal region, either directly or via the basal regulatory sequences. We are currently using 3C technology (Dekker et al., 2002, Science 295:1306) to identify spatial, long range, in cis interactions within the 100 kb b1 chromatin domain.
The hepta-repeat region is more nuclease sensitive in the high than in the low b1 expression state. In the high expression state each repeat contains two nuclease hypersensitive regions. Interestingly, these regions flank a ~200 bp fragment spanning two repeats that is only methylated in the low expression state. This suggests that a DNA methylation sensitive protein binds the 200 bp fragment in the high but not in the low expression state. We are currently studying the chromatin structure changes correlating with b1 paramutation in more detail.

P133

Role of chromatin proteins in paramutation at the B gene in maize

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Paramutation is an interaction between two alleles in which the expression of one allele is altered. The altered state is meiotically and mitotically heritable. In maize, paramutation has been demonstrated at 4 loci: B, Pl, P1, and R. We are using a reverse genetics approach to study the role of putative chromatin proteins in paramutation at B. B encodes a transcription factor that activates the anthocyanin biosynthetic pathway, and activation can be easily scored by observation of vegetative plant pigment. Two alleles of B participate in paramutation, B-I and Bí. Transgenic lines have been with inverted repeat constructs designed to reduce expression of the ~130 putative chromatin proteins that we are targeting. We crossed each transgene-induced RNAi line sequentially with B-I (paramutable), and then Bí (paramutagenic) testers, the resulting T3 progeny were scored for plant color. If a transgenic family segregates dark plants, paramutation has been affected and the gene targeted by the inverted repeat construct in that transgene may be involved in paramutation. This poster provides a summary of our progress to date.

P134

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

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Pl-Blotched is a stable epiallele of purple plant1 (pl) that leads to a variegated pattern of anthocyanin pigmentation that contrasts markedly with the uniformly purple pigmentation seen in plants with the Pl-Rhoades allele. Spb is a semi-dominant modifier that increases pigmentation of Pl-Blotched plants. At the molecular level, the increased pigment production is due to increased pl mRNA levels and corresponding increases in mRNAs for the genes encoding the anthocyanin biosynthetic enzymes. In addition, Spb leads to changes in the pattern of DNA methylation at several methylation sensitive restriction sites in Pl-Blotched. These observations led us to propose that Spb increases Pl-Blotched expression epigenetically by altering the chromatin structure of Pl-Blotched. To test this hypothesis, the effect of Spb on Pl-Blotched chromatin structure is being investigated through two types of assays: DNaseI sensitivity assays and chromatin immunoprecipitation (ChIP) assays. Our prediction is that the DNaseI sensitivity of Pl-Blotched plants with Spb will be intermediate between the closed, DNaseI resistant chromatin structure of Pl-Blotched (without Spb) and the open, DNaseI sensitive chromatin structure of the fully expressed Pl-Rhoades allele. Similarly, in ChIP assays, we expect that Pl-Blotched with Spb will have a distinct pattern of histone modification, relative to both Pl-Rhoades and Pl-Blotched without Spb. The results of our analysis should help us better understand the mechanisms that regulate the epigenetic state of Pl-Blotched.

P135

The Elongator histone acetyl transferase complex has a function in cell proliferation during organ growth in plants

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The "histone code" represents the interplay between the different post translational modifications of the core histones, including methylation and acetylation and provides a signal for downstream processes, such as transcription and DNA repair. Elongator is a histone acetyl transferase (HAT) complex, consisting of six subunits (ELP1 to ELP6), that co purifies with the elongating RNA Polymerase II (RNAP II) in yeast and humans. Here, we demonstrate that point mutations in three Arabidopsis thaliana genes, encoding homologues of the yeast Elongator subunits ELP1, ELP3 (HAT), and ELP4, were responsible for the phenotypes of the elongata2 (elo2), elo3, and elo1 mutants, respectively. The elo mutants are characterized by narrow leaves and reduced root growth that results from a reduced cell division activity. Morphological and molecular phenotypes show that the ELONGATA (ELO) genes function in the same biological process and the epistatic interactions between the ELO genes are explained in view of the proposed model of complex formation in yeast. Furthermore, we position the plant Elongator complex genetically in the process of RNAP II mediated transcription downstream of Mediator. Our data indicate that the Elongator complex is evolutionarily conserved in structure and function. In addition, in multicellular plants, Elongator acquired a new function in the regulation of cell proliferation during organ growth. The project was supported by the European Training Network DAGOLIGN (HPRN-CT-2002-00267).

P136

The nature of allelic interactions affecting pl1 paramutation

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Paramutation and potentially similar epigenetic processes have been documented in maize. As the purple plant1 (pl1) locus encodes an R2R3 myb-like transcription factor required for control of anthocyanin biosynthesis, changes in pl1 activity are monitored with a visual assay. Most maize pl1 alleles are genetically stable, however the Pl1-Rhoades allele exhibits dynamic instability. Pl1-Rhoades can exist within a spectrum of regulated states, ranging from weakly expressed (referred to as Pl') to highly expressed (referred to as Pl-Rh). Typical of paramutation, the Pl-Rh state invariably changes to a heritable Pl' state in Pl-Rh / Pl' heterozygotes. The nature of paramutagenic activity, the property of Pl' to facilitate change of Pl-Rh in trans, is not understood. Conceptually, this allelic interaction is mediated by either physical contact between chromosomes or by a diffusible material. Taking a genetic approach to test these hypotheses, loss-of-function Pl1-Rhoades derivatives were generated and subsequently characterized. Genetic segregation tests revealed that most loss-of-function derivatives still obtain paramutagenic activity. DNA sequence analysis shows that some of these Pl1-Rhoades derivatives are protein nulls, illustrating that PL1 protein is not required for paramutagenic activity. One working model involves siRNAs generated by Pl1-Rhoades facilitating degradation of homologous pl1 transcripts. Consistent with this model, phenotypic expression of pl1-W22, an allele which typically confers solid pigmention in plant anthers, is trans-silenced by a paramutagenic, loss-of-function Pl1-Rhoades derivative. Unlike Pl1-Rhoades, genetic segregation tests show that pl1-W22 does not acquire paramutagenic activity. Similar segregation analyses demonstrate that two partially functional Pl1-Rhoades derivative alleles fail to acquire paramutagenic activity. Comparisons of RNA species of these non-paramutagenic derivatives and Pl1-Rhoades should determine if pl1 RNA is required for paramutagenic activity. These data are so far consistent with a model where a form of pl1 RNA mediates paramutagenic activity. Establishment of pl1 paramutation may thus share some features of RNA mediated homology-dependent gene silencing.

P137

cis-acting Sequence Requirements for Establishment and Maintenance of Silencing in 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 darkly pigmented and highly expressed P1-rr (red pericarp and cob) state is heritably silenced by a low expressed and lightly pigmented P1-rrí (patterned pericarp and pink cob) state. The distinguishing feature of paramutation is that a newly silenced P1-rrí is not only heritable but also paramutagenic -- capable of silencing another active P1-rr allele. Earlier we showed that transgenes carrying the 1.2 kb enhancer fragment of the P1-rr regulatory region efficiently induced silencing of the endogenous P1-rr allele (Sidorenko and Peterson, 2001, Plant Cell:13, 319-335). Here we present results of transgenic analysis that further dissect the paramutagenic 1.2 kb fragment. Our data obtained from testing eight deletion constructs indicate that the center of epigenetic activity is located within a 405 bp sub-fragment. Even though this fragment was able to induce P1-rrí silencing, it did not confer high heritability of the silenced state; heritability was reduced whether the inducing transgene remained in the genome (81%) or was segregated away (29%). Recovery of P1-rr expression in the presence of transgene suggests that either transgene become not paramutagenic or P1-rr became insensitive to silencing effects of an inducing transgene. This is in striking contrast with full, 100%, heritability of P1-rrí silencing observed for transgenes carrying the full length 1.2 fragment. Therefore, our data suggest that while the 405 bp sub fragment is sufficient to induce silencing, the sequences flanking this sub-fragment are important to maintain silencing through generations. This provides the first evidence that establishment and maintenance of p1 paramutation are conferred by different cis-acting sequences and might involve distinct control mechanisms.

P138

A Maize Root Transcriptome Map

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Drought is a serious problem in maize. Much research has been conducted on drought response in the maize shoot but not in the root. 22,533 root expressed-sequence-tags (ESTs) were sequenced from segments in the first 20 mm of well-watered and water-stressed roots (http://genome.rnet.missouri.edu/Roots/SeqUnigeneSummaryTable.html). These EST represent approximately 7100 maize unigenes expressed in the root transcriptome. Molecular barcodes were used to enable us to incorporate kinematic interpretation into analysis of the unigene information. Our objective is to map genes from the root transcriptome and to develop genetic and genomic resources that can be used to study drought response in maize and other plants. Twenty-four percent of the 7100 root unigenes could be electronically mapped by alignment of the sequences against sequences used previously for genetic or physical mapping in maize. The remaining 5300 unigenes without prior map information were assessed for the presence of simple sequence repeat markers using the SSR Finder software (http://www.maizemap.org/bioinformatics/SSRFINDER/SSR_Finder_Download.html). 880 previously untested and unmapped primer pairs representing 840 unigenes were designed. 150 root unigenes were genetically mapped onto the Integrated B73 x Mo17 (IBM) population and 150 were also located to BAC clones using a set of 6-dimensional BAC pools. As a second round of electronic mapping, the 5000 remaining unigenes were blasted against whole BAC and BAC-end sequences. A map containing all of the electronically and experimentally mapped root transcriptome unigenes was produced and the kinematic information has been integrated into the display. Alignment of this map with QTL, mutant, and expression information will aid in our understanding of maize root response to water-stress. This research was funded by NSF-DBI-0211842.

P139

Analysis of unequal sister chromatid and homolog exchange at a tandem duplication of the *a1* locus: rates and patterns of recombination breakpoint resolution

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The maize genome contains large numbers of tandemly arrayed nearly identical paralogs (NIPs). Novel alleles can be generated from tandem duplications via unequal pairing and recombination with either chromatids or homologs. Recombination experiments conducted by JR Laughnan (1949, 1951, 1961) suggested that the naturally occurring *A1-b* allele consists of a tandem duplication of *a1* components (alpha and beta). These experiments also suggested that at low frequencies, unequal pairing between *A1-b* and its chromatid or a homolog generate recombinants that have lost the beta component and retained the alpha component, which conditions a pale aleurone phenotype. Sequence analysis of alpha and beta genomic clones revealed that beta is a typical wild-type *A1* allele. In contrast, the alpha component contains a 5.4-kb insertion between exons two and three.
To test the hypothesis that the rate of recombination between alpha and beta differs depending on the choice of repair template (chromatid vs. homolog), three genotypes: *A1-b*/*a1::rdt*, *A1-b*/*ax-1* (a deletion of *a1*); and *A1-b*/*A1-b*, were crossed by *a1* resulting in the isolation of pale recombinants. Nine times more "loss-of-beta" recombinants were isolated when both homolog and chromatid templates were present (*A1-b*/*a1::rdt* and *A1-b*/*A1-b*) than when only the chromatid was available for pairing (*A1-b*/*ax-1*).
To test the hypothesis that patterns of recombination breakpoint resolution differ depending on the repair template used, fine-structure mapping of recombination breakpoints was conducted. For pale recombinants isolated from *A1-b*/*a1::rdt*, repair templates were distinguished by using linked genetic markers. Our results demonstrate that the choice of repair template has a pronounced effect on the distribution of recombination breakpoints across *A1-b*.

P140

Cloning of GaMS-1 male-sterile mutant: Linking genetic and physical maps

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Sexual plant reproduction is a fundamental process that involves the coordinated action of a large number of genes. The increasing number of sequence information and the availability of detailed genetic maps has pressed scientists to pay more attention to phenotypic traits and mutants, in order to integrate the existing data with functional ones. Here we present the fine mapping of a gametophytic male-sterile mutant: gaMS-1, previously located to a 1.3 cM region of bin 2.03 of maize. The use of molecular markers bracketing the mutated gene led to the identification of a maize BAC contig covering the complete genomic region. We integrated public data about the physical map to include EST and BAC-end sequences to verify BAC alignments and to reduce the region of interest, as much as possible.
At the same time, the molecular markers around GaMS-1 have been used to identify the rice syntenic chromosomal region. A single BAC contig of rice was detected corresponding to the complete maize region. Thanks to the high level of sequence similarity, some of the maize markers could be identified in rice too, allowing the reduction of the region of interest to 2 rice BAC clones. The available gene annotations were used to identify all transcripts present on those BAC clones. Currently, we are in the process of verifying the presence of those transcripts in the maize syntenic region to reduce the list of candidate genes to be analyzed.
The availability of large segregating populations and the exploitation of the sequence polymorphism present in the maize ESTs and BAC-ends will allow reducing, even further, the region of interest. Finally, comparing the expressed sequences of mutant and wild type genotypes we could gain valuable information leading to the identification of the mutated gene.

P141

Genome-wide scan for structural polymorphisms in diverse maize germplasm

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Genomic and evolutionary processes have shaped the patterns of genetic polymorphism throughout the maize genome. Maize descended from a segmental allotetraploid event ~11 MYA (Gaut and Doebley, 1997), and since that time has undergone a doubling in genome size from retrotransposon insertions and rearrangement of homoeologous chromosome pairs into segmental duplications (SanMiguel et al., 1998; Gaut, 2001). Even today, the maize genome experiences restructuring events that cause instability in local genome organization and gene content, as evidenced by a recent comparative sequence analysis of the bz genomic region from two different North American maize lines that revealed structural diversity within an allelic region of the same species (Fu and Dooner, 2002). Not only did allele structure differ in both pattern and composition of retrotransposon clusters, but 4 out of 10 allelic genes were also absent in one of the maize lines. A method for characterizing intraspecific variation in gene content at the whole genome level would prove useful in providing a better understanding of the extensive structural diversity present in maize. Here we describe a method we are developing to detect and score the presence/absence and copy number of genes in diverse maize lines via direct hybridization of labeled genomic DNA to maize 70-mer oligonucleotide arrays (NSF Maize Oligonucleotide Array Project: UA, UW, and TIGR).

P142

The Maize Genome Contains A High Frequency Of Nearly Identical Paralogs (NIPs)

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As a segmental tetraploid (Gaut and Doebley, 1997) the maize (Zea mays L.) genome contains large numbers of paralogous gene pairs. Over time these paralogs have diverged in sequence identity on average by 5-10%. Recent sequencing efforts that targeted the gene-rich regions of the maize B73 genome have yielded over 850,000 Genome Survey Sequences (GSSs). These sequences were assembled into 114,173 Maize Assembled Genomic Islands (MAGIs) using the method of Emrich et al. (2004). NIPs (Nearly Identical Paralogs) are defined as paralogous sequences that exhibit >98% identity. In silico analyses of the GSSs during genome assembly, coupled with novel wet lab validation strategies, have revealed that over 1% of maize genes have a NIP. Some NIPs are closely linked genetically; others are genetically unlinked. Preliminary analyses of both Arabidopsis and sorghum suggest that both genomes have a lower observed rate of NIPs than that of maize. These results demonstrate that the maize genome has more recently experienced a high rate of gene duplications and/or homogenization, which may have contributed to the plasticity of the maize genome. For example, NIPs provide a mechanism for the maize genome to contain more than two “alleles” of a given locus and may therefore contribute to the success of long term selection experiments. The presence of NIPs will complicate the assembly of the forthcoming maize genome sequence. In addition,NIPs can interfere in the development of genetic markers derived from “SNPs” that are actually paralogous differences.

P143

A High-Density Genetic Map Of Maize Transcripts

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A genetic map of the maize genome containing over 3,500 gene-based markers has been constructed using MultiMap software. The PCR-based transcript (IDP) markers are derived from ESTs or Maize Assembled Genomic Islands (MAGIs, http://www.plantgenomics.iastate.edu/maize/), which were assembled from over 850,000 B73 Genome Survey Sequences (GSSs) using a parallel processing pipeline (Emrich et al., 2004; Fu et al., 2004). Pairs of 3' UTR primers are designed upstream of polyA sites EST sequences while intron-spanning primers are designed based on in silico determined gene models of MAGIs via GeneSeqer-facilitated MAGI/EST alignments or ab initio FgeneSH predictions (Yao et al., 2005). Polymorphisms between B73 and Mo17 are detected via gel electrophoresis or a new technology that has been adapted to genetic mapping, Temperature Gradient Capillary Electrophoresis (TGCE). TGCE can detect IDPs and SNPs, even in the absence of prior knowledge of sequence polymorphisms. A new software tool, GRAMA (Genetic Recombinant Analysis and Mapping Assistant) was developed to streamline the analysis of TGCE mapping data. Draft genetic maps have been generated using both IDP markers with and without ~ 2,000 markers generated by other projects. These genetic maps are displayed using the CMap module of GMOD at: http://maize-mapping.plantgenomics.iastate.edu/. A demonstration of this web site will be provided. ISU's IBM_IDP+MMP_bd (ver4) map contains 1,447 new genic markers and 2,029 markers previously mapped by the MMP (http://www.maizemap.org) and has a total length of 3,604 cM that extends the lengths of chromosome arms 1S, 3L and 9S as compared to the Missouri's IBM2 map (Feb 2003 version). Supported by NSF DBI-0321711.

P144

A radiation hybrid system for the genetic and physical mapping of the maize genome

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The oat-maize radiation hybrid (RH) mapping project is developing RH lines from chromosome addition lines (oat plants that carry a single maize chromosome). Physical maps produced will complement existing genetic maps. Here, we report on the progress made this year. First, a number of new chromosome addition lines have been developed. Included among these are the first fertile addition line carrying an intact chromosome 10, a line carrying a maize B chromosome, and addition lines derived from maize inbreds B73 and Mo17. Second, radiation hybrid mapping panels have been produced for several maize chromosomes and approximately 100 sequences have been mapped onto each panel. Third, uses of these materials for mapping are expanding beyond mapping low-copy sequences. Centromeres for several chromosomes are being localized to very small regions, and the distribution of repetitive sequences along chromosomes is being studied. This material is based upon work supported by the National Science Foundation under Award No. 0110134.

P145

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

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The IBMRIL population has not only served as the first viable bridge between the genetic and physical realms of the maize genome, but has also been widely and successfully used for discovery and functional analysis of QTL. In two cases, we have localized alpha=0.1 bootstrap confidence intervals to single BACs. Separately, we have shown that small physically defined regions of the genome pleiotropically affect multiple phenotypes, providing new functional hypotheses for these QTL. The critical steps in constructing this bridge were the four generations of intermating of the lines prior to recombinant inbreeding. Based on the successes of the IBMRILs and also on the natural limitations of having only one such resource to fill these keystone research niches, we introduce the INKRIL population, which was intermated four times and consists of >500 lines for which inbreeding has commenced. The 500 lines have been assayed for linkage and genetic purity at four independently assorting regions in the genome. The per line levels of recombination are equivalent to the IBMRILs. The purity assays have revealed no evidence of residual heterozygosity in the inbred parents and no evidence of pollen contamination. North Carolina 89 appears to have been derived from Mexican central plateau germplasm, based on its hairy sheath, non-stiff stalk and shallow root system. It has yellow-orange kernels. Kansas 55 is derived from the drought tolerant Pride of Saline, has hairless leaf blades, a stiff stalk, deep rooting, white kernels, and has excellent combining ability. More than 65% of 384 SSLP markers assayed have shown a polymorphism for this wide cross and transgressive segregation of traits not obviously different between the parents is widespread. We discuss our effort to positionally clone macrohairless1 using this resource, highlight several genomics utilities and traits for QTL studies, and present a strategic plan for resource development.

