

**44th ANNUAL
MAIZE GENETICS
CONFERENCE**

**PROGRAM
and
ABSTRACTS**

**14-17 MARCH 2002
THE HYATT ORLANDO
KISSIMMEE, FL**

This conference receives financial support from:

The National Science Foundation

Eukaryotic Genetics Program
Integrative Plant Biology Program
Plant Genome Research Program,

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WE THANK THESE CONTRIBUTORS FOR THEIR GENEROSITY

General Information

Meals All meals will be served buffet style. ***Your name badge is required for meal service!*** Those who did not register for a room and meals package and would like to eat with the group can purchase the meal package at the Maize Genetics Conference Registration Desk, and should do so by 11 AM Friday so that we will have an accurate count for meals.

Breakfast Friday and Saturday 7:00 - 8:30 AM; Sunday 7:30 - 9:00 AM; Trellis Court in the hotel lobby
Lunch Friday and Saturday 12:20 - 1:30; Hibiscus Court, or Trellis Court in case of rain
Dinner Thursday 6:00 - 7:30 PM; Friday 6:00 - 7:30 PM; Saturday 5:30 - 7:00 PM; Hibiscus Court, or Florida Exhibit Hall East in case of rain

Beverage breaks Beverage breaks will be in the poster area (Florida Exhibit Hall West); coffee, tea and soft drinks are available at no charge during beverage breaks.

Talks and Posters All talks and workshops will be presented in Paradise Ballroom III & IV. Posters will be presented in Florida Exhibit Hall West. Posters should be hung on Thursday evening and should stay up until the end of the Sunday morning session if possible; they need to be removed by Sunday noon.

Evening at Disney/Epcot Monsanto Company's Beautiful Science experience at Innoventions at Epcot invites guests from around the world to "Take A Closer Look" at plant biotechnology and the important benefits it can bring. Buses will depart the Hyatt Orlando Saturday at 7:15 PM. The evening is sponsored by Monsanto Company and the Maize Genetics Conference for all conference attendees. Guests of attendees who want to attend can purchase tickets for \$20 at the Conference Registration Desk. The evening includes refreshments, the IllumiNations Laser Show and a tour of Monsanto's Beautiful Science and Nestle's Living with the Land.

Informal meeting places Informal socializing will take place Thursday, Friday and Saturday nights in the poster area (Florida Exhibit Hall West). Refreshments will be provided until 2 AM. After 2 AM each night, we will have a private suite (location to be announced) for socializing. These are private party rooms and alcoholic beverages may be brought in. However, please stay in these rooms if you are carrying drinks and dispose of your trash and bottles in the private party room. ***Please do not take any glass to the pool area as broken glass will necessitate draining the pool!***

Steering Committee

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

Sarah Hake, co-chair	Torbert Rocheford	Karen Cone (ex-officio, treasurer)
Sue Wessler, co-chair	Patrick Schnable	Christine Chase (ex-officio, Florida coordinator)
Gunter Feix	Lynn Senior	Mary Polocco (ex-officio, abstracts coordinator)
David Jackson	Dave Weber	Marty Sachs (ex-officio, Wisconsin coordinator)
Bob Meeley		

Acknowledgements In addition to the Steering Committee, many others contributed to the success of this conference. The meeting was outsourced to the MU Conference Center and ably handled by Lucy St. John. Doreen Bowers, Hyatt Orlando Catering and Convention Services Manger, provided excellent assistance with local arrangements. John Baier, Senior Biologist at the University of Florida, generously gave his time to provide computer assistance for the sessions. Steve Schroeder, Programmer Analyst, University of Missouri, provided the abstract submission form, updated this year to include editing by submitters, and graciously assisted with their presentation in the program.

The Next Maize Genetics Meeting The 45th Annual Maize Genetics Conference will be held March 13-16, 2003 at the Grand Geneva, Lake Geneva, WI. Local organizer is Marty Sachs, msachs@uiuc.edu.

44th Annual Maize Genetics Conference

March 14-17 2002

Thursday, March 14

6:00-7:00 PM DINNER

7:00-7:15 PM **Announcements**

Name: **Sarah Hake**

EVENING SESSION

7:15-9:15 pm

Chair: **Pat Schnable**

- 7:15-7:45 PM **Jerry Kermicle, University of Wisconsin, Madison, WI**
Oliver E. Nelson (1920-2001): Maize Geneticist Extraordinaire
- 7:45-8:30 PM **Jeff Dangl, University of North Carolina, Chapel Hill, NC**
Molecular Control of Host-Pathogen Interactions-Parasitizing the Weed
- 8:30-9:15 PM **Robert Schmidt, University of California, San Diego, CA**
Genes Controlling Floret and Inflorescence Development in Maize
- 9:15 PM Informal Poster Viewing Hang Posters Thursday Night

BEER & SNACKS AVAILABLE IN POSTER AREA UNTIL 2:00 AM

Friday, March 15

7:00-8:30 AM BREAKFAST

Session I

GENOMICS I

8:30-10:10 am

Chair: **Torbert Rocheford**

- 8:30-8:45 **Karen Cone, University of Missouri, Columbia, MO**
iMap: The Integrated Genetic and Physical Map of Maize
8:45-8:50 Discussion
- 8:50-9:05 **Susan Latshaw, University of Florida, Gainesville, FL**
Optimization of MuTAIL-PCR to Amplify Mutator-Flanking Sequences in Maize
9:05-9:10 Discussion
- 9:10-9:25 **Thomas Brutnell, Cornell University, Ithaca, NY**
Regional Mutagenesis Utilizing Activator (Ac) in Maize
9:25-9:30 Discussion
- 9:30-9:45 **Doreen Ware, Cold Spring Laboratory, Cold Spring Harbor, NY**
Gramene: A Resource for Comparative Grass Genomics
9:45-9:50 Discussion
- 9:50-10:05 **Mingsheng Chen, Clemson University, Clemson, SC**
An Integrated Physical and Genetic Map of the Rice Genome
10:05-10:10 Discussion

10:10-10:40 am - BREAK WITH BEVERAGES

Friday, March 15 Cont'd

Session 2 **GENOMICS II** **10:40-12:20 am** Chair: **Gunter Feix**

- 10:40-10:55 **Cedric Feschotte, University of Georgia, Athens, GA**
Two New Families of Transposases Are Responsible for the Origin and Amplification of MITEs
10:55-11:00 Discussion
- 11:00-11:15 **David Braun, University of California, Berkeley, CA**
Utility and Distribution of Conserved Noncoding Sequences (CNS) in the Grasses
11:15-11:20 Discussion
- 11:20-11:35 **Patrick Schnable, Iowa State University, Ames, IA**
High-Throughput Mapping Tools for Maize Genomics
11:35-11:40 Discussion
- 11:40-11:55 **Anusha Dias, Ohio State University, Columbus, OH**
Duplication and Functional Divergence of R2R3 Myb Regulatory Genes in the Grasses
11:55-12:00 Discussion
- 12:00-12:15 **Viviane Jaenicke, Max-Planck Institute, Leipzig, Germany**
Investigation of Key Genes in Maize Domestication from Archaeological Samples
12:15-12:20 Discussion

12:20-1:30 PM - LUNCH

1:30-3:00 pm - POSTER SESSION - Contributors will be at EVEN-NUMBERED Posters

2:30-3:00 pm - BEVERAGE BREAK

Session 3 **MAIZE TRANSGENICS WORKSHOP** **3:00-4:40 pm** Chair: **Sue Wessler**

- GMO 3:00-3:15 **Kan Wang, Iowa State University, Ames, IA**
Agrobacterium-Mediated Maize Transformation Using Standard Binary Vectors
3:15-3:20 Discussion
- 3:20-3:35 **David Somers, University of Minnesota, St. Paul, MN**
Transgene Locus Formation in Plants Genetically Engineered Using Microprojectile Bombardment
3:35-3:40 Discussion
- 3:40-3:55 **Curt Hannah, University of Florida, Gainesville, FL**
Super-Modified Shrunken2 Increases Wheat and Rice Yield 38% and 28%
3:55-4:00 Discussion
- 4:00-4:15 **Richard Clough, ProdiGene, College Station, TX**
Recombinant Protein Expression in Seed Is Increased in High Oil and Opaque 2 Germplasms
4:15-4:20 Discussion
- 4:20-4:35 **Stephen Moose, University of Illinois, Urbana-Champaign, IL**
Gain-of-Function Analyses of Glossy15 and its Role in Regulating Phase Change in the Leaf Epidermis
4:35-4:40 Discussion