P146

Analysis of Maize Anther Development Using the 21K Maize Oligo Array

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Our research focuses on identifying markers and studying gene expression and regulation profiles for anther development in maize. Within the anther a subset of cells are specified for meiosis, which represents one of the most important yet poorly understood stages in plant biology. There are several maize cDNA microarrays available, however, genome-scale expression profiling of maize using cDNA arrays is often hindered by cross hybridization due to among gene family members. Here we introduce the first maize oligonucleotide array results. We developed the probe set in collaboration with Agilent; the array contains ~21,000 60-mer probes (Version 1) designed from the PlantGDB Dec 2003 maize EST assembly. Version 2 contains updated designs and probes for ~400 meiosis-related genes (designated by Lisa Harper and Zac Cande). For the development study our staging system is based on organ size. Multiple anther stages, pollen, tassel somatic tissues, and juvenile leaves have been sampled. The large number of similar stage anthers within a tassel permit more detailed dissection than is feasible with most other plants. To illustrate the array platform and our analysis methods we will present results from a pilot study on 3 inbred lines and 4 tissue types. Analysis of male sterile mutants in these backgrounds is presented in a separate poster. Our goal is to identify genes that are candidates for stage-specific markers as well as "constitutive" genes. Comparison of the transcriptomes of anther stages should help pinpoint genes associated with the switch from mitosis to meiosis.

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P147

Applications of oligonucleotide microarrays for polymorphism detection and comparative genomic hybridization.

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A NimbleGen oligonucleotide microarray was designed to investigate the utility of oligonucleotide microarrays for polymorphism detection and comparative genomic hybridization. The MF_AZM4 GSS contigs were aligned with the TIGR EST contigs and the resulting alignments were analyzed using POLYBAYES to identify putative polymorphisms. The resulting 1,785 INDEL and 16,406 SNP polymorphisms were used to design oligonucleotide probes for both allelic variants. Each probe was present on the array twice resulting in a set of 72,764 oligonucleotide probes that were used for polymorphism validation. In addition to validating putative polymorphisms, we were also interested in identifying novel polymorphisms through the use of single feature polymorphism (SFP) discovery. Toward this objective a set of 12 probes per sequence was designed for 13,135 B73 derived MF_AZM4 sequences. The signals derived from this set of 157,620 probes were used to identify putative polymorphisms and insertion/deletions present in several maize inbreds. Hybridizations were performed with genomic DNA from several maize inbreds hybridized with varying conditions. An analysis of the number of probes detected using different conditions as well as the validation rate for polymorphisms is presented. We also report data regarding the discovery rate of novel polymorphisms using SFP probe sets as well as the applicability of this technique for performing CGH between maize inbred varieties.

P148

Biological materials from the Rice Genome Resource Center

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The availability of a complete genome sequence makes rice a unique model plant for analysis of cereal crops. Additional biological materials which could serve as indispensable tools for functional genomics have been also been generated through various projects on rice genomics. These resources have been made available to the scientific community through the Rice Genome Resource Center (RGRC) to enable rapid and successful progress in research that will lead to thorough understanding of rice and other cereal crops. The genetic stocks currently available include Tos17 insertion mutant lines, plant materials for genetic analysis and rice full-length cDNA clones. The Tos17 mutant lines are specific gene-disrupted mutants generated by Tos17 retrotransposon and can be used to clarify the function of specific rice genes. The genetic analysis materials include backcross inbred lines (BIL), chromosome segment substitution lines (CSSL) and doubled-haploid lines (DHL) from japonica / indica crosses which are extremely useful tools for quantitative trait loci (QTL) analysis of many agronomically important traits. The rice full-length cDNA collection consists of 30,000 fully-sequenced clones which could be used for functional characterization of the genome as well as for expression profiling using a microarray. As the next challenge in cereal genomics will focus on characterization of about 40-60,000 genes predicted in rice and their counterparts in other cereal crops, as well as in applying the information in crop breeding, an unlimited access to rice DNA and seed stocks will provide a broad community of scientists with the necessary materials for formulating new concepts, developing innovative researches and making new scientific discoveries in cereal genomics.

P149

Brazilian Maize Genome Initiative

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Maize is one of the most important cereals worldwide.However, its large genome size has somehow inhibited/delayed the launching of a whole genome sequencing project up to recently. The complete sequence of the rice genome obtained by IRGSP has been seen as a gold standard model for genome structure in the grasses and will accelerate the understanding of many genetic structures in other cereals, including maize.Nevertheless, the uniqueness of the maize genome and high polymorphic genotype structure has pointed to a necessity of a large genome sequencing effort. We proppose to sequence a minimum tiling path covering a region of 1 Mb on maize bin 3.04, covering the rootless (rt1) gene. Submitted to a grant propposal opportunity for well stablished research groups through the PRONEX-FAPERGS(Program for supporting groups of excellence in research)funded by the Ministry of Science and Technology - Brazil, the Brazilian Maize Genome Initiative is aimed at contributing to the scientific community efforts to unveal the secrets of the maize genome.

P150

CIMDE: A community IBM genetic mapping service

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CIMDE (http://www.maizemap.org/CIMDE/cIBMmap.htm) is a public genetic mapping service for any member of the community using the IBM-94 mapping population. This map population is a 94-line subset of the 302 high-resolution recombinant inbred lines that were derived from B73 and Mo17 inbreds; and were used to create a genetic resource for anchoring the BAC contigs (Arizona) to chromosomal locations. Seed for the IBM-94 are available from the Stock Center (http://w3.ag.uiuc.edu/maize-coop/IBM-Stocks.html). DNA is available from the University of Missouri-Columbia (http://www.maizemap.org/dna_kits.htm). The community may download scores for this population at MaizeGDB and compute their own maps; alternatively, they may submit scores to the CIMDE interface at the University of Missouri-Columbia and receive, sometime over the following 2 weeks, an approximate map coordinate. Either tab-delimited files or "hand entry" of individual scores may be used to submit data. The submitter may use the interface to edit scores at any time, including weeks after submission. RI line identity is controlled by scoring data for 3 selected SSR markers. There are also controls for marker syntax. All data are stored in a local laboratory information management system described by Sanchez-Villeda et al 2003 Bioinformatics 19:2022-2030. There is no limit to the number of markers that may be submitted at one time. Map computation uses the build and place commands of Mapmarker; loci are ordered onto a framework of 250 loci previously mapped onto the high resolution IBM population. With permission of the submitters, map data submitted to the CIMDE interface is supplied to the MaizeGDB as the community cIBM map. This map is periodically incorporated into the IBM neighbors representation. At this time over 400 loci, from some 11 research groups, have been mapped by this tool. Current support is provided by the USDA-ARS and the University of Missouri-Columbia.

P151

Candidate gene association for genes influencing kernel starch composition in maize.

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Starch is a major component of mature maize kernels, along with oil and protein. It is made up of several separable glucose polymers: Amylose is a linear polymer which affects starch gelatinization; Amylopectin is a highly branched polymer with a high molecular weight which affects starch pasting; and phytoglycogen is a soluble branched polymer. Depending on the specific needs of production, maize breeders would want to modify starch properties to affect pasting, viscosity. Recent studies have made use of near-infrared (NIR) spectroscopy to determine maize kernel composition of starches, oils, and proteins. It is a rapid and non-destructive technique with very little sample preparation, making NIR ideal for this study. The connection of genotype to phenotype will allow the identification of useful alleles for improvement of grain quality in maize. Association mapping and linkage disequilibrium provide an interesting approach to dissecting the genetics of complex traits for crop improvement. This work focuses on associating starch traits, such as amylose/ amylopectin ratio, starch viscosity and pasting, with sequence variations. This study uses 288 diverse maize lines in an association mapping approach to locate DNA polymorphisms within 15 candidate genes identified as influencing kernel composition, starch synthesis and starch metabolism. The genes were PCR amplified and sequenced in both directions in the diverse inbreds. We will present the preliminary results of association analyses. This work focuses on starch traits such as starch content, amylose: amylopectin ratio.

P152

Cell wall genomics update: High-throughput screening for secondary cell wall defects using NIR spectroscopy

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The plant cell wall is of major importance because of its role in providing support to tissues, defense against pests and pathogens, and as a source of food, fibers, and green chemical feedstocks. Plant cell walls are composed of independent but interacting networks of carbohydrates, proteins, and aromatic substances. Interacting with this complex matrix are several hundred proteins that carry out many functions, including wall assembly and disassembly, but the precise function of the majority of these proteins is poorly understood.

The cell wall genomics project is a multi-institutional NSF-supported program to uncover and characterize cell wall related genes in maize and Arabidopsis, using both forward and reverse genetics approaches. Putative maize cell wall mutants are identified among F2 families of the UniformMu population. As part of this project, each year 1,000 families are subjected to a high-throughput chemical screen to identify changes in secondary cell wall composition using near infrared (NIR) spectroscopy.

The NIR screening process has been adapted to accommodate environmental variation in the field, and operator-related variation during the acquisition of the NIR spectra. A custom-designed computer program allows communication between commercial software packages. A number of mutants with altered cell wall composition have been identified. Some have very distinct visual phenotypes, but the majority have subtle or no apparent visual phenotypes, demonstrating the value of the NIR screening. Since NIR spectra provide limited detail on the chemical composition, confirmed mutants are being subjected to detailed chemical and cytological analyses in order to define the function of the targeted genes. Genes of interest are cloned using the PCR-based Mu-TAIL procedure.

These combined efforts will eventually result in a detailed picture of the many aspects of cell wall biogenesis. Resulting data will be made available to the scientific community on the following web site: http://cellwall.genomics.purdue.edu

P153

Collinearity of the Alpha Zein Genes Between Different Inbred Lines of Maize (Zea mays, L.).

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It has been tacitly assumed that the order of genes is preserved within the same species providing the foundation for genetic maps. Genomic analysis of the major storage proteins in maize endosperm, the alpha zeins, has shown that their genes are distributed in 7 loci, most of them amplified in roughly tandem arrays that arose in the last few million years. These arrays are sometimes arranged over larger distances and interrupted by the presence of non-zein genes and transposable elements. Recent sequence analysis of one locus has shown that non-allelic copies of zein genes are present in BSSS53 and B73 and that also some of the non-zein genes are unique in their position for one of the two inbreds. Furthermore, expression levels of hybrids compared to their parental inbreds did not exhibit a typical dosage response of the expression levels of the non-allelic genes, suggesting lack of complementation and sometimes overdominance. In addition, allelic gene copies that have in one the parents, a premature stop codon, can exhibit a negative dominance over the other intact allelic copy. Given such unusual structural chromosomal organization and gene expression and its potential implication in breeding and agriculture, additional loci of alpha zein genes from the two inbreds have been investigated and will be discussed.

P154

Confirmation and Analysis of Germinal Insertions Identified by Mutail Blast Clusters

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The UniformMu population is the first large scale inbred Mutator transposon population. We have isolated over 2000 independent seed mutants from this pedigreed population and have analyzed Mu-flanking sequences from a subset of 130 diverse seed mutants. We have previously described a bioinformatic clustering strategy to identify the nonredundant sequences in the resulting 34,253 MuTAIL quality trimmed reads. MuTAIL sequences are clustered using BLASTN of all available cereal cDNAís and maize genomic sequence, and the identified blast clusters are annotated by BLASTX analysis of protein databases. At least 34% of the identified Mu insertions are in or near exon sequences, facilitating selection of candidate genes associated with the mutant phenotype. Blastclusters containing sequences derived from single or multiple MuTAIL libraries distinguish novel and parental insertions, respectively. The blast cluster resource is accessible at UniformMu.org and is presented so that candidate genes can be identified with confidence and with minimal time input before subjecting them to an expanded linkage analysis. Because MuTAIL libraries were derived from lines selected for loss of Mu activity using the bz1-mum9 marker, insertions identified by blast clusters are expected to be germinal and therefore heritable. To confirm heritability and to identify candidates for the causative insertions, we performed an analysis of non-parental blast clusters in MuTAIL libraries derived from two mutant lines, 02S0111 and 02S0114. A total of 16 insertions were annotated as being in or near genes and present in at most one other MuTAIL library. All 16 Mu insertions were confirmed to be heritable. One sequence, bc856, is annotated as alliin lyase and may co-segregate with the small kernel/embryo defective (smk/emb) phenotype in 02S0111. The population is being expanded to test linkage. These results confirm the efficiency of Mu-off selection based on the bz1-mum9 marker, and the effectiveness of candidate selection by blast clustering.

P155

Consortium for Maize Genomics: Progress Towards an Effective Large Scale Whole Sequencing Strategy for the Maize Genome

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The maize genome will be the next plant to benefit from a whole genome sequence. However, its large size and expectation that upwards of 80% of the genome is represented by repetitive elements makes this a difficult endeavor. Two NSF funded maize genome sequencing pilot projects have been undertaken to examine the structure of the maize genome and enable the maize genome community to develop strategies to sequence maize. One project provided genome sequence from over 100 maize BAC clones and over 450K maize BAC ends. The second project examined two reduced representation sequencing methodologies, methyl-filtration (MF) and High Cot selection (HC), for their ability to target maize gene rich regions. Approximately 900,000 reduced representation sequence reads were obtained and analyzed for gene content and gene space coverage. Analysis of the 900K reduced representation sequences suggest that these methods reduce the genome by 5 fold, and that >90% of the genes in maize are represented within these sequences with an average coverage of 70%; suggesting that MF and HC are effective at capturing maize gene sequences. Furthermore, 292 additional maize BACs, including BACs selected by the maize community, were shotgun sequenced to 5X depth and assembled. We have performed detailed analysis of the ability of several genome sequence assemblers to assemble maize BAC shotgun data at several levels of coverage, and we have examined the effects of adding MF and HC sequences to BAC shotgun. This analysis has suggested an effective strategy to sequence the maize genome, which involves a balance of several genomic sequencing methods. An overview of the analysis, our experimental findings and our suggestion for an effective sequencing strategy will be presented.

P156

Consortium for Maize Genomics - Towards Assembly of the Maize Genome

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Nearly 80% of the maize genome is comprised of retrotransposon and repetitive DNA sequences with the genes distributed within unique sequence “islands” in an “ocean” of repeats. Our goal is to investigate rapid and cost-effective approaches to reconstruct the maize ëgene spaceí for gene discovery as an alternative to sequencing the entire genome.

We have evaluated two genome filtration approaches - methylation-filtering (MF) and high Cot selection (HC) which effectively capture genic sequences from the maize genome. In total, we have generated nearly 1 million paired-end sequence reads from approximately 250,000 methylation-filtered clones and 250,000 high Cot clones. Overall, these filtering approaches provide a seven-fold enrichment of gene sequences relative to the whole genome shotgun approach. The sequences derived from the MF and HC clones are assembled collectively to generate the AZMs (Assembled Zea mays sequences). The AZMs are annotated using a combination of homology searches and gene prediction programs. We are in the process of incorporating additional MF reads (The Cold Spring Harbor Laboratory) and gene-rich reads from whole genome shotgun sequences (The Joint Genome Institute) to enhance the gene space coverage in the latest version of the AZMs.

We have also evaluated the effectiveness of a skim approach to target gene-enriched regions of the maize genome by shotgun sequencing 282 BACs, including BACs selected by the maize community. Due to the highly repetitive nature of the maize genomic sequences, we first tested and determined the performance of different methods of contig assembly and scaffolding. The assembled BAC sequences are also annotated by homology searches and gene predictions, to enhance our understanding of the gene structure and organization of the maize genome.

Results of these analyses are presented at the TIGR Maize Database website (http://www.tigr.org/tdb/tgi/maize) and the Consortium for Maize Genomics websites (http://maize.danforthcenter.org, http://www.maizegenome.org). A detailed overview of the project and the results derived from our analysis will be presented.

P157

Detection of genes under selection during the domestication of maize from sequence data

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Maize was domesticated about 6,000-10,000 years ago from its wild progenitor, teosinte (Zea mays ssp. parviglumis). During the domestication event, important morphological changes occurred to lead to the cultivated form. Consequently, a certain number of genes involved in traits adapted for agriculture are expected to have been strongly selected. We investigated sequence diversity data for 100 regulatory genes (ESTs) in maize landraces and in teosinte to detect selection, and thus identify genes that have been involved in the domestication process. We chose the 100 ESTs from a set of 10,000 ESTs by preferentially pick those that have low diversity in current inbred lines of maize. A set of 50 randomly chosen ESTs were also sequenced in maize landraces and in teosinte to serve as a control for the domestication bottleneck effect on sequence diversity. These data will allow us to perform classical selection tests but also apply more specific tests that include elements of the precise evolutionary history of maize.

P158

EST analysis of transition stage maize embryos

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The transition stage of maize embryos is characterised by a change from the radial symmetry of the globular embryo to the bilateral symmetry of the coleoptilar embryo. It also marks the end of seemingly unorganised cell proliferation and the onset of protoderm differentiation and meristem formation. To gain further insight into this key stage of early embryo development polyA+ RNA was isolated from micro-dissected transition stage embryos and the corresponding cDNA amplified by non-specific PCR prior to cloning into a standard vector. A DACS analysis of 586 clones identified 28 sequences presented more than twice in the analysed sample. These sequences were used as probes on colony lifts to normalise the library. Sequencing was mainly done from the 5' end but a minority of clones was also sequenced from the 3' end. After quality control of the chromatograms 2496 sequences were used for clustering. 1583 TUG (tentative unigenes) were obtained and annotated. 232 TUG potentially corresponded to novel genes since they were not present in the combined Genoplante and Genbank EST databases with an e value below -20. 50 TUG were chosen for expression profiling by quantitative RT-PCR. Two TUG corresponding to genes with unknown function were strongly expressed in 7 DAP kernels but not at any other stages during kernel development or in any other tissues of the maize plant. They were good candidates for fulfilling key roles in transition stage maize kernels. These results show that highly specialised libraries are a means to discover novel ESTs and genes even in species for which extensive EST data are already available.

P159

Effects of phosphorus on the growth of root and shoot in diverse maize inbred lines

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Phosphorus (P) is among the major macronutrients required by plants. Phosphorus deficiency is one of the major abiotic stresses that limit plant growth and crop productivity in acid soils of temperate and tropical regions. Phosphorus plays a critical role in energy conservation and carbon assimilation via inorganic phosphate (Pi) that is necessary for coupling the light and dark reactions in photosynthesis. As a consequence, Pi availability affects also processes that require an adequate carbon supply such as nitrogen and sulphur assimilation. Phosphate is also a constituent of key molecules such as nucleic acids and phospholipids. Although phosphorus is abundant in the lithosphere, the physicochemical properties of inorganic phosphate (Pi) make more than 80% of Pi immobile and not readily available for plant roots. Since little Pi is available in most soils, P fertilizers are applied to crops. However, the use of P fertilizers is unsustainable and may cause pollution. Our objective was to test 240 inbred lines of maize, representing much of the diversity in maize in controlled greenhouse conditions in order to identify P-uptake and P-use efficient lines. We measured and compared root and shoot biomass, root growth, and P content. Phenotypic data obtained on 4 weeks-old inbreds grown in high and low phosphorus media will be presented. In this study our final goal is to use these phenotypic data in association mapping with candidate genes. For this purpose, we are currently sequencing several genes involved in phosphorus uptake and metabolism.