6:00-7:30 PM - DINNER

Friday, March 15 Cont'd

Session 4 **EVENING SESSION** **7:30-9:00 pm** Chair: **David Jackson**

7:30-8:15 **Ingo Potrykus, Swiss Federal Institute of Technology, Zurich, Switzerland**
Towards Nutritional Optimization of a Major Staple, Vitamins, Iron, and Essential Amino Acids

8:15-9:00 **Virginia Walbot, Stanford University, Stanford, CA**
Mu Element Transposition: Regulation and Applications in Gene Tagging

BEER & SNACKS AVAILABLE IN POSTER AREA 9:00 pm - 2:00 am

Saturday, March 16

7:00-8:30 AM BREAKFAST

Session 5 **REPRODUCTION** **8:30-9:50 am** Chair: **Lynn Senior**

8:30-8:45 **Elene Valdivia, Penn State University, University Park, PA**
Functions of the Maize Group-1 Pollen Allergen, Zea m1
8:45-8:50 Discussion

8:50-9:05 **Wojtek Pawlowski, University of California, Berkeley, CA**
Genetics of Homologous Chromosome Pairing in Maize Meiosis
9:05-9:10 Discussion

9:10-9:25 **Joerg Bantin, University of Hamburg, Hamburg, Germany**
Maize and Tripsacum as Model Systems to Compare Gene Expression Pattern After Fertilization and Pathenogenesis
9:25-9:30 Discussion

9:30-9:45 **Kirsten Bomblies, University of Wisconsin, Madison, WI**
The Maize FLORICAULA/LEAFY Homologs (ZFL1 and ZFL2) Control Floral Identity and Determinancy
9:45-9:50 Discussion

9:50-10:20 am - BREAK WITH BEVERAGES

Session 6 **GENE REGULATION & FUNCTION** **10:20-12:20 am** Chair: **Robert Meeley**

10:20-10:35 **Cliff Weil, Purdue University, Lafayette, IN**
Ac Transposition and the Mre11/Rad50 Complex
10:35-10:40 Discussion

10:40-10:55 **Charles Carey, University of Arizona, Tuscon, AZ**
Cloning of Pale Aleurone Color 1, a WD40 Repeat Protein Regulating the Anthocyanin Pathway
10:55-11:00 Discussion

11:00-11:15 **Harley Smith, University of California, Berkeley, CA**
Selective Interaction of Plant TALE Homeodomain Proteins Mediates High DNA-Binding Affinity
11:15-11:20 Discussion

Saturday, March 16 - Cont'd

Session 6 cont'd

- 11:20-11:35 **Rita-Ann Monde, University of Oregon, Eugene, OR**
Tha8: A New Factor Involved in the Delta-pH Thylakoid Protein Targeting Pathway
11:35-11:40 Discussion
- 11:40-11:55 **Mary Frank, University of California, San Diego, CA**
Three Brick Genes Function in a Common Actin-Dependent Pathway
11:55-12:00 Discussion
- 12:00-12:15 **Jay Hollick, University of California, Berkeley, CA**
Trans-Silencing Interactions at the Purple Plant Locus
12:15-12:20 Discussion

12:20-1:30 pm - LUNCH

1:30-3:00 pm - POSTER SESSION - Contributors will be at ODD-NUMBERED Posters

2:30-3:00 pm - BEVERAGE BREAK

Session 7 GRASS WORKSHOP 3:00-4:55 pm Chair: Sarah Hake

- 3:00-3:15 **Andrew Paterson, University of Georgia, Athens, GA**
Sorghum and Its Value to the Maize Community
3:15-3:20 Discussion
- 3:20-3:35 **Daryl Pring, USDA - University of Florida, Gainesville, FL**
Development of AFLP Markers and Mapping of Fertility Restoration Genes in Sorghum
3:35-3:40 Discussion
- 3:40-3:55 **Surinder Chopra, Penn State University, University Park, PA**
Analysis of Transposon Induced Mutations in the Regulator of 3-Deoxyflavonoid Pathway and Its Implication in Understanding the Phytoalexins Synthesis in Sorghum
3:55-4:00 Discussion
- 4:00-4:10 **Wusirka Ramakrishna, Purdue University, West Lafayette, IN**
Sequence and Physical Map Analyses of the Maize and Sorghum Rp1 Complex Uncovers Numerous Sites and Unexpected Mechanisms of Local Rearrangement
4:10-4:15 Discussion
- 4:15-4:25 **Rentao Song, Waksman Institute, Rutgers, Piscataway, NJ**
Mosaic Organization of Orthologous Sequences in Grass Genomes
4:25-4:30 Discussion
- 4:30-4:40 **Michael Freeling, University of California, Berkeley, CA**
Fractionation of Genes and Cis-Acting Elements (Maize/Rice CNSs) in Chromosomal Regions Following Ancient Duplications in Grass Lineages
4:40-4:45 Discussion
- 4:45-4:55 **Nancy Cullen, Monsanto Corporation**

5:30-7:00 pm - DINNER

7:15-11:00 pm - DISNEY EPCOT ADVENTURE (7:15 pm Departure - 11:00 pm Return)

BEER & SNACKS AVAILABLE IN POSTER AREA 11:00 pm - 2:00 am

Sunday, March 17

7:30-9:00 BREAKFAST

Session 8 KERNELS & METABOLISM 9:00-10:40 am Chair: Karen Cone

9:00-9:15 Alfons Gierl, Lehrstuhl für Genetik Tech Universität München, Garching, Germany
Inspection of Maize Metabolism by NMR

9:15-9:20 Discussion

9:20-9:35 Marina Dermastia, University of Florida, Gainesville, FL
Cytometrical Evidence that the Loss of Seed Weight in the Miniature1 Seed Mutant of Maize Is Associated with Reduced Mitotic Activity in the Developing Endosperm

9:35-9:40 Discussion

9:40-9:55 Hajime Sakai, Dupont, Newark, DE
Giant Embryo: A Genomic Approach to Dissect Genetic Circuits Controlling Embryo/Endosperm Size

9:55-10:00 Discussion

10:00-10:15 Stein Erik Lid, Agricultural University of Norway, As, Norway
The Maize Defective Kernel 1 (dek1) Gene Encodes a Membrane Protein of the Calpain Gene Super Family

10:15-10:20 Discussion

10:20-10:35 Bryan Gibbon, University of Arizona, Tucson, AZ
Proteomic Analysis of Modified Opaque 2 Maize Endosperm

10:35-10:40 Discussion

10:40 FINAL ANNOUNCEMENTS

11:00 ADJOURN

ABSTRACTS – TALKS AND POSTER PRESENTATIONS

PLENARY TALKS

Robert Schmidt	Genes Controlling Floret and Inflorescence Development in Maize
Ingo Potrykus	Towards Nutritional Optimization of a Major Staple, Vitamins, Iron and Essential Amino Acids
Virginia Walbot	<i>Mu</i> Element Transposition: Regulation and Applications in Gene Tagging

TALKS

Fri 8:30 am

Genomics I

- 1 Cone, Karen iMap: The integrated genetic and physical map of maize
- 2 Latshaw, Susan Optimization of MuTAIL-PCR to amplify Mutator-flanking sequences in maize
- 3 Brutnell, Tom Regional Mutagenesis Utilizing Activator (Ac) in Maize
- 4 Ware, Doreen Gramene: A Resource For Comparative Grass Genomics
- 5 Chen, Mingsheng An integrated physical and genetic map of the rice genome

Fri 10:40 am

Genomics II

- 6 Feschotte, Cedric Two new families of transposases are responsible for the origin and amplification of MITEs.
- 7 Braun, David Utility and distribution of conserved noncoding sequences (CNSs) in the grasses
- 8 Wen, Tsui-Jung High-Throughput Mapping Tools For Maize Genomics
- 9 Dias, Anusha Duplication and Functional Divergence of *R2R3 Myb* Regulatory Genes in the Grasses
- 10 Jaenicke, Viviane Investigation of key genes in maize domestication from archaeological samples

Fri 3:00 pm

Maize Transgenics Workshop

- 11 Wang, Kan Agrobacterium-mediated maize transformation using standard binary vectors
- 13 Hannah, Curt Super-Modified Shrunken2 Increases Wheat and Rice Yield 38% and 28%.