P160

Functional and comparative genomic analysis of aluminum tolerance in Zea mays

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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 upon biotechnology. In this project we will identify and characterize Al tolerance genes and their associated mechanisms in maize. Our study populations are derived from diverse sources; thus we should capture a wide range of tolerance levels and useful alleles. First, we used QTL mapping to characterize the genetic basis for Al tolerance in the Intermated B73 x Mo17 (IBM) population. A 5-factor model explains approximately 60% of the variance observed, where Mo17 donates 3 of the 5 loci. These three factors apparently act epistatically. Physiological experiments and fine-scale mapping are underway. Second, we used comparative (in silico) mapping analyses to place our results from the IBM population in context with mapping results from a South American population developed by collaborators at Embrapa Maize and Sorghum, and also with rice. Third, we have initiated experiments to test the range of Al tolerance responses observed in diverse maize materials, in support of future association analyses. Fourth, we have initiated microarray experiments with greatly contrasting genotypes to clarify how Al stress affects patterns of gene expression and to assist in candidate gene identification. This work is supported by NSF Plant Genome Award DBI #0419435 (PI: Kochian).

P161

Genomic Screening for Domestication and Improvement Genes in Maize

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Maize (Zea mays ssp. mays) was domesticated from teosinte (Zea mays ssp. parviglumis) through a single domestication event in southern Mexico between 6,000 and 9,000 years ago. This domestication event resulted in the original maize landrace varieties that were spread throughout the Americas by Native Americans, leading to adaptation to a wide range of environmental conditions. Starting with landraces, 20th century plant breeders selected inbred lines of maize for use in hybrid maize production. Both domestication and crop improvement involved selection of specific alleles at genes controlling key morphological and agronomic traits, resulting in reduced genetic diversity relative to unselected genes. The loss of genetic diversity in maize varieties involves not only directional selection, but also population bottlenecks and hitchhiking effects. Here we sequenced 1,095 maize genes from a sample of 14 inbred lines and chose 35 genes with zero sequence diversity as potential targets of selection. These 35 genes were then sequenced in a sample of diverse maize landraces and teosintes. Hudson-Kreitman-Aguadé tests and coalescent simulations of domestication were performed to test for selection. Four domestication and four improvement genes were identified as significant for selection by both analyses. Genomic screens for evidence of selection identified genes of potential agronomic importance even when gene function and the phenotype of interest are unknown.

P162

Genomic Structure of the Dicer-1 Homologue Gene in Maize (Zea mays, L.)

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Dicer-1 is a dsRNA-specific RNase that has been shown to play an important role in RNA-mediated gene silencing. Several studies have suggested that the regulation of gene expression by miRNAs occurs in a conserved pathway. We have found that certain miRNAs that are regulated by Dicer1-1 homologue (DCL1) in Arabidopsis, are also conserved in maize. Using a probe developed from a partial cDNA of a DCL1 homologue from maize, we detected two putative copies in the maize genome by Southern blot analysis; fingerprinting results of individual clones confirmed this result. Two BAC clones, from a B73 BAC library, containing the two putative DCL1 homologues were sequenced. Only one of the two clones had a gene encoding a conserved DCL1 protein. The second clone contained only a partial sequence homology to the DCL1 and is not expressed. Therefore, the cDNA is likely to represent the gene of the first clone. However, when this clone was annotated and its genes aligned with rice, a conserved gene was found in rice, but did not appear to be in the same syntenic regions of the two genomes. Further studies are required to determine the function of this putative DCL1 in maize.

P163

Global expression analyses of genes involved in meristem organization and leaf initiation

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All above ground organs of higher plants are ultimately derived from specialized organogenic structures called shoot apical meristems (SAMs). The SAM exhibits distinctive structural organization, marked by tissue zonation and cell layering. The structure of plant SAMs is correlated with their function, such that new leaves are initiated from the peripheral zone of the SAM and the central zone replenishes new meristematic cells that are lost during organogenesis. Experiments are proposed to identify and analyze genes required for meristem function and early stages of leaf development in maize. Laser dissection microscopy is a powerful technique that permits the isolation of RNA from specific cell types within fixed plant tissues. RNA collected from 1,000-10,000 cells is sufficient for use in microarray analyses of global gene expression. The relatively large size of the maize vegetative meristem, approximately 250 meristematic cells are recruited into the incipient maize leaf, renders this plant especially tractable for this experimental system. The laser microdissection/microarray technique is being used to capture cells from specific domains of the maize meristem and newly-formed leaf primordia for use in comparative analyses of global gene expression. The differential expression patterns of candidate genes will be verified by real time RT-PCR and in situ hybridization of transcript accumulation in maize tissues. These experiments will microdissect gene expression patterns in meristems and leaf primordia, and promise to provide novel insight into mechanisms of plant development.

P164

High Resolution Physical Map, Sequence Composition, and Genome Organization of Maize

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We developed a framework for sequencing the 2,365 Mb maize genome (inbred B73). A high information-content fingerprinting (HICF) physical map was built and integrated with the genetic/physical map derived from the Maize Mapping Project (www.genome.arizona.edu/fpc/maize). We generated 472,682 BAC end sequences (BESs) equivalent to 305 Mb (13% of genome) as sequence tagged connectors (STCs) to link other sequences to the map. To probe the maize genome organization, we sequenced 100 randomly selected BACs (14.38 Mb), in addition to a 56 BAC tiling path (8.66 Mb) from Chr#9L. In lieu of finished data, manual curation provided high quality assemblies, thereby greatly improving resolution of subsequent analyses. Comparison of BESs and BACs to an updated repeat-database (de novo repeat detection from BACs) revealed a repeat content of 58-66%, of which 96% are retroelements. The ratio of class I retroelements to class II DNA transposons was 50:1, in contrast to rice (2:1). About 92% of 457 genes predicted on the 100 BACs were supported by EST data. Almost half of the genes lacked a homolog in close relatives like sorghum and rice; only 11% were conserved in Arabidopsis. Gene density varied from 6 to 107 genes per Mb without any pronounced distribution pattern of genes relative to repetitive sequences. Exon number varied from 1 to 31, the medium value being 3 exons per gene. Though the largest gene, related to disease resistance, is as large as 59 kb the average gene size is only 3 kb. Gene-enrichment procedures did not result in a sharp division of genes and intergenic regions, but confirmed the high degree of duplicated, paralogous genes within the maize genome. Coding potential of BESs indicated the transcriptome to be 7.5% (177 Mb). Average gene length of 3 kb suggested a total of 59,000 genes (1 gene/40 kb).

P165

Identification of genes involved in UV-B stress responses by transcriptome profiling

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Understanding the mechanisms plants use for acclimation to ultraviolent radiation stress is key in predicting plant responses to our changing environment. Maize inbred line B73 plants were grown in sterilized vermiculite in a greenhouse until the majority of the plants were at the three leaf stage. Plants were then divided into groups of approximately 20 plants for treatments of one, two, four, eight, or twelve hours of UV irradiation at a dose rate of 0.024 W m-2. Control groups were placed the same distance under an identical pair of UV bulbs that were covered with Mylar to block all UV-B radiation and harvested at the same time points. Arrays were hybridized with labeled control and UV RNAs from each time point, with four replicates at each time point and dye switch duplicates. Using transcripts from pools of leaf tissue, cDNA microarrays were used to monitor UV-induced alterations in gene expression to gain a better understanding of global gene expression in response to UV-B. Array data were standardized using a variance stabilizing procedure accounting for the many sources of systematic variation that affect measured gene expression levels. Examination of the effects after irradiation at different times indicated that different suites of genes are expressed at specific times in response to UV-B. The number of genes expressed late only (after 12 hours of UV-B exposure) was substantially larger than genes expressed early only (after 1 hour of UV-B exposure). Further studies using network and profile analysis will be used to identify the mechanisms involved in this type of stress response and the progression of plant responses over time.

P166

Identification of loci under selection in a maize population recurrently selected for quantitative resistance to northern corn leaf blight

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Recurrent selection (RS) is often used in crop improvement to increase the frequencies of favorable alleles and allelic combinations. Molecular analysis of allele shifts under RS can reveal quantitative trait loci (QTL) and allow favorable alleles to be identified from a rich pool. Full-sib S1 RS was conducted by the International Maize and Wheat Improvement Center (CIMMYT) in eight diverse tropical maize populations to improve the level of quantitative resistance to northern corn leaf blight (NCLB). Through four cycles of RS significant increases in resistance were achieved in each cycle; the mean area under the disease progress curve decreased by an average of 17% per cycle across all cycles and populations.
For one population, we performed a genome-wide scan with simple sequence repeat (SSR) markers to identify loci exhibiting significant differences in allele frequency shifts between the initial and final populations. Among the more than 100 loci tested, 10% were found to deviate from expectations attributable to drift, suggesting the effect of selection. Effects of selection could be related to NCLB response but could also be due to acknowledged selection for yield and rust resistance or to unrecognized factors. As an initial effort to identify loci likely to be under selection for NLCB resistance, we compared the results of our analysis with those of a summary of published NCLB QTLs, multiple disease QTLs, and major genes for NCLB resistance (Ht genes). Several loci showing evidence of selection coincided with genomic regions previously associated with quantitative and/or qualitative resistance.
To pinpoint the signal of selection and further characterize the chromosomal response to RS, we saturated selected chromosomal segments with additional SSR markers. Here, we report the latest results from saturating multiple chromosomal segments putatively under selection.

P167

Identification of ultraconserved sequences in grass genomes

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The nearly finished rice genome, ~500,000 sorghum methylation filtration (MF) genome survey sequences (GSSs) and ~2 million maize GSSs are powerful resources for comparative analyses of grass genomes. After we built Maize Assembled Genomic Islands (MAGIs) and Sorghum Assembled genoMic Islands (SAMIs; http://www.plantgenomics.iastate.edu/maize), we are able to identify 130 non-overlapped maize genomic fragments with the length >=200 bp that have identical (100%) homologs in sorghum genome. In the further comparisons including rice genome, more than 40 highly similar homologs (95% over 200 bp) were identified across these three grass genomes. Here we also present and discuss the function assignments, RNA secondary structures and potential significance of these ultraconserved sequences.

P168

Isolation and functional characterization of a flavonoid 3í hydroxylase corresponding to the red aleurone 1 locus of maize

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Anthocyanins are synthesized via the flavonoid biosynthesis pathway which produces a variety of pigmented and non-pigmented secondary metabolites. Several intermediate steps of biosynthesis of flavonoid compounds require activity of cytochrome P450 dependent enzymes. A functional red aleurone1 (pr1) locus is required for the purple (cyanidin) aleurones, whereas a mutation in pr1 leads to accumulation of red pigment (pelargonidin). Previously, Larson, Bussard and Coe (Biochem Genet. 1986, 24:615-624.) showed that functional pr1encodes or regulates a flavonoid 3í-hydroxylse (F3íH) activity responsible for B ring hydroxylation. In the anthocyanin biosynthesis pathway, this F3íH activity would thus be required to convert dihydrokaempferol (DHK) to dihydroquercitin (DHQ). We have isolated and characterized maize genomic and cDNA sequences which encode for a putative F3íH. Sequence characterization shows that the maize F3íH is a member of the super family of cytochrome P450s that catalyze NADPH- and O2-dependent hydroxylation reactions. Genetic characterization of three pr1 mutant alleles shows that the red aleurone phenotype is linked with the isolated sequence. Using a SNP marker assay, the f3íh gene maps to the pr1 position on chromosome 5. In addition to the transcriptional regulation of pr1 by transcription factors r1 and c1 in aleurones, our results indicate that pr1 expression in pericarp is regulated by the pericarp color (p) locus consistent with a role in 3-deoxyflavonoids or phlobaphene synthesis in pericarp and cob glumes. Overall, results obtained from transcript analysis, functional complementation and linkage mapping studies confirm that the putative sequence encodes for a F3íH corresponding to the pr1 locus of maize.

P169

Junction-linked primers for mapping repetitive elements

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We are developing a PCR based marker system that can map a specific insertion of a repetitive element within a block of repetitive sequence. Most existing molecular marker techniques, such as SSRs and transposon display, target specific low-copy sequences or a collection of sequences that may include repetitive sequences. These procedures are not will suited to map an individual insertion of a repetitive element. Junction-linked primers use one PCR primer located within the repetitive element being mapped and a second primer located at the junction between the repetitive element and adjacent sequence. This approach works even when the adjacent sequence is also repetitive. We have succeeded in amplifying specific sequences from over 25 insertions of repetitive elements. Several of these sequences have been sequenced and mapped using the IBM mapping lines.

P170

Large Scale Transformation and Trait Screening in Corn

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All transgenic crop products currently on the market are based on the expression of plant or other genes in the crop plant. These products are based on genes that have been discovered over years of intensive study in model systems. This approach to product discovery requires a large investment in model systems and basic plant biology, an investment that is lost if the gene fails to perform in the crop plant. We have taken a fundamentally different approach to product discovery based on the production and screening of large numbers of transgenic corn plants. In this genetic style approach, genes are selected and used to produce a small number of independent transgenic corn plants. The plants are then screened through a battery of assays that are closely tied to the desired product description. Cost intensive optimization and mode of action studies are then focused on genes that have been demonstrated to perform effectively in the crop plant.

P171

Linkage mapping of 1454 new maize candidate gene loci

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Bioinformatic analyses of maize EST sequences have highlighted large numbers of candidate genes putatively involved in agriculturally important traits. To contribute to ongoing efforts towards mapping of these genes, we used two populations of intermated recombinant inbred lines (IRILs): (1) IBM, derived from B73◊Mo17 and publicly available from the Maize Genetics Cooperation Stock Center, and (2) LHRF developed from F2◊F252 to map genes monomorphic on IBM, and publicly available from INRA, France. 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 gene 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, actual meiotic centiMorgan distances were calculated taking into account the number of intermating generations undergone by the IRILs. The actual sizes of our framework maps were 1825 cM for IBM_Gnp2004 and 1862 cM for LHRF_Gnp2004. All genes mapped on LHRF_Gnp2004 were also projected on a consensus map IBMconsensus_Gnp2004. This work was carried out in the frame of the French Genoplante plant genomics consortium.

P172

Microarray Resources for Maize

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The Maize Micorarray Project (http://www.maizearray.org) was initiated to provide low-cost, comprehensive, public sector long-oligonucleotide microarrays for gene expression analysis in Zea mays L. The overall objectives are: 1) Produce an array with 70-mer oligonucleotides for the >30,000 identifiable unique maize genes which should allow better discrimination among the gene duplications common in maize relative to cDNA arrays, and provide a hybridization service, both based on a cost recovery model. 2) Provide a website to distribute microarray information, and document expression data generated by the project with links to rice and maize genome annotation. 3) Perform expression profiling with a subset of maize tissues to provide a baseline of data and detailed protocols for the community. 4) Utilize the flexible NimbleGen system to experimentally refine oligonucleotide design for the next generation of 70-mer arrays which will achieve better discrimination among gene family members and gene duplication. 5) Develop web based experimental design and analysis tools, as well as design tutorials, compatible with the TIGR data curation tools. An array with 57,452 70-mer oligonucleotides is currently available as a slide pair for $125. To date, over 900 sets of arrays have been distributed to researchers in the U.S., England, Italy, China, Mexico, and Switzerland. A Sybase relational database, Zeamage, has been constructed to store expression data generated from this project. The schema is based on existing microarray projects at TIGR but has been modified to address the multiple types of data in this project. Three design tutorials for the more common types of microarray experiments are now available on the project website, as are data curation tools. Experimental protocols for the array have been developed and optimized via designed experiments and are also available on the website. Two workshops (May 10-15, December 12-17) have been held at the University of Arizona and a total of 23 maize researchers have participated.

P173

Microarray analysis of maize bundle sheath and mesophyll photosynthetic differentiation

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In C4 plants, such as maize, Rubisco accumulation is spatially restricted to sites rich in CO2 so that carbon fixation is favored and photorespiration is reduced. To do this, the leaves of C4 plants possess two distinct photosynthetic cell types and display a structural syndrome termed Kranz anatomy. Carbon fixation, catalyzed by the enzyme PEPC, occurs initially in mesophyll (M) cells resulting in the formation of four carbon (C4) compounds. These C4 compounds are subsequently transported into adjacent B cells, the site of Rubisco accumulation, where decarboxylation releases CO2 and generates a high CO2 environment.

Cell-type specific differences in chloroplast development are fundamental to the differentiation of B and M cells. The chloroplasts of B cells are larger than those of typical C3 plant species and are arranged centrifugally bringing them in close proximity to the adjacent M cells. M cell chloroplasts are smaller and randomly distributed throughout the cell. Transcipts encoding several Calvin cycle and C4 carbon shuttle enzymes, as well as those encoding a number of components of the light-harvesting machinery, are known to accumulate to different levels in B and M cells. However, the full scope of differential gene expression between B and M cell types is, as yet, undefined.

We have used DNA microarrays, provided by the maize gene discovery project (University of Arizona), to profile B and M cell types. We have developed an ANOVA based approach for use with this multi-treatment microarray data set and will present the initial results of this analysis.

P174

Mining genome data to reconstruct gene families controlling plastid isoprenoid biosynthesis in the Poaceae

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Plant isoprenoids, such as carotenoids, tocopherols, sterols, hormones, and phytoalexins, are all derived from the five-carbon isopentenyl pyrophosphate (IPP) which is biosynthesized by two independent pathways located either in the cytosol or plastid. Cytosolic IPP is produced via the mevalonate pathway while plastid IPP is synthesized by the non-mevalonate 1-deoxy-D-xylulose-5-phosphate (DOXP/MEP) pathway. Our research laboratory has been investigating regulation of the plastid-localized carotenoid biosynthetic pathway which utilizes IPP as a substrate. Since substrate availability will impact flux through the downstream biosynthetic pathway, we have focused attention on the plastid IPP (MEP) biosynthetic pathway. To characterize the MEP pathway with regard to the gene families encoding enzymes for IPP biosynthesis, we are using a bioinformatics approach to analyze publicly available genomic sequences and sequences that we have determined from BAC genomic DNA isolation. To put these results in the context of a more general model applicable to the Grasses (Poaceae), we are further comparing these results among various subfamilies of the Poaceae. Multiple sequence alignments provided an estimate of gene family composition and EST analysis revealed tissue-specificity of expression. Phylogenetic trees were also utilized to classify paralogous gene family members across species. Characterization of these gene families will contribute to developing rationale strategies of metabolic engineering of plant isoprenoid content.