14 Clough, Richard Recombinant protein expression in seed is increased in high oil and *opaque 2* germplasms.

15 Moose, Stephen Gain-of-Function Analyses of *glossy15* and its Role in Regulating Phase Change in the Leaf Epidermis

Sat 8:30 am

Reproduction

16 Valdivia, Elene Functions of the Maize Group-1 Pollen Allergen, *Zea m1*

17 Pawlowski, Wojtek Genetics of homologous chromosome pairing in maize meiosis.

18 Bantin, Joerg Maize and *Tripsacum* as model systems to compare gene expression pattern after fertilization and parthenogenesis

19 Bomblies, Kirsten The maize FLORICAULA/LEAFY homologs (ZFL1 and ZFL2) control floral identity and determinacy.

Sat 10:20 am

Gene Regulation and Function

20 Weil, Cliff *Ac* transposition and the Mre11/Rad50 complex

21 Carey, Charles Cloning of Pale Aleurone Color 1, a WD40 repeat protein regulating the anthocyanin pathway.

22 Smith, Harle Selective interaction of plant TALE homeodomain proteins mediates high DNA-binding affinity

23 Monde, Rita-Ann *Tha8*: a new factor involved in the delta-pH thylakoid protein targeting pathway.

24 Frank, Mary Three Brick Genes Function In A Common Actin-Dependent Pathway

25 Hollick, Jay Trans-silencing interactions at the purple plant locus

Sat 3:00 pm

Grass Workshop

27 Pring, Daryl Development of AFLP markers and mapping of fertility restoration genes in sorghum

28 Chopra, Surinder Analysis of transposon induced mutations in the regulator of 3-deoxyflavonoid pathway and its implication in understanding the phytoalexins synthesis in sorghum

29 Ramakrishna, Wusirika Sequence and physical map analyses of the maize and sorghum Rpl complex uncovers numerous sites and unexpected mechanisms of local rearrangement

30 Song, Rentao Mosaic Organization of Orthologous Sequences in Grass Genomes

Sat 9:00 am

Kernels and Metabolism

32 Gierl, Alfons Inspection of Maize Metabolism by NMR

33 Dermastia, Marina Cytometrical Evidence that the Loss of Seed Weight in the *miniature1* Seed Mutant of Maize is Associated with Reduced Mitotic Activity in the Developing Endosperm

- 34 Sakai, Hajime GIANT EMBRYO: A Genomic Approach To Dissect Genetic Circuits Controlling Embryo/Endosperm Size
- 35 Lid, Stein Erik The maize defective kernel 1 (*dek1*) gene encodes a membrane protein of the calpain gene super family
- 36 Gibbon, Bryan Proteomic analysis of modified opaque 2 maize endosperm

POSTER ABSTRACTS

BIOINFORMATICS

- 1 Buckler, Edward TASSEL: a software package for Trait Analysis by aSSociation, Evolutionary, and Linkage.
- 2 Fang, Zhiwei Comparative Bioinformatic Analysis of Genetic Maps From Rice and Maize
- 3 Jiang, Cizhong Phylogenetic and Computational Analysis of Myb Gene Evolution and Regulation
- 4 Moose, Stephen Evaluation of Phylogenetic Footprinting to Identify Promoter Regulatory Elements in Maize and Rice Genes
- 5 Pechanova, Olga Proteomic Analysis of Maize Rachis Tissue in *Aspergillus flavus* Resistant Inbreds
- 6 Polacco, Mary MaizeDB 2002
- 7 Rauh, Bradley Analysis of Diverse Germplasm using Microarrays
- 8 Springer, Nathan Relationships among maize (*Zea mays*) and Arabidopsis SET domain proteins
- 9 Tiffin, Peter Sequence diversity in three defense-related genes in North American inbred lines, land-races, and teosinte
- 10 Vincent, Leszek Controlled Pollinations Of Maize
- 11 Vincent, Leszek The Plant Ontologytm Consortium (POC) And Plant Ontologies

BIOCHEMICAL GENETICS

- 12 Colleoni, Christophe Insight into the roles of debranching enzymes in starch biosynthesis from carbohydrate profiling of an allelic series of *sugary1* mutants.
- 13 Cross, Joanna ADP-glucose pyrophosphorylase activity from maize-potato mosaics
- 14 Dinges, Jason Analysis of a null *zpu1* mutation indicates a role for the pullulanase-type debranching enzyme in both the synthesis and mobilization of maize starch
- 15 Frey, Monika Two glucosyltransferases are involved in detoxification of benzoxazinoids in maize