P175

Molecular Marker Development for Map-based Cloning in Maize

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The complex nature of the maize genome poses many challenges for the cloning of genes using a map-based strategy. However, understanding and utilizing the publicly available sequence information to its full potential can provide an effective means to navigate the genome and capture a gene of interest. One of our focuses is to develop a variety of markers that will aid in the map-based cloning process. BAC-end, EST (expressed sequence tags), and genomic sequences are the primary sources for our marker development. The most common types of polymorphisms found among these sequences are SSRs (simple sequence repeats) and SNPs (single nucleotide polymorphisms). CAPS (cleaved amplified polymorphic sequence) and genotype-specific PCR markers are developed from SNPs. In addition, SNPs are directly used as markers using new technologies such as “Pyrosequencing”. We will present a comprehensive guide to illustrate the sources of available sequence information (database etc.) and the types of markers that can be developed by all of these resources for map-based cloning in maize.

P176

Molecular Population Genetics of Maize Domestication

(submitted by Qiong Zhao <qiongzhao@wisc.edu>)

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The domestication of maize resulted in a dramatic change in its plant architecture and inflorescence development as compared to its wild ancestor, teosinte, with the most remarkable difference being in their female inflorescence (ear) structure. Only a few genes have been uncovered to be associated with selection during domestication, such as tb1, dwarf8, and the starch pathway genes, etc. One group of interesting genes as candidates targeted by selection is the MADS-box genes, the key regulators of inflorescence and flower development. Besides the MADS-box genes, there are many other genes regulating plant vegetative and reproductive development and thus forming another set of candidates as targets for selection. We sequenced about 40 maize MADS-box genes and some other developmental genes in a common set of maize inbred lines, maize landrace and teosintes to investigate how selection shaped the diversity of MADS-box genes and other plant developmental genes during the domestication of maize. The proportion of selected genes in MADS-box gene family will be compared to that found in a set of loci chosen at random to see whether MADS-box genes are a specific target of selection during maize domestication. We will also test the hypothesis whether it is more likely for genes at a specific developmental stage to become targets of selection.

P177

Molecular and Functional Diversity in the Maize Genome

(submitted by Wei Zhao <zhaow@cshl.edu>)

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In 2001, maize became the number one production crop in the world. Current US maize production is almost four times wheat and rice production combined. Maize is also the single most diverse crop species, containing tremendous variation in morphological and physiological traits and extensive DNA sequence polymorphism.

We have previously determined the genetic relationships among maize and teosinte germplasm, examined how average loci evolve, and developed methods for relating nucleotide diversity to phenotypic effects. Our new objective is to understand how selection has shaped molecular diversity in maize and then relate molecular diversity to functional phenotypic variation.

How has selection shaped molecular diversity? To address this, 4000 loci will be screened for selection evidence, and then 1000 loci will be studied extensively by doing SNP surveys across diverse maize and teosinte. A range of tests of selection will be used to identify genes showing positive, diversifying and purifying selection. The identified genes will be those involved in domestication, agronomic improvement, and local adaptation.

How does this molecular diversity relate to functional trait variation? A wide range of maize and maize-teosinte linkage and association mapping populations will be created that capture a tremendous range of diversity. These populations will be genotyped for SNPs and candidate genes and phenotyped for domestication, agronomic and developmental traits. This will permit high-power and high-resolution dissection of a wide range of traits, and relate the molecular diversity to functional variation.

Further information and data produced by the project can be obtained at the project website, www.panzea.org.

P178

Physiological and molecular analysis of nitrogen uptake and utilization in maize

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Nitrogen (N) is considered to be one of the most important factors limiting crop production in most agricultural systems. Nitrogen use efficiency (NUE) is defined as grain yield per unit of N supplied and includes N uptake, assimilation, and remobilization. Conventional breeding methods have been used over the past decades in combination with heavy nitrogen fertilization to achieve high yields. Since nitrogen use efficiency for cereal production (including maize) is approximately 50%, the excess of N adds unnecessary expenses to corn production, as well as increases the risk of environmental pollution. It is therefore necessary to view sustained and enhanced agricultural yields in relation to the quality of the environment by increasing NUE. An increase of NUE in maize would lead to substantial savings in N fertilizer costs for maize growers. Furthermore, N metabolites are remobilized from leaves to provide nutritional resources for pod filling and seed content; the increase of NUE will therefore add value to maize varieties with higher grain yield and better protein content. At the molecular level, a number of genes involved in NUE are relatively well characterized to date. We used a fertigation system in controlled greenhouse conditions and two levels of N (high and low) to test a set of 300 diverse inbreds (described at www.maizegenetics.net) that captures a great amount of maize diversity. The classification of the inbreds obtained from N-use indexes will be presented. We are currently sequencing and testing a number of key genes involved in different pathways of N uptake and N metabolism for association analyses in the diverse inbreds.

P179

Quality Assessment Of Maize Assembled Genomic Islands (MAGIs) And Experimental Verification Of Predicted Novel Genes

(submitted by Yan Fu <yanfu@iastate.edu>)
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Recent sequencing efforts have targeted the gene-rich regions of the maize (Zea mays L.) genome. We report the release of an improved assembly of Maize Assembled Genomic Islands (MAGIs) and first assess its quality using both computational and biological approaches. Comparisons to maize BAC sequences suggest that over 95% (157/165) of MAGIs are correctly assembled and the misassembly can be caused by not only highly homologous transposons but also nearly identical non-transposon genes. Because the rates at which GSS junction-testing PCR primers (90-92%) amplify genomic DNA are not significantly different than those of control primers (~91%), we conclude that a very high percentage of genic MAGIs accurately reflect the structure of the maize genome. This quality assessment will provide helpful information for the impending maize and other complex plant genome projects. EST alignments, ab initio gene prediction and sequence similarity searches of the MAGIs are available at http://www.plantgenomics.iastate.edu/maize. This assembly contains 46,688 ab initio predicted genes, of which 16,093 lack significant BLASTN hits (E-value cutoff: 1e-10) to maize transcripts. The expression of almost half (628/1,369) of a sample of the predicted genes that lack expression evidence was validated via RT-PCR. Our analyses suggest that this assembly of the maize gene space has "tagged" over 6,900 genes that lacked evidence of transcription and almost 700 of these genes do not exhibit similarity to the Arabidopsis or rice genomes or other genome databases. This study also first report the large-scale application of RT-PCR for the verification of predicted monocot genes.

P180

Recombination, Rearrangement, Reshuffling and Divergence in a Centromeric Region of Rice

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Although several plant and animal genomes have been sequenced, the sequences of most centromeres remain incomplete and/or were not analyzed in depth. To shed light on the organization and evolution of centromeric DNA, we have carefully analyzed the 1.9-Mb sequence of centromere 8 (CEN8) of Oryza japonica (c.v. Nipponbare). Thirty-two LTR-retrotransposon families (including 10 new ones) were identified in CEN8, totaling 245 elements and fragments that account for 67% of the region. The ratio of solo-LTRs to intact elements in CEN8 is ~1:1, significantly lower than previously reported in non-centromeric regions. Interestingly, the ratio of solo-LTRs to intact elements in the core of CEN8 is higher than in any other regions investigated in rice, in apparent disagreement with a predicted lower frequency of recombination in centromeric regions. Comparison of CEN8 of O. japonica and its orthologous segments from O. indica (c.v. 93-11) indicated that ~15% of the intact retrotransposons and solo-LTRs were inserted into CEN8 after the divergence of indica and japonica from a common ancestor. In addition, a 212-kb subregion in CEN8 was found to be composed of three large and highly rearranged tandem repeats. Phylogenetic analysis revealed recent extensive rearrangements and reshuffling of the CentO satellite repeats, apparently by processes that have led to dramatic variation in copy numbers of satellite repeats in different centromeres of rice. Although frequent centromeric DNA rearrangements were found, we also observed that single nucleotide changes in the centromere were running at a somewhat lower rate than in non-centromeric regions.

P181

Root Growth Maintenance During Water Deficit: Physiology, Genomics and Proteomics.

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Roots play a vital role in water and mineral acquisition. Under drought conditions, roots can adapt to allow continued growth. At the same time root to shoot signals are transmitted. Under the same drought conditions, shoot growth is inhibited. The Plant Root Genomics Consortium (www.rootgenomics.org) was formed to further our understanding of the molecular mechanisms occurring in the root system particularly under water stress conditions. Combining whole plant physiology, genetics, genomics, and proteomics approaches we hope to identify the genes and biochemical networks responsible for root growth maintenance and root to shoot signaling under water stress. The project is utilizing a kinematic approach to further understand the spatial and temporal distribution of these genetic and biochemical factors in drought response. 22,533 root ESTs were sequenced from segments in the first 20 mm of well-watered and water-stressed maize roots. These EST represent approximately 7100 maize unigenes expressed in the root. Embedded oligonucleotide tags enable us to add kinematic interpretion to analysis of the transcript information. 1800 root unigenes can be located on the IBM neighbors map by prior information. 150 more have been mapped experimentally on the IBM and another 150 were physically mapped using BAC pools bringing the total number of mapped genes to more than 2000. Electronic mapping by sequence alignment to full-length BAC and BAC end sequence is contributing additional transcriptome map information. Microarray analysis has identified 1924 genes that are differentially expressed in response to water stress in the first three mm of the root; of these 1244 were up-regulated. Characterization of cell-wall profiles and xylem sap in drought-tolerant, drought-sensitive, and ABA-deficient maize lines will contribute to a more complete understanding of root growth maintenance and root to shoot signaling under water stress. This research was funded by NSF-DBI # 0211842.

P182

SNP Genotyping for Diversity and Mapping Studies in Maize and Teosinte

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SNPs are powerful markers for population genetic studies, and linkage and association mapping experiments. SNP have become increasingly available from sequencing data and they are particularly abundant in maize. We designed SNP marker assays within both low-copy EST sequences and known genes for high throughput genotyping in teosintes, landraces, and inbreds. The SNPs were discovered by scanning alignments of EST sequences from 32 diverse maize and teosinte inbreds. Nearly 1000 SNP assays within more than 500 ESTs or genes have been developed to date. A collection of 800 teosinte plants and 278 maize inbreds were genotyped for the markers. Polymorphism indices within the maize inbreds and teosinte as well as the map positions of the markers will be presented. SNP assays including context sequences will be available at www.panzea.org and www.wisc.edu/teosinte.

P183

Sequence analysis of the P1-wr gene cluster

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Here, we exemplify how gene amplification results rather in the divergence of regulatory mechanisms than in subfunctionalization. Recently, it has been shown that the sizes of gene families in plants are much higher than in other organisms. The p1 gene encodes a myb-homolog that is the major transcriptional activator of the phlobaphene biosynthesis pathway and is thought to have arisen by a duplication event from the p2 gene. Both genes are very similar in their coding regions, but differ in their regulatory sequences. In addition, the P1-wr allele consists of multiple genes in tandem fashion. One P1-wr repeat isolated from the inbred line W23 has already been sequenced and analyzed. However, the composition of the entire locus and its relation to the p2 gene remained unknown. Therefore, we have isolated the entire locus comprising the p1 and p2 and their flanking genes within a 300kb region from inbred line B73. A total of eight repeats have been sequenced that are arranged in a head-to-tail tandem array. The overall structure of one repeat unit is almost identical to the previously sequenced P1-wr gene from W23. The deduced aa sequences of P1-wr are very similar to all p1 and p2 proteins known so far. Therefore, the p alleles demonstrate how changes in regulatory sequences along with conserved coding regions can alter tissue-specific expression. Few polymorphisms among different P1-wr repeats indicate a rather recent amplification. Interestingly, sequences flanking the P1-wr repeats show high similarity to p2. Further analysis revealed a truncated P1-wr repeat displaced from the main cluster by 68 kb of retrotransposable elements. Based on our analysis, we present a model explaining the evolution of the P1-wr cluster from a single myb-homolog to the present multigene complex.

P184

Sequencing a 1.3 Mb contig spanning the rf1 fertility restorer locus as a prototype to assess complex-genome coverage strategies

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In T-cytoplasm maize, cytoplasmic male sterility (CMS) is attributed to the presence of the unique mitochondrial gene, T-urf13. Full suppression of T-urf13-mediated CMS is directed by the combined action of dominant alleles of the nuclear (fertility restorer) genes, rf1 and rf2a. To facilitate a candidate approach towards identification of the rf1 gene, three B73 BAC libraries were used to create a physical map of 794 clones from the centromeric region of chromosome 3 anchored to the rf1 locus. A minimum-tiling path of 14 contiguous BACs covering 1.3 megabases were shotgun sequenced, assembled and finished to completion for annotation and display in the GBrowse viewer. Eighty-seven percent has been identified as repetitive sequences, with most transposable elements found in large nested clusters spanning up to 200 kb with insertion chronologies of ~0.01 to ~2.98 million years. GeneSeqer, Fgenesh, and GeneMark.hmm were used to predict consensus locations and structures for 53 genes. Thirty-seven of these are positioned in gene clusters with as many as 8 members. Two hundred fifty-four GSS assemblies (including MAGIs, TIGRís AZM and PlantGDBís GSS) aligned to the 1.3 Mb contig, 36 of which aligned to predicted genes. Two hundred eighteen GSS assemblies aligned to regions not predicted as genes, revealing that only 15% of GSS contigs align to genes in this region. Seventeen predicted genes did not correspond to any GSS assembly indicating that at least in the centromeric region of chromosome 3, finished sequence can provide a significant number of previously undescribed gene predictions.

P185

Thirty putative MIR genes of maize and their relationship to rice and sorghum sequences

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Here we contribute to the characterization of non coding RNAs in maize, by identifying thirty genomic sequences (MIR genes) which putatively code for 5 different microRNAs (miRNAs). miRNAs are short RNAs, which are involved in post-transcriptional gene regulation, and a single miRNA can be produced from several different longer primary transcripts that fold into a stem-loop structure.

Starting from sequences of 8 rice pre-miRNAs, we amplified maize genomic sequences. Amplicons were cloned and sequenced and based on sequence homology and on the conservation of their most stable predicted secondary structure, were classified as MIR genes for 5 miRNAs. These findings were confirmed, consolidated and extended by data mining in publicly available maize genomic databases. To provide further data for evolutionary studies on MIR genes in cereals, Sorghum bicolor genomic clones containing putative MIR genes were also considered, and sequence homology was determined by reciprocal best BLAST hit approach. Expression profiles of these 5 miRNAs were analysed by RNA gel blot analysis of RNA purified from different maize tissues in different genotypes. Finally, MIR genes were assigned to maize chromosomes utilizing oat-maize addition lines.

P186

Towards identification of a platform for routine SNP genotyping in corn

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Single nucleotide polymorphisms (SNPs) are abundant and widely distributed in many genomes investigated to date. Use of SNPs is on the rise in several genomes due to many discovery efforts, large scale availability of sequence data in public domain and the amenability of SNP assays for automation. Availability of a reliable SNP detection platform is crucial for the validation, screening, mapping and downstream application of SNP markers. More than twenty different low to high throughput SNP detection technologies are currently available in the market. These technologies are primarily based on principles such as primer extension, allele-specific PCR, oligonucleotide ligation, mini-sequencing and some type of hybridization. While choosing a cost-effective, high throughput technology (capable of analyzing millions of samples) could be achieved with minimal effort, a clearly dominant SNP platform has not emerged for low to medium throughput needs. In order to identify an efficient and suitable platform in this category, we have evaluated the SNP technologies from Pyrosequencing (Biotage), Third Wave Technologies, Applied Biosystems and Promega using several corn SNPs. Some of these technologies could be easily upgraded to high throughput modes with minor changes in assay formats and incorporating multiplexing feature. The criteria used for the comparison of these technologies included the ease of assay design, assay preparation time, run time, data analysis and interpretation, throughput, cost and equipment needed. An overview of these technologies and the results obtained using corn SNPs against selected criteria will be presented.

P187

Transcriptional Expression Profiling During Maize Anther Development in Normal and Male Sterile Mutants

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This study proposes to identify transcript changes at the transition from somatic to germinal development in maize anther tissue using microarray hybridization expression profiling. Instead of microdissecting somatic and pre-germinal cells, we are using a "genetic ablation" strategy by obtaining transcriptional profiles of key developmental stages of selected meiotic male sterile (ms) mutants. For the initial analysis we are using: multiple archesporial cells1 (mac1), male sterile23 (ms23), and male sterile converted anther (msca1). For each ms, dissected stages of normal siblings (ms/+) are used for comparison. Because the ms were available in different inbred backgrounds, we are introgressing into W23 and conducting additional experiments that will delineate differences between the original background, BC1, BC2, and W23 in several anther stages, juvenile leaves, and pollen (findings are presented in a separate poster). A detailed study of anther development in W23 will incorporate both microarray hybridization and proteomics. By filtering out gene expression relating to background-related variables, we should pinpoint transcripts involved in the fundamental shift between somatic and germinal development. Lists of differentially expressed candidate and marker genes identified by expression profiles will be verified by Real Time RT-PCR. We are using a new 60-mer Agilent oligoarray that provides ~21,000 unique in situ synthesized probes drawn from the December 2003 PlantGDB maize EST assembly, including probes for ~400 meiosis-related genes designated by Lisa Harper and Zac Cande. Hybridization of a mixture of one Cy3 and one Cy5-labeled sample allows evaluation of gene-specific expression differences. The various statistical methods used to evaluate results will be presented.

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P188

Transcriptome characterization of apical and basal regions of maize root growth zone under water deficit condition

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Plant roots play a vital role in water and mineral acquisition, and are essential for plant growth and development. Under drought conditions, roots can adapt to continue growth while at the same time producing and sending early warning signals to shoots, which inhibit plant growth above ground. A high density maize oligo array was used to characterize the transcript profiles in the growth zone of well-watered and water-stressed maize primary roots to discover region-specific responses to water deficits. The expression profiles were compared to identify genes contributing to root growth maintenance and these responding to water deficits (region1). Also, we will identify genes responsible for progressive inhibition of root elongation under water-deficit condition (region 2), and compare the differential gene expression in the root region of progressive inhibition of root elongation under water stress (region 2) with the normal growth deceleration under well-watered root region (region 3). Maize seedlings were grown for 24hr and transplanted to high (-0.03 MPa) or low water potential (-1.6 MPa) conditions in vermiculite medium, and harvested at 48hr after transplanting. Labelled pairs of cDNA probes (Cy3 and Cy5) from four biological replicates of water stressed root segment 1 (3 mm) with well-watered root segment 1 were hybridized to 57K maize oligo arrays and scanned. The cross-slides variation was evaluated using GeneSpring and R script for CV filter. Spots intensity was normalized using R program and the expression data analyzed through the Mixed Linear Model. Differentially expressed genes identified by microarrays were validated by quantitative RT-PCR. Gene annotation and ontology analysis revealed some of these differentially displayed genes have been previously implicated in other plant species under water deficits, whereas others may reflect novel pathways or genetic content involved in maize root growth maintenance and root region-specific responses to water deficits.

P189

Use of high density maskless 70mer NimbleGen microarrays to determine transcriptional orientation of 9364 unique maize sequences

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The goal of the Maize Oligonucleotide Array Project is to develop and manufacture low cost long oligonucleotide microarrays for the maize community. A fixed build of maize ESTs was completed by TIGR at the outset of this project. There were 9346 contigs and singletons in this build for which transcriptional orientation could not be determined with confidence via bioinformatic analysis. The goal of this study was to evaluate the likely orientation of those sequences empirically using NimbleGen microarrays. The oligonucleotide design for each of the 9346 sequences was originated by Operon. These sequences were represented on the array by oligos in the sense and the antisense orientation. RNA from 5 different maize tissues (11 dap endosperm, 7 day-old seedling shoot, 7 day-old seedling root, Black Mexican Sweet suspension culture, and HiII callus) was used to interrogate the array. An ANOVA model was used to determine significance of gene expression as well as probable orientation. 30% of the elements had no significant expression in any of the tissues tested. Of the significantly expressed sequences, 22% were detected in only one orientation while 22% were observed only in the opposite direction for at least one tissue. In addition, 25% of the sequences showed significant expression for both orientations in one or more tissues in. 1% of the oligos showed differential orientation across tissues. This data will be presented in the context of approximately 44,000 assemblies of “known orientation”. This analysis was used to determine transcriptional orientation of the oligos that are present on the spotted arrays distributed by the Maize Oligonucleotide Array project.

P190

Young primary roots as a model for heterosis studies in maize

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Heterosis or hybrid vigor describes the phenomenon that crosses between two genetically different homozygous plants often result in more vigorous F1-plants. Heterosis in maize (Zea mays L.) was first described at the beginning of the 20th century and is manifested already in early plant development. The objective of this study is to test the suitability of the young primary root as a model for heterosis studies and the use of this model system for microarray analyses. Morphological studies of the German flint lines UH002 and UH005 and the dent lines UH250 and UH301 and their reciprocal hybrids 5 days after germination revealed that the primary roots of hybrids were significant longer and the root cortex cells are enlarged compared to the corresponding midparent values of the parental inbred lines. Primary root width of the hybrid plants was, however, not increased. In addition, hybrids displayed an increased lateral root density per cm of primary root compared to the inbred lines as detected by the Feulgen staining technique. Lateral root length is at the same time point not enlarged.
Transcriptome profiling of seedling roots 3.5 days after germination before differences between inbred and hybrid roots become manifested is under way. At this developmental stage root hairs which are a marker for cell differentiations have been formed, whereas lateral roots have not been initiated. Identification and characterization of genes that are differentially expressed in the parental inbred lines in comparison to the reciprocal hybrids will contribute to the understanding of the molecular basis of heterosis.

P191

An Update on Gramene QTL Data Module

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We continue to curate and develop the QTL data module within Gramene. The current release of the QTL database houses trait and map position information for more than 8015 QTL. This includes most published QTL from rice, pearl millet and foxtail millet and some other QTL from maize, oat and barley. We are developing a set of standardized trait terms that are currently classified into nine different trait categories and used to organize and cluster the QTL. These trait terms form the basis of Grameneís Trait Ontology. Besides the previous simple searching and trait category browsing options, a new "QTL Power Search" interface has been developed to help users do more comprehensive queries to the database. We will continue to curate QTL studies for all cereal crops in coordination with other species databases such as MaizeGDB and GrainGenes. In the future, we plan more comprehensive curation for existing QTL, including methods of trait evaluation, descriptions of the environment, QTL statistics, allelic interactions, germplasm and population information, as well as the development of a repository for raw segregation data and phenotypic scores underlying QTL studies. Users are encouraged to participate in the improvement of the phenotype search through suggestions for search utilities and visual displays, as well as direct data submission and curation.

P192

Association Between Molecular Markers Diversity and Phenotypic Diversity in Barley Species

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Conservation of crops and wild relativesí diversity requires an understanding of the way genetic diversity is maintained in their populations, and the way it evolved favoring different characters and adapting to different environments. Genetic Diversity can be measured by different criteria, including molecular markers and phenotypes. However, molecular markers appear to be poor indicators of heritable variation in adaptive traits. The objective of the research is to understand how diversity is partitioned in populations of wild and cultivated species; and to understand the association between molecular markers diversity and phenotypic diversity. The species used in this study were Barley (Hordeum vulgare), Wild Barley (H. spontaneum), and Foxtail Barley (H. jubatum). Twenty breeding lines of each of 20 Barley Breeding Programs in North and South America, Europe, and Australia were included. Twenty natural populations of Wild Barley collected from the Middle East and twenty natural populations of Foxtail Barley collected from the Midwest USA were included. Molecular markers diversity was measured with Simple Sequence Repeats (SSR) in each species. A set of 632 SSR markers was screened, and 80 were selected based on polymorphisms and genome coverage. The phenotypic diversity will be measured at standard morphological traits in the field during the seasons 2004 and 2005. The data will be analyzed with classical population genetics and quantitative analysis. Among population differentiation at molecular markers (FST) and at quantitative traits (QST) will be measured. The comparison of differentiation of the population at molecular markers and at quantitative traits allows testing if the trait has undergone selection, and the type of selection acting (divergent or stabilizing). Association between the molecular markers and the phenotypes will be measured by a comparison of QST with FST, and with an Association Mapping study in Barley. Results from the molecular analysis of Barley will be presented.

P193

Comparison of Phenotype and Genotype-based Selection Procedures for Multiple Disease Resistance in Maize

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Marker-assisted selection (MAS) has been advocated as a superior method for selection of traits with low heritability or high screening costs. Theory suggests that marker-based selection of major gene resistance in the absence of the pathogen will be effective. Improved levels of selection efficiency have been demonstrated in Phaseolus beans and rice using markers linked to major genes. However, less obvious is the utility of molecular-markers linked to quantitative trait loci (QTL) controlling resistance in more complex partial-resistance systems or for pyramiding disease resistance factors. Successful pyramiding of resistance requires merging good trait data and genetic map into an adapted genetic background. Our objective is to examine the effectiveness of combining candidate QTL to three maize pathogens using phenotype and marker-based selection procedures. This study elected to combine resistance for northern leaf blight (Exserohilum turcicum), gray leaf spot (Cercospora zeae maydis) and maize streak virus that are destructive maize (Zea mays L.) diseases. The basis of resistance to the diseases has been reported and a number of QTL associated with resistance have been identified. The consensus QTL across populations on chromosomes 3 (3.06), 5 (5.04), and 8 (8.06) for northern leaf blight, 1(1.04) for maize streak and 4 (4.08) and 2 (2.09) for gray leaf spot were used as candidate QTL for MAS. Partially inbred families were evaluated for resistance and rated on plot basis. Both phenotypic and marker-based selections were made for resistance for each disease at 1.0 standard deviation units from the mean for further evaluation with randomly selected families representing non-selection. Our results todate validate the effect and location of QTL controlling resistance to each disease. These results will demonstrate whether or not SSR based selection techniques being adopted in developing countries provide effective and economical opportunities for breeders confronting multiple diseases in their target environments.

P194

DNA Markers for Maize Genetics, Breeding, and Seed Production

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Great economic significance of maize and its some biological features stimulate high intensity of genetics and breeding researches. Important aspect of maize breeding is the characteristic of the initial genetic material used for new forms creation. The differentiation and identification of maize lines and hybrids and its genetic relation definition is important for maize improvement process. Genotypes differentiation and testing for their distinctiveness between themselves make several critical demands (requirements) upon the used marker systems, statistical receptions and technological procedures: independence of marker expression of environmental effects, high discrimination potential, stable reproducibility.
Last time various methods of DNA-profiling on basis PCR began to apply for genotypes characterization. They are more effective than traditional morphological comparisons and methods with use of protein systems. DNA-profiling data with the pedigree information and knowledge about key polygenic traits of agronomical significance may provide the most objective scientific and practical base for genotypes description and registration.
PCR-methods selecting depends on breeding and seed production problems. We used various PCR-methods for investigations of different genome regions for the decision of following tasks: (1) genetics variability estimation of maize genotypes by RAPD-, AP-PCR- and ISSR-PCR-techniques; (2) definition of inbreds and hybrids genetic uniformity (purity) by SSRP-analysis; (3) testing of simple hybrids paternity (hybrid genotyping) by SSR-PCR; (4) registration of genotypes as formulas that show allelic structure of SSR-loci for maize genotypes cataloguezation; (5) reconstruction of historical events for likelihood and pedigree definitions; (6) prognosis of heterotic level in simple hybrids and parental pairs choosing for high yield hybrid creation.
The most important problem of breeding is variety creation with biotic and abiotic environment factors resistance in particular with resistance to fungus disease. A stalk and ear rot caused Fusarium is widely widespread maize disease: the infected maize is lower yielding and contains toxins that dangerous to animal and human health. We identified some genome regions controlling the resistance to Fusarium infections for PCR-markers system creation of resistance genotypes screening.

P195

Detecting epistasis with double-introgression near-isogenic lines of maize

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Detecting epistatic interactions in typical mapping populations is confounded by segregation of the genetic background. This problem can be reduced through the use of near-isogenic lines (NILs). Objectives of this study were to test for evidence of epistatic interactions, their frequencies, and magnitudes in both inbred and hybrid combinations of maize. Using marker-assisted selection, 127 double introgression near-isogenic lines (dNILs) were developing using B73 as the recurrent parent and two introgressions from the donor parent Tx303. Epistasis was detected by comparing dNILs to their specific parental single-introgression NILs. After the first year of testing, 54 dNIL inbred lines (43%) exhibited epistasis for all six agronomic traits measured. Epistasis for only one trait was detected in 32 lines (25%), and epistasis for two traits simultaneously was detected in 17 lines (14%). In the hybrid trials, 37 dNILs (29%) exhibited epistasis for eight of the ten agronomic traits measured. Twenty-five lines (20%) exhibited epistasis for one trait while 10 lines (8%) exhibited epistasis for two traits simultaneously. Only 5 epistatic genetic combinations were in common between the inbred and hybrid lines.

P196

Diversity-based approaches to exploring genetic phenomena and quantitative traits in maize

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Twenty-seven maize inbred lines representing a substantial portion of known maize genetic diversity were crossed using a half-diallel mating scheme to form 351 maize hybrids. The diversity-based approach, in concert with the half-diallel design, provides germplasm for populations that lend to the evaluation of multiple alleles at each locus during quantitative trait locus discovery, and provides an excellent scheme for the study of epistasis and other genetic phenomena. A large amount of molecular genetic information is already known about the inbred parents of the population. Three hundred forty nine of the 351 members of the half-diallel hybrid population were grown in the winter maize nursery for preliminary phenotypic evaluation and to begin the derivation of recombinant inbred line populations representing each cross of the half-diallel. Observations indicate substantial phenotypic variation is present for a large number of traits within the population. Pilot analyses based on these observations are presented. The half-diallel hybrid population will be planted in replicated yield trials in Missouri and at least two locations in North Carolina during the summer of 2005 for evaluation and the collection of phenotypic data. It is hoped that materials from this project will develop into tools for genetics research within the maize community. Those interested in collecting phenotypic data in the hybrid material or future generations should contact Steve Szalma. Seed from selected F1, F2, and subsequent generations will also be made publicly available as resources allow.

P197

Epicuticular wax components affect Lepidopteran-feeding behavior

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Fall armyworm (FAW) causes significant yield loss in maize through the destruction of the whorl-stage leaf. Several quantitative trait loci (QTL) for larval-feeding damage during the whorl-stage have been identified. One of these co-localized to a QTL for juvenile leaf number and corresponds to the location of the Gl15 gene. Gl15 is an apetala-2 type transcription factor that controls the juvenile-to-adult phase change in maize leaves. Statistical comparison of the high and low larval damage tails of the mapping population indicates they are significantly different for number of juvenile leaves; resistant lines having fewer juvenile leaves. The goals of this study are to validate the effect of Gl15 in reducing larval-feeding damage and to investigate the role of structural genes in the Gl15-mediated pathway on larval-feeding damage. Eleven glossy mutants were examined in a leaf-feeding preference test with neonate FAW larvae; Gl1, Gl3, Gl7, Gl8, Gl11, Gl13, Gl14, Gl15, Gl18, Gl19, and Gl22. The mutants were tested relative to Mp705 a line with low whorl-stage larval-feeding damage and Va35, a line with high whorl-stage feeding-damage. Leaf damage was measured digitally using AlphaEase software as a percentage of the total leaf area. Gl15 displayed reduced FAW feeding damage as compared to both Mp705 and Oh28 confirming that it is a resistance factor. Gl8 had reduced FAW damage compared to the susceptible control but was not significantly different from the resistant control indicating that it may confer partial resistance to feeding damage. Gl7, Gl14, and Gl19 showed increased FAW damage as compared to both Mp705 and Oh28 indicating they may confer susceptibility to larval damage. The data indicate that consistituents of the epicuticular wax influence larval feeding damage. Assessment of a series of naturally-occuring alleles of these genes would allow for development of markers in maize improvement.

P198

Expression of CBF3 under the stress inducible promoter Rd29A using split-seed explant to enhance drought and cold tolerance in maize

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Efficient, genotype-independent tissue culture systems are the ideal targets for genetic engineering efforts in any crop species. We demonstrate here an efficient regeneration system based on a novel explant, split-seed. By splitting the seed longitudinally, three different sources of undifferentiated cells namely: Scutellum, Coleoptilar ring and Shoot apical meristems, can be simultaneously targeted to enhance the regeneration and or increase the ability of DNA transfer. Split-seed explants were used to initiate callus on induction medium supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2, 4-D), or cultured on multiple shoot induction medium supplemented with various concentrations of 6-benzylaminopurine (BAP) and 6-furfurylaminopurine (Kinetin). Callus induction frequency exceeded 92% and adventitious shoots formation frequency was 76% with a range between 2-26 shoots per explant within a period of 2-3 weeks. We further wanted to explore split-seed explant for the genetic transformation of maize. The C-repeat/dehydration-responsive element binding (CBF3/DREB1A) transcription factor under the stress-inducible promoter Rd29A was transformed into split-seed explants via biolistics transformation. Previous reports have shown that the expression of CBF transcriptional factors family enhances drought and cold tolerance in various plant species (A. Pellegrineschi et al 2004, Glimour SJ et al. 2000, Kasuga M et al. 1999, Sang-Choon Lee et al. 2004, Stockinger EJ et al. 1997 and Yamaguchi-Shiozaki K et al. 1994). Since moisture stress is one of the major factors affecting maize crop productivity, our main objectives are to incorporate the split-seed regeneration system with genetic transformation to study the effects of CBF3 expression in maize and to enhance drought and cold tolerance in maize. We identified 18 putative transgenic plants by PCR. For the first time, we report a robust and efficient regeneration protocol coupled with a high frequency transformation system in maize using split-seed explant.

P199

Genetic Diversity and Selection for Amino Acid Genes and Content in Diverse Maize

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The objective of crop improvement is to enhance a trait phenotype through breeding and/or biotechnology. Allelic variation in gene(s) underlying the trait is critical for crop improvement. However, a priori knowledge of the genes underlying the trait and degree of allelic variation is usually limited. A key factor impacting the degree of allelic variation is whether the gene has been the target of selection, either during domestication or crop improvement. If a gene has been the target of selection, there may be limited genetic variability, even within diverse germplasm. In a prior study comparing genetic diversity in maize inbreds and teosinte accessions for ~1800 randomly selected genes, we observed that a number of selected genes were in amino acid biosynthetic pathways. We analyzed the amino acid composition of seeds from seven teosinte accessions and 27 maize inbred lines. While there were limited differences among the maize inbreds, there were significant differences between teosinte and maize for several amino acids. We have sequenced a large number of candidate genes in amino acid biosynthetic pathways to identify targets of selection. We have examined the sequence diversity and will discuss the relationship between diversity, selection, and amino acid content in our set of diverse maize and teosinte.

P200

Genetic Mapping and Analysis of QTL Affecting Tassel Branch Number in Maize

(submitted by Michael Lee <mlee@iastate.edu>)

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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, designated LE-37 (long ear) and SE-40 (short ear), have approximately 2 and 11 tassel branches, respectively. F2 plants were genotyped at 179 simple sequence repeat (SSR) and restriction fragment length polymorphism (RFLP) loci. Tassel branch number data were collected on F2 plants at Ames, Iowa in 2000, and on their F2:3 lines in replicated trials at four Iowa locations in 2001. Quantitative trait loci (QTL) were mapped in both F2 and F2:3 populations, using composite interval mapping (CIM). Four QTL explaining 23% (R2 = 0. 23) of the phenotypic variance among F2 plants were detected on chromosomes 1, 2, 3, and 5. Eleven QTL on chromosomes 1, 2 (2 QTL), 3 (2 QTL), 4, 5 (3 QTL), 6, and 7 accounted for 67% (R2 = 0.67) of the phenotypic variance, and 72% of the genetic variance among F2:3 lines across four locations in Iowa. Two QTL near bnlg1297 on chromosome 2 and phi330507 on chromosome 5 were consistently detected in both F2 and F2:3 generations. QTL x environment interactions were significant (P

P201

Genetic Variation for Maize Seedling Root Growth under Water-Deficit Stress

(submitted by Tina Wambach <twambach@uoguelph.ca>)

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Genotypes often differ in their responses to environmental change, resulting in a change in their relative rank when grown in different environments. We have investigated the magnitude of GxE interaction for a critical trait in the plant response to water-deficit stress, namely the maintenance of root growth, using a locally adapted population of inbred lines. Employing a hydroponic system to characterize primary root growth rates of seedlings under several water-deficit stress treatments, we found that the rank of genotype growth rates changes across conditions and that genotypes vary most for recovery from water-deficit stress rather than during water-deficit stress. A genomic survey of 122 loci within the lines identified two loci on the top of chromosome 10 that strongly correlate with growth rate during stress recovery. We are initiating global gene expression profiling experiments and introgression of the top of ch10 into a uniform genetic background to characterize the genes and loci that may explain the observed GxE interaction.

P202

Genetic diversity in corn inbreds and its relationship to heterosis in single cross hybrids using RAPD markers

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Random amplified polymorphic DNA (RAPD), a PCR-based technique was used to study the genetic diversity of 24 corn inbreds and to invesigate the relationship of genetic distance with heterosis in their 20 single cross hybrids. Seventeen 10-mer primers were used giving a total of 91 reproducible products, out of which 68 were polymorphic. Genetic distance among pairs of inbred lines was estimated using Jaccard's similarity coefficient and it ranged from 0.19 to 0.32 with an average of 0.25. A dendrogram was constucted using the unweighted pair group method with airthmetical averages (UPGMA). Cluster analysis divided the 24 inbreds into two main groups of 23 inbreds (having six sub groups) and one inbred. The genetic distances (GD) were correlated with heterosis in single cross hybrids for important agronomic traits including grain yield. No linear correlation was observed between the two. Like previous studies, our results sugggested that RAPDs may be used to investigate relationship among corn inbred lines but they are of limited usefulness for predicting the heterotic performance of single cross hybrids.

P203

Genetic variation for the phenotypic effects of ploidy change

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Changes in ploidy level have a wide variety of effects on organisms, ranging from altered morphology such as increases in seed size to various levels of sterility. In maize, individuals with ploidies ranging from haploid (1x) to octaploid (8x) have been generated previously. The rationale for this study was to determine the relationship of genetic background and ploidy on morphological characteristics by producing ploidy series from several diverse inbred lines. We examined four lines to determine the extent of variation for the types of morphological changes that are associated with ploidy changes. Haploids and tetraploids were generated in our laboratory from the diploid inbred lines A188, B73, Oh43 and W22. The haploids were produced by crossing the four lines by haploid inducer stocks. The tetraploids were generated by nitrous oxide gas treatment of newly formed zygotes. The tetraploids were identified by subsequent screening of the resulting seedlings via root tip chromosome counts. The tetraploids identified were self pollinated for increase and these progeny were used in the comparison. Using these materials, we investigated the nature of morphological changes associated with alterations in ploidy at the 1x, 2x, and 4x levels using a randomized field study. Ploidy level and genetic background have strong effects on plant morphology. ANOVA revealed a significant interaction between these two factors, indicating that genetic background can modify the response to ploidy change. However, some characteristics, such as adult height, are reduced in both haploid and tetraploid derivatives of all inbred lines examined.