- 16 Grsic-Rausch, Slobodanka The CIF/VIF-related proteins in maize: Search for invertase inhibitor homologs
- 17 Kozaki, Akiko Identification of putative binding sites for the maize ID1 transcriptional regulator.
- 18 Licciardello, Nicholas Characterization and functional analysis of a maize cDNA encoding betacarotene hydroxylase
- 19 Miguel, Cervantes-Cervantes Characterization of the maize gene family encoding geranylgeranyl pyrophosphate synthase, a key enzyme in biosynthesis of plant isoprenoids
- 20 Park, Woong June Characterization of maize nitrilase genes and enzyme activities: implications for auxin biosynthesis
- 21 Schneerman, Martha Molecular Analysis of Zein Accumulation in the Illinois Protein Strains
- 22 Woodruff, Dana Evaluating the Growth of *Aspergillus flavus* with Flavanoids and their Derivatives

CYTOGENETICS

- 23 Golubovskaya, Inna Discovery and characterization of genes controlling meiosis in maize
- 24 Harper, Lisa A Cytogenetic Map of Maize
- 25 Kaszas, Etienne Centromere structure in intact and rearranged maize chromosomes derived from oat-maize additions lines.
- 26 Koumbaris, George 3D FISH analysis of the structure of maize chromosome 9
- 27 Lee, Michael Increased Recombination After Water-Deficit Stress In Maize
- 28 Mroczek, Rebecca Cytogenetic Analysis Of The Distribution Of Gypsy- And Copia-Like Retroelements In Maize.
- 29 Stack, Stephen Crossing over on maize chromosomes is related to the pattern of synapsis and the distribution of early recombination nodules
- 30 Weber, Gerd Frequency and physical localization of the major repetitive DNA sequences in maize

CYTOPLASMIC INHERITANCE

- 31 Chourey, Prem Gene expression analyses during pollen maturation in S-cytoplasmic male-sterile (S-CMS) and -fertile genotypes in Maize.

DEVELOPMENTAL GENETICS

- 32 Al, Reinoldus H. Maize endosperm development; aleurone organization. Three allelic mutant lines with disorganized aleurone layers.
- 33 Arthur, Kirstin The Rho GTPase RopB and Maize Pollen Function.

- 34 Barrell, Philippa A screen for non-reduction mutants in maize
- 35 Betz, Stephanie Chimeric mitochondrial open reading frames unique to cms-S maize and their hypothetical role in pollen collapse
- 36 Bommert, Peter Molecular cloning and characterization of CLAVATA1-like genes from *Zea mays*
- 37 Cartwright, Heather The Brick and Pangloss Genes Affect the Polarized, Asymmetric Divisions of the Stomatal Complex
- 38 Chuck, George The control of spikelet meristem identity by the branched silkless1 gene, a new member of the maize AP2 gene family
- 39 Colasanti, Joe Loss of indeterminate1 gene function affects leaf cell structure
- 40 Costa, Liliana The globby (glb) mutation affects early kernel development
- 41 Deleu, Wim The maize MADS-box gene ZMM6 distinguishes between the sessile and pedicellate spikelet and affects inflorescence branching.
- 42 Dorweiler, Jane *Mediator of Paramutation2-1* mutation perturbs normal development and disrupts transgene silencing
- 43 Fellner, Martin Tolerance of neighbor proximity in corn may involve light- and auxin-regulated development of leaf angle
- 44 Hay, Angela Characterization of the dominant mutation Wavy Auricle in Blade (Wab)
- 45 Henderson, David ragged seedling*2 affects medio-lateral and dorsiventral patterning in maize leaves
- 46 Hochholdinger, Frank Comparative proteomic analysis of lateral root initiation in maize
- 47 Inada, Noriko Morphological characterization of maize coleoptile mutant lco1-R
- 48 Ji, Jiabing NARROW SHEATH 2 is a NITRILASE 4 ñ like protein required for the recruitment of a lateral domain in the maize leaf
- 49 Juarez, Michelle Adaxial/Abaxial polarity specification in Maize leaf development
- 50 Kaplinsky, Nicholas Combinatorial control of meristem identity in the maize inflorescence.
- 51 Kessler, Sharon Xcl1 causes delayed periclinal cell divisions in developing maize leaves leading to cellular differentiation by lineage instead of position.
- 52 Kladnik, Ales Three-Dimensional Model Of Maize Endosperm
- 53 Lauter, Nick Developmental and genetic analyses of macrohair initiation