P204

Identification of QTL Responsible for Resistance to Fusarium Ear Rot and Fumonisin Contamination in Maize (Zea mays)

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Fusarium verticillioides and F. proliferatum are fungal pathogens of maize that cause ear rot and contaminate the grain with fumonisins, a family of mycotoxins that adversely affect animal and human health. Maize inbred lines GE440 and NC300 were identified in preliminary studies as potential sources for resistance to fumonisin contamination and Fusarium ear and kernel rot. Two mapping populations, GE440 ◊ FR1064 and NC300 ◊ B104, were used to identify loci associated with ear rot and fumonisin contamination resistance. Our research is focused on answering two key questions: (1) Do some QTL for ear rot resistance also confer resistance to fumonisin contamination?, and (2) Are QTL for these traits consistent across populations? In 2002, the GE440 ◊ FR1064 population was grown at Mt. Olive, NC and Haubstadt, IL, and the NC300 ◊ B104 population was grown at Clayton, NC. In 2003, both the GE440 ◊ FR1064 population and the NC300 ◊ B104 population were grown at Plymouth, NC and Clayton, NC. Populations were replicated twice both years at all locations. Primary ears were inoculated with a mixture of three isolates each of F. verticillioides and F. proliferatum. Inoculated ears were rated for the percentage of kernels rotted. The grain was then ground, bulked by plot, and evaluated for fumonisin concentration using ELISA. SSR markers were used to fingerprint both populations. The genetic map was constructed with Mapmaker/Exp version 3.0. QTL analysis was performed using SAS and Windows QTL Cartographer version 2.0.

P205

Identification of Quantitative Trait Loci Important for Maize Aluminum Tolerance Using the Intermated B73 x Mo17 Population

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Aluminum (Al) toxicity is a global problem limiting agricultural yields on acid soils. Al tolerance in maize is genetically complex. Al-activated root tip citrate exudation is a well-characterized Al tolerance mechanism in maize, but does not completely explain the observed range of tolerance when a number of maize genotypes are compared. In this study, a quantitative genetic analysis of Al tolerance in the Intermated B73 x Mo17 (IBM) population revealed five genomic regions important for Al tolerance. Three of these QTL combine to explain 42% of the variation in Al tolerance as determined by the net seminal root length (NSRL) phenotypic index. Mo17 contributes the superior allele at three of the five genomic locations, while B73 contributes the superior allele at the other two. Because Mo17 has much higher rates of Al-induced citrate release, the B73 QTL may represent genes conferring Al tolerance through novel mechanisms not associated with Al-activated organic acid exudation.

P206

Identification of maize grain yield QTLs using an identical-by-descent limited recombinant inbred line population

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A novel mapping population structure (identity-by-descent (IBD) limited recombinant inbred lines) has previously been proposed for quantitative trait loci mapping, which involves reducing the amount of genome surveyed therefore permitting smaller population sizes and fewer molecular marker screened. The primary objectives of this study are: to use the IBD limited recombinant inbred lines (RILs) in testcross condition to identify QTLs for corn grain yield, and to explain these QTLs in terms of its underlying physiological components (such as photosynthesis, leaf area index, general stress tolerance). A population of 128 IBD limited RILs were developed from a sister line cross between CG60 and CG108 (both Iodent) and each were crossed to CG102 (Stiff stalk). Molecular marker data indicates that the two sister lines CG60 and CG108 are 69.6% identical by descent, while significantly differing in grain yield. The non-IBD regions were present as small linkage blocks. Although only 30% of the genome was segregating between the parents, the RILs exhibited a wide range of phenotypic variation for grain yield, from 94.1 to 141.4 bu/ac, with evidence of negative epistatsis and no RIL yielding as much as higher yielding parent CG108.

P207

Identifying a natural suppressor of cell death in maize: implications for gene discovery, diversity evaluation and beyond

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Penetrance and expressivity of Mendelian mutants is often influenced by the genome in which they reside. In maize, this is most evident with disease lesion mimic mutants (les) which spontaneously form symptoms resembling infectious encounters. For instance, the same les mutation may have a lethal phenotype in one background but a largely benign one in another. We took advantage of this exquisite sensitivity of les mutants to genomic background to genetically delineate this ever-present variable. An F2 population was developed between les23 (a recessive mimic) and Mo20W, an inbred known to suppress lesions associated with many les mutations. About 575 les23 mutants from this population were evaluated for overall symptom severity as well as for days (after planting) when lesions first appeared on them. They were genotyped using 103 SSR markers and a QTL mapping approach was used to establish associations between the genotype and the phenotype. This analysis resulted in the identification of a major suppressor of cell death (Slm1) that accounted for 70-90% of the variation present in the population. A few minor QTL were also identified that together with Slm1 completely suppress cell death associated with les23 lesions. Implications that this study has for gene discovery and for identifying useful variation in diverse germplasm will be discussed.

P208

Inheritance of panicle architecture in sorghum

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The sorghum panicle provides a parallel to the maize ear as a model of morphological change in response to human selection. Races of domesticated sorghum show striking variation in panicle size, shape, and branching. Since sorghum and maize diverged only ~12 MYA and the genetic basis of panicle variation in sorghum is little understood, we are focusing our initial efforts on the co-localization of sorghum panicle QTL and maize inflorescence mutants.

To study the inheritance of panicle architecture in sorghum, a set of 125 RILs was measured in three environments for 11 panicle traits, including branch number, density, and length. QTL analysis was conducted using genotype data from the high-density molecular map previously constructed for these RILs. Relatively few QTL were significant across all environments. Candidate genes from maize and other grass species were mapped in sorghum, and the results of their co-localization with panicle QTL will be presented. We also observed co-localization of sorghum and maize QTL in regions where no obvious candidates exist. Scanning-electron microscopy of sorghum panicles is being undertaken in order to associate the observed phenotypic variation with specific developmental stages and refine the candidate gene search.

P209

Inhibition of Pathogenic E. coli O157:H7: Analysis of Transgenic Maize Expressing Colicin E7

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Escherichia coli O157:H7 is a highly virulent pathogenic bacterium that naturally resides asymptomatically in the digestive tract of cattle. Manure contamination of foods and water has resulted in multiple food-borne outbreaks. Pre-slaughter control strategies include using probiotic E. coli strains capable of inhibiting pathogenic E. coli. Inhibition by E. coli strains is mediated by colicins, however success is dependent on the colicinogenic bacteria to colonize and produce the colicin in the gastrointestinal tract. This project takes a novel approach to circumvent these limitations by developing a transgenic maize line that produces colicin (E7) that could be used as feed. A construct was designed that was used for biolistic bombardment into HiII maize callus tissue. This construct included the entire colicin E7 (1763 bp) gene driven by a constitutively expressing promoter (CaMV35). Following transgene introduction, maize callus tissue was analyzed for transgene insertion (PCR) and copy number (Southern) to establish ideal candidates for plantlet development. Maize plants have been established and are currently being studied. Analysis includes detection and integration of the colicin E7 transgene as well as mRNA expression. Additional resources including polyclonal colicin E7 specific antibodies and an inhibitory activity assay have been developed. These resources will aided in detection of protein expression in maize plant and protein activity against the pathogenic E. coli strain.

P210

Mapping QTLs associated with Late-Season Cold Tolerance in Maize

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When exposed to low night temperatures (~4C) during the grain-filling period, leaf photosynthesis in maize is reduced (20-40%). To further our understanding of the genetics and physiology underlying this response, a QTL mapping project involving two related inbred lines, CG60 and CG105 (both Iodent), was undertaken. Since measuring photosynthesis reduction is complicated and time consuming we have developed a novel population structure for QTL mapping by exploiting the concept of Identical-by-descent (IBD). IBD estimates for these two inbred lines have been established using SSR markers; they share 71% of their genomes, thus limiting the amount of genome to scan for QTLs and reducing the size of the population required. Forty-seven recombinant inbred lines (RILs) were developed for the mapping population. They were evaluated in a field hydroponic system, consisting of 22-L pails filled with ëTurfaceí, supplied with nutrient solution. Treatments consisted of exposure to a 4∞C night and a field control at silking and 4 weeks post-silking. Leaf photosynthetic rate was evaluated during the day subsequent to the cold night. SSR markers are currently being used to saturate the non-IBD regions of the genome. Molecular markers combined with field measurements will be used to identify QTL associated with the response to low night temperatures during the grain-filling period in maize.

P211

Mapping When Phenotype Measurements Are Not Well Behaved: Comparison of Recursive Partitioning with Composite Interval QTL Mapping

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Standard methods for QTL analysis use a summary measure such as the mean of the trait measurement from each line. The phentoype measurements are assumed to be normally distributed. In practice, a good fit to a normal distribution is rare.
Recursive partitioning is a powerful algorithm that investigates the association among various predictor variables and a response (or multiple responses). Some advantages recursive partitioning offers over other conventional algorithms are 1) it does not assume the relationship between the response variable and the predictor (marker) variables is linear; 2) missing values are easily implemented into the analysis without the problem of imputing values; 3) it can handle data sets where there are many more predictors, p, than sample size, n; 4) associations among predictor variables are not an issue, in fact, recursive partitioning will uncover the associations and interactions between the various predictor variables; 5) in the case of QTL analysis, the number of QTLs need not be known apriori nor any assumptions made regarding interactions between QTLs.
We compare recursive partitioning and regression-based methods using simulations. We then compare analyses of data on Fusarium ear rot susceptibility in an BC1F2 and an RIL population, and ear fill in one RIL data set, using recursive partitioning, single-marker ANOVA, and composite interval mapping. We develop guidelines for use of recursive partitioning versus the current standard, composite interval mapping. Recursive partitioning is generally applicable for genome scanning; the method is most useful for situations in which 1) there is no map available (as in polyploid species or early in genotyping efforts), 2) the trait data have non-normal distributions and/or the trait data are poorly described by summary measures such as means, and 3) in mapping populations that have high marker density or have small numbers of lines such that the number of lines is less than the number of markers.

P212

Mapping of genes for antibiosis to green rice leafhopper, Nephotettix cincticeps Uhler, in exotic germplasm of rice

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Green rice leafhopper (GRH), Nephotettix cincticeps Uhler is a serious insect pest of rice in East Asia. The leafhopper causes either direct damage to the rice plant by sucking the sap or by transmitting several viral diseases. The genetic resources of wild relatives have many agronomically beneficial traits for breeding such as yield and resistance to diseases and insects. The wild relatives for resistance to insect pests have significantly higher genetic diversity than the cultivated rice. Three exotic accessions of rice, Oryza glaberrima Steud. (IRGC104038), Oryza nivara (IRGC105715) and Oryza rufipogon Griff. (IRGC104630) with GRH resistance were selected by antibiosis test. The BC1F1 populations obtained by backcrossing with a susceptible Japonica variety Taichung 65 (T65) as a recurrent parent were evaluated for resistance to GRH and were used for QTL analysis using simple sequence repeats (SSR) markers. A total of seven QTLs for antibiosis were detected on seven different loci in three cross combinations. The introgression lines derived from a cross between resistant accessions and T65 were used for mapping QTLs of resistance genes. Further SSR analysis using BC4F2 population of O. rufipogon revealed that a new resistance gene, Grh5 (Green rice leafhopper resistance 5), was located on the distal region of the long arm of chromosome 8. Similarly, SSR analysis revealed that a resistance gene (Grh6) was located on the short arm of chromosome 4 in O. nivara. The near isogenic lines (NIL) for Grh5/Grh5 and Grh6/Grh6 genotype were more than nymph mortality with 90%. The NILs carrying Grh5 and Grh6 which show higher resistance to GRH than resistant cultivars are new promising genetic resources in future breeding program in rice.

P213

Mappingof opaque 2 modifier genes in maize endosperm

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Opaque2 modifier (mo2) genes can convert the soft, starchy endosperm of an opaque 2 (o2) mutant to a hard vitreous phenotype. By systematically introgressing mo2 genes into o2 germplasm, plant breeders in South Africa and CIMMYT in Mexico were able to develop several hard endosperm o2 mutants that they designated “Quality Protein Maize,” or QPM. These new QPMs materials have the phenotype and yield potential of normal maize, but maintain the high lysine content of o2. The development and widespread use of QPM germplasm has been slow; in part, this is because of the technical complexity of introducing multiple mo2 loci, while maintaining a homozygous o2 locus and monitoring amino acid composition. Genetic mapping of modifier genes could accelerate their transfer to commercially valuable germplasm and would facilitate their isolation and molecular characterization. We used Bulk Segregant Analysis (BSA) in two different segregating F2 populations and mapped o2 modifier loci using SSR DNA markers. Several SSR markers in bin 7.02, which includes the gene encoding the 27-kD gamma zein storage protein, were tightly linked with the modified kernel phenotype. This result suggests the 27-kD gamma-zein is directly involved in the process of endosperm modification, or a modifier gene could be tightly linked to the genes responsible for 27-kD gamma-zein synthesis. A second locus was identified in bin 9.02 that is linked to o2 modification based on several SSR markers. Work is in progress to investigate the role of the 27-kD gamma-zein protein in o2 modification and the identification of candidate genes in bin 9.02.

P214

Novel Mapping Population Structure - Identity-by-Descent Limited Recombinant Inbred Lines

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Improvements in our ability to associate phenotypes with specific genomic regions [quantitative trait locus (QTL) mapping] have come through two avenues: increases in the number of molecular markers, and advances in statistical methods for mapping. In plants, the traditional approach has been to design mapping populations specifically for a trait by selecting parents that maximize the genetic variation for that trait. Unfortunately because the entire genome needs to be surveyed the populations tend to be quite large (>200 individuals), limiting the types of traits that can be examined (e.g., photosynthesis). An alternate approach is to minimize the amount of the genome that needs to be surveyed, while still exploiting the power of linkage and genetic variation. Materials developed for this purpose would enable researchers to phenotype considerably fewer individuals without loss of precision or power. By exploiting the concept of identical-by-descent (IBD) we have developed and identified a set of materials in which only ~30% of the genome needs to be surveyed for a QTL of interest. The starting genetic materials consisted of 3 hybrid families (Pioneer3902, Pioneer3929, Pioneer3790) of 6 inbred lines each. Identity-by-descent estimates for each inbred pair have been established using SSR markers and significant genetic variation for grain yield has been detected in 2 of the 3 inbred line families.

P215

QTL Mapping of Telomere Length-Regulating Factors

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

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Telomeres are specialized nucleoprotein complexes at the ends of linear chromosomes. They have essential functions in genome stability, meiotic chromosome behavior, and solving the end-replication problem. Quantitative Trait Locus (QTL) mapping is a powerful method for identifying loci that control traits with complex inheritance. This approach works well with traits can be precisely measured and exhibit a high degree of heritability. We have initiated a QTL mapping project to identify genes that regulate variation in telomere repeat length. We are QTL mapping within the IBM population (302 RILs, >2,000 markers) using Southern blot hybridization data to determine telomere length (TTTAGGG tandem repeat copy number) phenotypes. A detailed analysis of multiple DNA preparations from four randomly chosen RILs revealed that mean telomere length was stable and uniform across multiple developmental stages and tissues. Preliminary QTL analyses of 250 RILs resulted in the detection of at least eight genetically defined regions that may account for some of the heritable variation in telomere length. Optimization of DNA extraction and gel electrophoresis procedures should improve the precision of our input data and further strengthen our ability to detect significant QTL. QTL mapping in the IBM population together with the emergence of a genetically-anchored physical map should allow us to identify candidate genes for telomere length homeostasis in maize.

P216

QTL analysis of root traits under two different water regimes.

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Roots play a primary role in the uptake of water. Development of an intensive and extensive root system is considered an adaptive behavior thought to promote survival during water limited conditions. The goal of our experiment was to identify, and compare the QTL involved in root architecture under well-watered and water-stressed conditions. Three experiments were performed. In all experiments, each replication used the same subset of 94 mapping lines from the intermated Mo17 x B73 (IBM) mapping population. In the well-water experiment, five replications were grown for 14 days. Trait data was collected for root and shoot mass, number of leaves, overall branching, primary root length, and the number of primary branches. In the water-stressed experiment, water was withheld at 14 days and trait data was collected 10 days later on the same traits plus relative water content. A third experiment was performed where four reps of well-watered and water-stressed plant pairs were measured when the soil water content was less than, or equal to 10%. QTL analysis was performed with QTL Cartographer version 1.16, with a genetic map created on Mapmaker Exp version 3.0 for Unix, utilizing genotypic data for 643 markers evenly distributed throughout the maize genome. A total of 67 QTL were identified based on results of 100 permutations of the data set. QTL were found for all traits in all three experiments. The 20 well watered, 31 water stressed, and 16 response QTL were distributed across 35 bins located on all 10 chromosomes. Investigation into the roles of these QTL will provide further insight into the genetic regulation of roots and their role in plant water relations. This research was funded by NSF-DBI-0211842.

P217

QTL and phenotypic analysis implies a role for the fasciated ear2 gene in controlling ear seed row number.

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The cob structure of maize is unique among the grasses, and is derived from the distichous inflorescence of teosinte. Whereas teosinte has alternating rows of seeds, modern maize lines have 8 to 18 rows arranged in polystichous phyllotaxy, and this was clearly a major yield factor in the development of maize as a crop. Seed row number is dependent on the number of spikelet pair meristems (SPMs) and it is tempting to speculate that the number of SPMs is directly dependent on the size of the inflorescence meristem (IM), however, we do not have any direct evidence for this assumption. Support for the hypothesis came from analysis of the fasciated ear2 (fea2) gene, which controls IM-size. fea2 mutants develop strongly enlarged IMs, and approximately double the number of seed rows.
To address whether allelic variation in the fea2 has an impact on seed row number, we performed QTL analysis using the integrated B73-Mo17 (IBM) recombinant inbred population. Seed row number was counted in cobs from approximately 250 lines (5-10 cobs/ line) in four replicates.
The mean row number of B73 was approximately 16, and for Mo17 was 11. The mean row number in the IBM families was 14, with a range from 10 to 20. We performed QTL analysis using QTL Cartographer with a strict probability level corresponding to a LOD score of > 2.5.
One of the most significant QTLs is located on chromosome 4, and overlaps with fea2, suggesting that allelic variation in fea2 may regulate row number. Further support for a link between IM size and seed row number comes from measurements of IMs in developing ears from a range of maize inbreds, where we saw a positive correlation with row number. We discuss a possible role for fea2 in controlling ear row number and inflorescence morphology.

P218

QTL mapping of resistance to Ustilago maydis in sweet corn

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Quantitative trait loci (QTL) for resistance to Ustilago maydis (common smut) were mapped and characterized in sweet corn. A population of 330 F5:6 families was generated by crossing a susceptible to a resistant inbred. F5:6 families were genotyped with 130 markers (SSR and SNP). The lines were evaluated in field trials under natural infection in Stanton, MN; Columbia Basin, WA; and Nampa, ID in 2001-2003. Resistance to U. maydis was highly heritable (h2= 0.67). 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 genotype by environment interactions. Three QTL were detected in all locations. Based on these results, marker assisted selection appears to be a suitable strategy for improving the resistance of sweet corn to U. maydis.

P219

Quantitative Trait Loci Associated with Resistance to Fall Armyworm and Southwestern Corn Borer Leaf Feeding Damage

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Southwestern corn borer (Diatraea grandiosella Dyar) and fall armyworm [Spodoptera frugiperda (J.E. Smith)] damage ratings were measured on a F2-derived population segregating for leaf feeding resistance following artificial infestation with neonates. Damage ratings for each insect were recorded in replicated trials at three locations. Linkage maps were developed using simple sequence repeat markers. Quantitative trait loci (QTL) and their interactions were estimated using multiple interval mapping analysis. Resistance to southwestern corn borer leaf feeding was fit to a model containing eight QTL and two interactions explaining 20% of the phenotypic variation. A model containing seven QTL and one interaction best fit resistance to fall armyworm leaf feeding damage, and it explained approximately 14% of the phenotypic variation. Three QTL located on chromosomes 6, 9, and 10 affect leaf feeding damage ratings of both insects with similar effects and gene action. Minor interaction effects were observed. QTL on chromosomes 1, 5, and 9 correspond to previously identified resistant regions affecting resistance to southwestern corn borer. Insect resistance genes including the mir family of genes located on chromosome 6 and the glossy15 locus on chromosome 9 fall within chromosomal regions of QTL predicted in this study. Multiple interval mapping reinforced previous documentation of the importance of QTL on chromosomes 1, 5, and 9 that reduce leaf feeding damage by southwestern corn borer in multiple environments. This study confirms that resistance to fall armyworm and southwestern corn borer involves many of the same QTL including, most significantly, the glossy15 candidate locus on chromosome 9. QTL data from a closely related population is being compared to these results in order to observe similarities in different environments.

P220

Response of Maize Primary Root Growth to Varying Degrees of Water Stress.

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Drought is a world wide common concern. Drought conditions cause a decrease in plant productivity reducing plant size and potential yield. Researchers are now looking to genetic diversity to help find answers on how to deal with drought. Maintenance of root elongation is an important adaptive response to drought conditions. To investigate the genetic diversity in the ability for root growth maintenance at low water potentials, ten maize inbred lines were selected. B73, B97, F2, FR697, IABO78, LO964, LO1016, MO17, OS420, and Polj17 were chosen because of previous drought and abscisic acid (ABA) studies or as parents of mapping populations. Seedlings with a primary root length of 10 to 22 mm were transplanted into clear Plexiglas boxes filled with vermiculite. The vermiculite had a water potential of -0.03 (well watered), -0.3 (mild stress), or -1.6 (severe stress) MPa. They were allowed to grow for 24 hours in well-watered conditions or 72 hours in the stress conditions. Measurements of primary root length were taken at three evenly spaced time points during the course of the experiment and a growth rate was determined by taking the change in length over the change in time. A statistical analysis was conducted using SAS to determine the differences between lines at each water potential. At -0.3 MPa, FR697 and LO964 are the most tolerant and B73 and Polj17 are the most susceptible. At -1.6 MPa, FR697 and LO1016 are the most tolerant and MO17 and B97 are the most susceptible. A comparison study between two of the extreme lines i.e. FR697 and B97 is in progress to gain better understanding, genetically and physiologically, of how plants are able to handle the environmental pressure of drought. This research was funded by NSF-DBI-0211842.

P221

Significant association between CLE genes and inflorescence architecture in maize

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Genes of the CLE family (clavata3-like) are essential to restrict the expression domain of WUS-like regulatory genes that allow the maintenance of stem cells in the meristem. Such pathways are thought to be responsible for differences in organ shape. Unlike the sequences from the other genes involved in these pathways, CLE sequences are poorly conserved across species. We think that these genes or gene regions could play a major role in plant phenotypic adaptation.
One year ago, we started our quest for the Zea mays Clavata3 gene. A number of CLE genes have been identified during this process. We report significant associations between polymorphisms at these genes and ear and tassel phenotypic variation.

P222

Survey of Aflatoxin Levels among Diverse Maize Germplasm

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A. flavus is an anamorphic soil-borne fungus that produces aflatoxin. Aflatoxin accumulation in maize poses a risk to mammalian health as well as causing large economic losses to producers. Water stress, high temperature, and insect damage are favorable conditions for infection to occur. The time of harvest can affect the amount of aflatoxin accumulation present. If grain dries slowly in the field the moisture content remains high enough to support continued fungal growth, aflatoxin production, and sclerotia production. Insects may also continue to feed during this time and spread the fungus. Once grain is found to be infected with aflatoxin few detoxification options are available. The FDA regulates the allowable toxin level to be 20 parts per billion (ppb) in grain for human consumption and 0.5 ppb in milk products. The best strategy to prevent aflatoxin accumulation is to develop 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. Two replications of each line were grown during 2003 and 2004. 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 an anti-aflatoxin rabbitIgG antibody. A subset of 86 lines from the Maize Diversity Project was also examined for aflatoxin accumulation. With the completion of this germplasm survey we hope to identify highly resistant and susceptible lines and establish a ranking for other lines whose aflatoxin resistance level is unknown.

P223

Identification and Analysis of Maize QTLs 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 and quantitative trait loci (QTL) for resistance to many diseases have been identified. However, almost nothing 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 (GLS) and Southern Leaf Blight (SLB), we are identifying and mapping new sources of resistance from diverse germplasm. In addition, we are developing materials and methods for the detailed characterization and fine-mapping of selected QTLs. We have developed controlled environment screens for both GLS and SLB and have examined parameters that effect host resistance such as light intensity and plant leaf maturity. We have identified several novel QTL for both diseases at both the seedling and mature plant stages. Furthermore, we have identified and made preliminary analyses of Near Isogenic Lines (NILs) differing in QTL for both GLS and SLB resistance. These results will be presented and discussed.

P224

The Power of the Joint Linkage and Association Mapping in Plants

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Linkage analysis and association studies are two common tools in dissecting the complex trait. Joint linkage and association mapping approach has recently been shown to have the power to fine mapping the quantitative trait loci (QTL) in cattle and human. Our objective is to develop the joint linkage and association mapping approach for plants using maize as a model species. The single nucleotide polymorphism (SNP) haplotype data of 26 diverse maize inbred lines are used to initiate a computer simulation. Simulation follows closely the procedures undertaken by an ongoing project to create a large-scale maize mapping platform. Twenty-five recombinant inbred line (RIL) populations, each of which has 200 RILs, are being derived from 25 diverse inbred lines each crossed to a common inbred. Different statistical models are compared for their power to detect QTL, false discovery rate, and resolution.

P225

The effects of combining regions of chromosomes 3, 6 and 10 on resistance to Maize dwarf mosaic virus (MDMV) and Sugarcane mosaic virus (SCMV).

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Genes controlling resistance to diseases caused by viruses in the family Potyviridae have mapped to maize chromosomes three, six and ten. To study the effects of the individual genes, near isogenic lines (NIL) were made that carry specific chromosomal regions from the highly resistant inbred line Pa405 in an otherwise susceptible background (inbred line Oh28). NIL W1 carries the region of chromosome 6 (bin 6.01 near umc85) that has the Mdm1, Scm1 and Wsm1 genes for resistance to MDMV, SCMV and Wheat streak mosaic virus (WSMV), respectively. NIL W2 carries a region near the centromere of chromosome three (bin 3.05 near umc102) that has the Scm2 and Wsm2 genes for resistance to SCMV and WSMV, respectively. NIL W3 carries the Wsm3 gene for WSMV resistance on chromosome 10 (bin 10.05 near umc44). Dosage effects and gene interactions were studied using intercrosses of the NIL and crosses of the NIL to the susceptible recurrent parent. All three NIL were highly resistant to WSMV, as were NIL x Oh28 hybrids. Thus, Wsm1, Wsm2 and Wsm3 each acted as single dominant genes. In contrast, W1 showed incomplete resistance to MDMV and SCMV in that limited symptoms appeared 14 and 18 days after inoculation, respectively. W1 x Oh28 hybrids were susceptible to SCMV and MDMV, but symptom development was delayed and initially limited in MDMV-inoculated plants. Neither W2 nor W3 was resistant to MDMV or SCMV. However, in W1 x W2 hybrids, MDMV and SCMV resistance was enhanced, with a lower percentage of infected plants and delayed symptom appearance relative to W1 x Oh28. In addition, MDMV but not SCMV resistance was enhanced in W1 x W3 hybrids. These results indicated that MDMV and SCMV resistance genes on chromosome 6 were dependent on allele dosage, and were affected by the addition of minor genes.

P226

Using Diverse Maize Germplasm to Investigate NUE

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With supplemental nitrogen fertilizers being applied to nearly every acre of maize grown, environmental issues are raised and the agriculturalistís 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 benefit the environment as well by reducing excess nitrogen. We have initiated a program to integrate physiological and genomic approaches to improve NUE. One approach is to investigate variation in NUE and component traits in diverse maize germplasm, and then associate this phenotypic variation with allelic variation in candidate genes. A core set of 25 inbred lines, selected to represent much of the allelic variation present in maize, were crossed to B73 and the resulting hybrids were evaluated for NUE and component traits. Evaluations were performed over two years at different N rates in an N-response nursery. The average NUE and the hybrid ranges of NUE decreased with increases in the nitrogen supply. There is considerable genetic variation within these hybrids in regard to the desirable characteristics of NUE which are vital to a hybrid with an improved NUE. These characteristics include a high grain yield without fertilizer addition, a high NUE at low rates of supplemental N, and a large magnitude of yield increase with N. The phenotypic variation for these traits will be utilized for identifying genes controlling NUE.

P227

A PCR based strategy to clone genes tagged by somatic mutagenesis

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This strategy will allow us to clone genes of interesting phenotypes directly from sectored leaves due to transposon insertion. During transposon tagging experiments using mu or Ac, a large number of somatic sectored but relatively few germinal mutant plants are generated. We plan to harness this tendency to efficiently clone dominant genes causing spontaneous expression of lesions resembling disease that are too severe to propagate and clone in a homozygous state or causing wildtype sectors in an otherwise lesioned background. However, as somatic sectors these phenotypes are not transmitted to the next generation and are often too small for traditional methods to isolate the gene behind the mutation. This experiment will use several PCR based techniques to profile, by either gel analysis or an automatic gene sequencer, transposon flanking regions between somatic sectors and nearby leaf tissue to clone the gene disrupted by the transposition event leading to the sector phenotype. The results of each PCR technique will be compared. The recovered putative gene fragment will be sequenced and BLASTed through the many available gene databases to identify it. If no match is found, the same PCR based approach used to find the gene fragment can be utilized to walk along the putative gene to recover more of its sequence to aid in identification.

P228

A two-component Activator/Dissociation tagging system in Maize

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A two-component Ac/Ds tagging program has been developed for regional mutagenesis in maize. An immobilized Ac element (Ac::im) containing a 12 bp deletion at the 5' end of Ac has been identified and characterized. Through genetic analysis Ac::im has been shown to display the typical negative dosage effect of Ac but is completely incapable of transposing. Ac::im mediates excision of numerous Ds elements at loci throughout the maize genome including r1, bz1, a1 and a2 and catalyzes germinal transposition of the Ds element present in r1-sc:m3 at an average frequency of 4.4%. Because Ac::im has been maintained in the W22 inbred it is an ideal source of transposase to mobilize Ds elements that have been converged into the W22 inbred. To this end, we have initiated the introgression of 17 previously characterized Ds insertions present at 11 loci that condition seed or plant mutant phenotypes into a W22 line. We have also developed a molecular screen to identify additional Ds insertions as deletion derivatives of physically and genetically mapped Ac insertions and have calculated the frequency of these events. These studies provide an essential foundation for developing a non-transgenic Ds gene-tagging platform in maize for use as a community resource.

P229

Amplification of mPing and Ping Transposable Elements in the rice cultivar Gimbozu.

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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, miniature inverted-repeat transposable elements (MITEs) are thought to be particularly important in the evolution of plant genomes because of their very high copy number (frequently >1,000 copies) and location preference in or near genes. MITEs are non-autonomous DNA transposons that can only transpose when provided with transposase from autonomous elements elsewhere in the genome.
The first active MITE called mPing, was independently isolated from rice by 3 groups. In this study we found that the copy number of mPing has increased from a single copy in tropical indica varieties to over 1,000 copies in the temperate japonica strain Gimbozu. Because this rapid amplification has occurred very recently and because mPing has been shown to insert preferentially into genes, we are interested in the new mPing insertion sites in the Gimbozu genome. We have characterized 327 mPing insertions and have found that, as with other MITEs, the newly amplified mPing elements insert frequently into genic regions. Several examples of mPing insertions in expressed genes are presented.
Transposase for mPing is thought to be provided by either Ping, from which mPing is derived by simple deletion, or by Pong, a closely related full-length element. To determine how Ping and/or Pong is involved in mPing mobilization, we assessed the copy number of Ping and Pong elements in the strains that show extreme variation in mPing number. We find a positive correlation of copy number between mPing and Ping, but not Pong, suggesting an important role for Ping in this family. This finding is surprising in light of the fact that mPing elements can amplify in indica cell culture lines which have no Ping elements.

P230

Centromeric retrotransposons: potential role of the integrase C-terminus in determining genomic distribution

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The plant CR retrotransposons are found only in plant centromeric heterochromatin. This distribution may be caused by targeted integration of CR retrotransposons into centromeric regions or by selection against retrotransposons from insertion into other chromosomal sites. A conserved domain was identified in the C-termini of CR retrotransposon integrases. We cloned this conserved domain and fused it to YFP. In vivo localization experiments showed a specific subnuclear distribution of YFP foci. The YFP foci were coincident with the localization of the heterochromatin-specific protein LHP1, which was fused to CFP. Our data suggest that the C-termini of CR retrotransposon integrases may recognize specific components of centromeric heterochromatin. We hypothesize that this interaction directs integration to the centromeres and results in the observed genomic distribution of these elements.

P231

Characterization of Maize Mre11 Genes

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Despite a dynamic genome occupied largely by active transposable elements, maize seems to cope extremely well with DNA damage, making it an interesting system in which to study DNA repair. Mre11 is a multi-functional protein and a major component of the two main DNA double strand break repair mechanisms in eukaryotes: Homologous Recombination and Non-Homologous End Joining. Mre11 null mutants are embryo lethal in mammals but not in plants, although in Arabidopsis they are associated with severe growth defects, sterility and chromosomal instability and fragmentation. We are interested in how Mre11 might contribute to maintaining the integrity of the maize genome. Maize has two Mre11 genes, Mre11A and Mre11B. We have found that the Mre11A gene is alternatively spliced and that one of the splice variants translates into a full length Mre11 protein similar to that in Arabidopsis, yeast and humans, while the second one is missing 177 amino acids of its C terminus. The shorter protein lacks a presumptive DNA binding domain. Mre11B, located in the 19kD zein cluster, is ~60% identical to mre11A at the protein level. We have now shown that Mre11A is expressed constitutively, while Mre11B is expressed in tassel, ear and root tissue but not in leaves. These observations raise the immediate questions of why Mre11B is absent in leaves and whether its expression pattern is related to the alternative splicing of Mre11A. In addition, we are examining what functional similarities and differences Mre11B might have with Mre11A, whether or not the two proteins physically interact and what the role of the truncated Mre11A protein is.

P232

Establishing enhancer detection and activation tagging as strategies to reveal gene function in maize.

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We are using the Ac/Ds and En/I transposon families to implement different strategies for activation tagging and enhancer detection in maize. The En/I-based activation tagging system includes a genetic construct that with three elements: an inmobile source of transposase under the control of the CaMV35S promoter, a mobile non-autonomous element comprising a tetramer of this same promoter, and 2 selectable markers to identify stable unlinked transpositionsó the bar gene conferring resistance to the herbicide basta and Su1 that converts the pro-herbicide R7402 into sulfonylurea, inhibiting plant growth. Our enhancer detection strategy is based on Ds-containing pGS1 plasmid, a construction designed by the group of Hugo Dooner that includes the bronze (Bz) gene interrupted by the transposon itself and C1 located inside the sequence of the transposon under the control of the CaMV35S promoter. The reporter gene uidA was inserted adjacent to a minimal CaMV35S promoter. We have tested a tissue culture regeneration protocol in three experimental genotypes (OíConnor-Sanchez et al. 2002 Plant Cell Rep 21: 302-312), and performed several transformation experiments through biolistics and Agrobacterium tumefaciens using embryogenic-organogenic callus derived from shoot tips of in vitro germinated seedlings. A first group of transposant lines has been generated and is currently under analysis to determine single stable insertions. The progeny of self pollinated plants will be genetically characterized through PCR and segregation analysis. The overall objective is to generate transposant plant populations useful for the scientific community and study genes involved in reproductive development.

P233

Heterosis and Combining Ability of Selected East African Maize (Zea mays L.) Populatins

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A diallele cross was made among seven East African maize populations and the resulting 21 crosses (excluding the reciprocals) and seven parents were evaluated in a randomized complete block design at two locations. The study was undertaken (1) to determine heterosis and combining ability of East African maize populations and (2) to select populations that could be used as suitable parents in a hybrid breeding program. Data were recorded on days to 50% silking, ear height, plant height and grain yield. All plots were hand harvested and shelled. The weight of shelled grain and moisture content of the grain were determined at harvest. Plot yield was adjusted at 12.5% moisture. The level of high parent yield heterosis manifested by the F1 progenies were used to determine genetic diversity among any two populations. Griffings (1956) Method 2 and Model 1 (fixed model) were used to estimate general and specific combining ability effects for each parent and their crosses, respectively. The study indicated wide genetic diversity among some of the populations as indicated by high level of yield heterosis over the high-parent. Kitale Composite B (KCB) & Abo-Bako, Ukuruguru Composite A (UCA) & Abo-Bako, and UCA & KCB were found to be the most genetically diverse populations showing high-parent yield heterosis of 55.3, 41.3 and 36.0 %, respectively. General combing ability (GCA) variance was found to be higher than specific combing ability (SCA) variance indicating the importance of additive genes in controlling all traits. KCB X Abo-bako and Bako Composite X A-511 showed significant and negative SCA effects for days to 50% silking, ear and plant height, and significant and positive SCA effects for grain yield and found to be ideal combinations for reciprocal recurrent selection and inter-population hybrid development program.

P234

Identification of Candidate Genes in Six Endosperm Mutants in Maize

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Identifying genes controlling development of the maize kernel is essential to both crop improvement (grain composition and nutritional quality) and the understanding of the mechanisms underlying seed development. Of the estimated 300 to 600 genes that have essential, non-redundant functions in maize kernel development (Neuffer & Sheridan, 1980), only a very small subset are cloned and analyzed. The uniformMu endosperm functional genomics project has generated more than 2000 independent seed mutants that are tagged by Robertsonís Mutator. This provides an excellent resource for identifying seed development genes. Using a combination of MuTAIL and co-segregation analysis, we have analyzed 16 endosperm mutants in a pilot experiment. This initial analysis has identified co-segregating elements in six mutants (emb-um1, emb-um2, emb-um3, smk-um2, smk-um3, and smk-um5). The candidate insertions are shown to be tightly linked to the mutant phenotypes in F2 populations ranging from 20 to 84 individuals. Among the six mutants, three are linked to Mu1; the others are linked to a Mu3, Mu8, and MuDR, respectively. MuTAIL sequence analysis of emb-um1 linked insertion indicated a mutation in a gene with strong similarity to prokaryotic translation initiation factor 3, thus designated as ZmIF3. ZmIF3 is recovered from maize endosperm cDNA libraries. The emb-um1 mutant has a defective embryo with a morphologically normal endosperm. If confirmed, this mutant suggests that ZmIF3 is essential for embryo formation, but not for endosperm development. We are using the 2000+ seed mutant collection to construct a mutant enriched reverse genetics tool for identifying confirming alleles for these and future cloned mutants.

P235

Inheritance of Husk Leaves in Maize

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Husk leaves extend from the ear husks of maize. They are typical of temperate sweet corn, where they facilitate machine-handling and removal of husks prior to canning. Most field corns lack or have very reduced husk leaves, and husk leaves are rare in tropical field or sweet corns. The inheritance of husk leaves was studied by generation mean analysis of progenes derived from crosses of the inbreds Hi38c1 and Ia453sh2. The Inbred Hi38c1 is a tropical Hawaiin supersweet that lacks husk leaves, and the Ia453 sh2 is a conversion to shrunken-2 of the Iowa sweet inbred and has typical long husk leaves. Husk leaf extention was scored in winter and summer seasons in Hawaii on a scale of 1(no husk leaves) to 9(very long extension). The parent's average scores were 1.18(Hi38c1) and 5.81(Ia453 sh2). The F1 hybrids averaged 3.78, while the F2 averaged 3.62. Backcross families averaged 2.82 and 4.85, respectively. Broad-sense heritability averaged 56.23% and narrow-sense heritability averaged 44.28%. The minimum number of effective gene loci, based on Castle and Wright formulas, was 1.10. It is concluded that a single major gene acting without dominance controls husk leaf extension in this material.

P236

Investigation of Mu transposition using proteomics

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The Mutator (MuDR/Mu) transposon family of maize comprises an autonomous element (MuDR) and several types of slave elements, all of which share ~215 bp terminal inverted repeat sequences and characteristically transpose late in development. There are two outcomes of Mu transposition: cut only or cut-and-paste in strictly somatic tissues and net replicative transposition in germinal tissue. Aside from the MuDR-encoded MURA and MURB proteins, other factors required for Mu transposition particularly those contributing to the developmentally specific behavior have yet to be identified. Our goals are to identify proteins found or missing from diverse terminally differentiating cells that could explain Mu activation, to define proteins in tassel somatic and pre-meiotic/gametophytic cells that could be associated with the different transposition mechanisms, and to determine if Mutator activity results in differential host protein accumulation or post-translational modification in somatic and/or gametophytic cells; this will be assessed using a proteomics analysis of sibling Mu active and suppressed genetic stocks. Proteins that play a general role in transposition, such as MURA and MURB, would be expected to be present in the Mu active sources for both somatic and germinal tissue. Differential expression of host proteins or the specific MURA and MURB proteins are expected in tissues utilizing the cut-and-paste or replicative mechanisms. For the analysis, protein samples from two carefully dissected tissues are prepared, labeled with fluorescent dyes, mixed, and resolved by two-dimensional gel electrophoresis. Approximately 3000 proteins are resolved on pH4-7 IEF/SDS-PAGE and an additional ~1000 high isoelectric point proteins (including many nucleic acid binding proteins) are resolved on pH6-11 IEF/SDS-PAGE. In this manner, protein spots can be systematically assigned as possible candidates for one or both transposition mechanisms. Ultimately, the role of candidate genes can be further investigated using molecular, biochemical, and/or reverse genetics approaches. Supported by a grant from the NIH.

P237

Microarray-based transcription analysis of maize repetitive elements in tissue culture reveals sense and antisense transcription

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Repetitive sequences comprise the majority of the maize genome and play an important role in genome organization. Our goal was to analyze the expression of a subset of characterized maize repetitive elements. To reach our goal, elements on a high density NimbleGen long oligonucleotide microarray were used to explore differences in transposon and repeat expression between tissue cultured cells and normal plant tissues. There were 400 sequences total, with each represented by a sense and antisense oligo. These sequences represent class I and class II transposable elements, centromere, neocentreme, telomere, and simple repeats. This probe set represents a large portion of the known repetitive sequences in maize, but is biased toward high copy and expressed elements. The microarrays were hybridized with polyA enriched RNA from BMS callus 25 years after initiation, HiII AxB callus 3 months after initiation, endosperm, shoots, and roots. BMS callus as compared to the other tissue types had the largest number of expressed sequences. Analysis of sense and antisense transcription revealed a number of probes that detected expression in both orientations and some probes showed tissue specific changes in the orientation of transcription. A total of 32 probes were differentially expressed in one or more tissues using a Bonferoni adjustment to control the false discovery rate, for a total of 86 significant differences. An additional subset of probes showing strong expression in tissue culture were chosen for further analysis to confirm the orientation and magnitude of expression detected in these microarrays.

P238

Mx-rMx, a family of interacting transposons in the growing hAT superfamily of maize

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More than half a century after the discovery of transposable elements, the number of genetically defined autonomous elements that have been isolated and characterized molecularly in any one species remains surprisingly small. Because of its rich genetic history, maize is, by far, the plant with the largest number of such elements. Yet, even in maize, a maximum of only two autonomous elements have been characterized in any transposon superfamily. This study describes the isolation and molecular and genetic characterization of Mx (Mobile element induced by X-ray), a third autonomous member of the hAT transposon superfamily in maize. Mx is 3731 bp long, ends in 13 bp terminal inverted repeats (TIRs) and causes an 8-bp duplication of the target site. Mx and rMx (responder to Mx), its 571-bp nonautonomous partner, define a classical family of interacting transposable elements. Surprisingly, the TIRs of Mx and rMx are only 73% identical and the subterminal sequences are even less so, suggesting that Mx and rMx may represent diverging transposable elements still capable of mobilization by the same transposase. Sequences that are closer to the ends of either Mx or rMx are present in the maize genome. Mx is predicted to encode a 674-amino-acid protein that is homologous to the Ac transposase. Although Mx and Ac are closely related, they do not interact. Other data suggest that maize may possess at least five families of hAT transposons that do not interact with each other. The possible origin of noninteracting transposon families within the same superfamily is discussed.

P239

Position-dependent escape from establishment of silencing.

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Silencing of transposons such as MuDR involves three successive stages: initiation, establishment, and maintenance. Mu killer is an inverted repeat version of MuDR that expresses a long hair-pin RNA molecule. This RNA is processed into small interfering RNAs that in turn degrade mudrA transcript, a post-transcriptional process associated with the initiation of silencing. In F1 plants carrying both Muk and MuDR, that post-transcriptional process leads to methylation of MuDR TIRs and transcriptional silencing of mudrA. In subsequent generations, mudrA remains transcriptionally inactive even in the absence of Muk. Thus, Muk is required for the initiation of silencing, but not for its maintenance. We have identified a MuDR element at a position where the initiation of silencing occurs, but where silencing is not maintained once Muk is lost. Based on these results, we hypothesize that maintenance of the silenced state can be dependent on the local sequence environment, with some environments being refractive to the establishment of a stable silenced chromatin configuration.

P240

Searching for Helitrons and Helitron- Related Transposable Elements in the Maize Genome

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Helitrons are a recently discovered family of transposable elements that apparently transpose through replication and strand replacement. Despite their abundance there is no direct genetic evidence or proof of the existence of an autonomous Helitron. We recently describe two maize mutants caused by recent Helitron insertion and provided the first evidence that an active Helitron may reside in the present day maize genome (Lal et al., 2003; Gupta et al., 2005). In this report, we have used the terminal ends of the mutant maize Helitron insertions and coding sequences of the rice Helitron as a query to discover putative Helitrons in the extant maize genome database. We demonstrate that Helitrons are abundant in the maize genome. Furthermore, maize Helitrons exhibit extreme size polymorphism due to the presence of pseudogenes apparently captured by these elements during their journey through the genome. Our data suggest that by frequently capturing cellular genes and multiplying them in different regions of the maize genome, Helitrons are continuing to play an important role in the evolution of the maize genome.

P241

Sex-Specific Differences in the Frequency and Timing of Reversion of PIF-Associated r1 alleles

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The Plant Instability Factor (PIF) family of class II transposable elements (TEs) in maize has garnered interest because of its timing of excision (perimeiotic), high target site specificity and very high copy number. Previously reported analyses of reversions of PIF-associated r1 alleles recovered from the female gametophytes revealed that PIF elements in the r1 locus excise primarily at or near meiosis and that repair of donor sites is similar to repair of donor sites following excision of other maize class II TEs. Here, we report on studies testing for sex-specific differences in the frequency, timing and repair of two PIF-associated alleles of the r1 locus, r-g:Y2902, which is comprised of a single r1 gene, and r-g:g2269, which is comprised of two r1 genes in a direct duplication. We find that the reversion frequency of both alleles is significantly higher in the male gametophyte than in the female gametophyte and that a significantly higher proportion of reversions are during or immediately prior to meiosis in the male gametophyte than in the female gametophyte. In contrast, we find no evidence for significant sex-specific differences in the types of molecular events associated with reversion. Precise and nearly precise excisions of the types typical of class II maize TEs predominate in both sexes. However, over 15% of reversions in both male and female gametophytes are associated with structures that could have arisen due to template-dependent gap repair of the donor site.

P242

Silenced Mutator elements become progressively and heritably reactivated through multiple generations in a mop1 (modifier of paramutation1) mutant background

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Transposons make up a sizable portion of most genomes. Given the mutagenic potential of transposons, it is not surprising that most organisms have evolved mechanisms to silence them. In maize, silencing of the Mutator family of transposable elements includes methylation of the terminal inverted repeats (TIRs) surrounding the autonomous element and loss of mudrA expression (the transposase) as well as mudrB (a helper gene involved in insertional activity). We have found that a mutant that suppresses paramutation in maize, mop1, also reactivates silenced Mutator transposons. In mop1 homozygous mutant individuals, Mu TIRs become hypomethylated immediately; however, mudrA expression and somatic reactivation is not observed until the silenced element has been exposed to mop1 for several generations. In subsequent generations individuals that are heterozygous for mop, or even wild type for the Mop1 allele, continue to exhibit hypomethylation at TIRs as well as somatic activity and high levels of mudrA expression. Thus, over time, mudrA silencing can be progressively and heritably reversed. Interestingly, mudrB expression is never restored, nor do we observe new insertions of Mu elements. These data suggest that that mudrA and mudrB silencing may be maintained via distinct mechanisms.

P243

Transposable Elements in the model Legume Lotus japonicus: abundance, diversity and intra-specific polymorphism

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Lotus japonicus (Lotus) belongs to the Leguminosae family, one of the largest plant family, among which are several agronomically important species, such as soybean and garden pea. Legumes provide the largest single source of vegetable protein in human diets and livestock feed. They form a symbiotic relationship with a member of the Rhizobium family of bacteria and aid in massive production of biological nitrogen. Lotus is an ideal model organism within the legume family to investigate legume-specific phenomena such as plant-microbe interactions and symbiotic nitrogen fixation because of its small genome size (~500Mb), short-life cycle (2-3 months) and the ease of genetic manipulation. For these reasons, a large scale sequencing project was initiated and 32.4Mb (6.9%) of genomic sequences is now available. This available sequence has permitted, for the first time, a survey of TEs within the legume family.
In this study, we used a computer-assisted approach to identify and characterize the transposable element (TE) content of Lotus by looking at the abundance (copy #) and diversity (lineages) of all the major TE types within the available Lotus sequences. In addition, transposon display technique (TD) was used to detect the intra-specific polymorphism and possible activity of recently amplified TEs between two ecotypes of Lotus.
Despite having a small genome, Lotus contains all the major TE types found in larger plant genomes including retrotransposons, miniature repeat transposable elements (MITEs), helitrons(rolling-circle transposons), and many families of transposase-encoding elements. Previously identified TE plant lineages as well as a couple new TE lineages were also identified. TD results, indicates less than 20% TE insertion polymorphism between the two ecotypes. Based on our findings, we estimate that ~20% of the Lotus genome consists of TEs. The information generated from our study will facilitate annotation of Lotus as well as other legume genomes, and aid in the development of TE insertion sites as markers.

P244

Transposon-Induced Rearrangements

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Transposable elements are known to generate a variety of genome rearrangements, including deletions, direct and inverted duplications, and translocations. Some rearrangements could occur by ectopic recombination between copies of transposons at non-allelic positions. Here, we show that rearrangements can also occur by alternative transposition reactions that involve the termini of different copies of the Ac/Ds family. At the maize p1 locus, the presence of multiple copies of Ac termini leads to various rearrangements: Ac 3' and 5' termini in direct orientation can undergo Sister Chromatid Transposition, leading to the formation of flanking deletions and inverted duplications (Zhang and Peterson, 1999. Genetics 153:1403-1410); while Ac termini in reversed orientation can undergo transposition reactions resulting in inversion, deletion, and other local rearrangements (Zhang and Peterson, 2004. Genetics 167:1929-1937). In each case, the rearrangement breakpoints are bounded by the characteristic footprint or target site duplications typical of Ac transposition reactions. These results show how alternative transposition reactions could contribute significantly to genome evolution by generating deletions, inversions, duplications, and other rearrangements, and by creating new genes through shuffling of coding and regulatory sequences.
We are attempting to reproduce these alternative transposition pathways in transgenic plants. The system utilizes transgene constructs containing maize Ac termini in direct or reversed orientation. The action of Ac transposase on the Ac termini generates deletions, with one end anchored at the transgene locus and the other end at various flanking sites. The deletion-inducing segment is itself inserted within a second transposon (I/dSpm) for mobilization throughout the genome. The construct contains markers (maize c1 and p1 genes) for detection of both I/dSpm transposition and Ac-induced deletions. In addition, the construct includes sequences to facilitate cloning by plasmid rescue and/or PCR-based approaches. The current state of the project will be presented.

P245

The Gramene Database

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Gramene (http://www.gramene.org) is a comparative genome mapping database for grasses, while using the rice genome as an anchor. Both automatic and manual curation are performed to combine and interrelate information on genomic and EST sequences, genetic, physical, and sequence-based maps, proteins, molecular markers, mutant phenotypes and QTL, and publications. There are currently nine main search modules within Gramene: Genome Browser, BLAST, CMap, Marker, Protein, Ontology, Mutant, QTL, and Literature. As an information resource, Gramene's purpose is to provide added value to data sets available within the public sector to facilitate researchers' abilities to leverage rice's genomic sequence and genetic information to identify and understand the characteristics of corresponding genes, pathways and phenotypes in the crop grasses, and to develop bioinformatic resources for the research community.

P246

Fertility restoration mechanisms in CMS-S of maize (Zea mays L.) revealed through expression differences identified by cDNA microarray and suppression subtractive hybridization

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Cytoplasmic male sterility (CMS) is thought to be due to an incompatibility of two genomes that results in pollen abortion. In CMS-S of maize (Zea mays L.), mitochondrial orf355-orf77 and the nuclear restorer of fertility interact to control fertility of gametophytes. Numerous studies demonstrated that Rf3 can regulate nuclear and mitochondrial gene expression and shows pleiotropic effects on the transcriptional level. Little is known, however, about the alteration of the global expression profile caused by Rf3 substitution of rf3 under S cytoplasm or about molecular fertility restoration mechanisms. In this study, cDNA microarray and suppression subtractive hybridization (SSH) were employed to reveal differentially expressed genes during pollen development by comparing a set of Rf3/rf3 near isogenic lines. A total of 137 tentative unique genes (TUGs) were identified at the transcriptional level. Based on functional category analysis, these TUGs were involved in a broad range of cellular and biochemical activities, including metabolism, cell structure, cell defense, and apoptosis, as well as signal transduction pathways. Northern blot using five representative clones as probes confirmed differential expression among S-(Rf3) and S-(rf3). Especially in S-(Rf3), the expression patterns of genes associated with electron or H+ conduction and anti-apoptosis genes (e.g., VDAC2, BI-1, cystatin, 14-3-3) are distinctly different from in S-(rf3). Together with normalization of cellular and biochemical activities in S-(Rf3), we proposed that Rf3 might regulate accumulation of nuclear and mitochondrial gene transcripts directly or indirectly to inhibit multiple PCD pathways in S-type cytoplasm allowing the normal developmental pathways to unfold.

P247

Revelation on early response and molecular mechanism of submergence tolerance in maize roots by microarray and suppression subtractive hybridization

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Submergence stress results in oxygen limitation in maize roots. Studying on submerging responsive genes, which were expressed in different tolerance-submergence inbred, is propitious to discover genes associated with submergence tolerance and elucidate the molecular mechanism of submergence tolerance. In the study, two inbred with different type of tolerance, Mo17 and Hz32£¬were used to reveal difference on early responsive genes in roots cell under submerging condition. The data from suppression subtracted hybridization (SSH) and cDNA microarray showed that a few of gene expressions were altered because of inducement of submergence. These genes are involved in a broad spectrum of biochemical, cellular, and physiological processes, such as glycolysis, energy metabolism, lipid metabolism, signal conduction, DNA transcription, protein biosynthesis and digestion, cell component and photosynthesis, and displayed six expression profiles. The spectrum of genes that were changed during the 0.5 h of treatment was significantly different from that of the genes induced later (2-4 h). In addition, molecular responses to submergence on gene expressions were also different between Hz32 and Mo17. Four transcription factors were expressed at different profile in Hz32 and mo17. The metabolic conversion from aerobic to anaerobic and other metabolic and physiological conversion were faster in Hz32 than that in Mo17. Based on the responsive genes and those function, we suggested that early response of maize roots to submergence stress may be a complex network involved in multiple physiological and metabolic pathways, and the regulation on transcriptional, translational and post-translational processes plays a critical role on metabolic adaptation of maize roots during early stage of submergence treatment.

P248

Development of Maize Ultra High-Density Gene Map Using Single Feature Polymorphisms Detected by GeneChip Microarray

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A high-density genetic map is an essential tool for map-assisted gene cloning and marker assisted breeding. We developed a custom designed maize GeneChip microarray to identify single feature polymorphism (SFP) within coding sequences at a genome scale. This GeneChip microarray has 1.3 million 25mer oligonucleotide probes for approximately 82,000 genes and EST clusters. Approximately 14400 SFPs, representing 1% of the total number of the screened features, were identified between B73 and M017. Using these hybridization polymorphisms as markers, a maize ultra high-density map was developed for the intermated B73 and Mo17 population (IBM). The current maize map consists of 4368 gene markers mapped by 10997 SFPs, anchored by 1127 previously published RFLP markers, and additional 362 SSR markers. Ninety-three percent of the studied SFPs can be validated by the segregation pattern of the associated known RFLP fragments. Further sequence analysis of these SFPs confirmed the associated single nucleotide polymorphisms (SNPs) in the probe regions. The integrated gene map provides a rich source of candidate gene markers for marker-assisted breeding, and useful tools for identification of genes that controls complex traits.

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