- in maize
- 54 Lee, Michael Genetic Analysis of Totipotency in Maize
 - 55 Lee, Michael Photoperiod Response and Two Genetic Pathways for Flowering in Maize
 - 56 Lisch, Damon Phenotypic effects of the mop1 mutation
 - 57 Ma, Zhengrong A mutation in chloroplast ribosomal protein S9 leads to embryo lethality in maize
 - 58 Mcsteen, Paula Expression profiling of maize inflorescence development
 - 59 Nardmann, Judith The maize homeobox gene MOM1 is expressed at the margins of growing coleoptile and leaf
 - 60 Osmont, Karen The genetic network controlling leaf development in maize: new and old players
 - 61 Pennington, Paul Douglas Imprinting in the Maize Endosperm
 - 62 Phelps-Durr, Tara Maintenance of Gene Silencing by the rs2 Gene in Maize
 - 63 Satoh, Namiko The SHOOTLESS2 gene is involved in both the initiation and maintenance of the shoot apical meristem in rice.
 - 64 Scanlon, Michael empty pericarp2 encodes a negative regulator of the heat shock response and is required for early stages of embryo development.
 - 65 Schreiber, Daniela N. The ZmMADS2-Transcription Factor Gene is Involved in Anther and Pollen Maturation
 - 66 Suzuki, Masaharu Shoot apical meristem mutants of maize embryo; application of Uniform Mu population.
 - 67 Tracy, Bill Altered timing of vegetative phase change and response to Puccinia sorghi
 - 68 Turbeville, Christopher Characterization of *Developmental Disaster 1 (Dvd1)*, a dominant mutation with reduced inflorescence branching and a pleiotropic disruption of vegetative development.
 - 69 Tyers, Randall A recessive allele of *rs1* suppresses the leaf phenotype of the *rs2* mutant.
 - 70 Vollbrecht, Erik Molecular and evolutionary analysis of *ramosa1* in inflorescence architecture
 - 71 Whipple, Clinton Conservation of B-class Gene Function Across 120 Million Years of Angiosperm Evolution
 - 72 Wierzba, Michael Identification Of Proteins Involved In Zein mRNA Trafficking In *Zea mays*
 - 73 Wisniewski, Jean-Pierre Two novel genes specifically expressed in the embryo sac and the basal endosperm transfer layer of maize

74 Yamawaki, Tracy Efforts to Clone Ramosa2

GENOME STRUCTURE/ SYNTENY

- 75 Bowers, John High Throughput Placement Of Overgo Markers On Bac Libraries Of Maize And 3 Other Grass Species
- 76 Carson, Chris Genetic Mapping of Maize Mutants with SSR Markers
- 77 Daviere, Jean-Michel Rp1 -a complex disease resistance locus in maize.
- 78 Davis, Georgia A High-Resolution Maize Map Based on a Randomly Intermated B73 x Mo17 Population.
- 79 Doebley, John The origin and structure of genetic diversity in maize as revealed by multilocus microsatellite genotyping
- 80 Elumalai, Rangasamy cDNA Microarrays for Maize Gene Discovery
- 81 Gardiner, Jack An Integrated Map for Maize
- 82 Ilic, Katica Structural evolution of an orthologous region in maize, sorghum and rice
- 83 Lai, Jinsheng Genomic organization and expression pattern of the duplicate factors Zmfie1 and Zmfie2 in maize
- 84 Ma, Jianxin Genic rearrangements of Wx1 homologous regions in barley, maize, pearl millet, rice, sorghum and diploid wheat revealed by comparative sequence analysis
- 85 Musket, Theresa IBM Maize Community Resources
- 86 Okagaki, Ron Low and Medium Resolution Radiation Hybrid Maps for Maize Chromosomes 2 and 9
- 87 Paterson, Andrew Sorghum and its value to the maize community.
- 88 Rabinowicz, Pablo D. Maize And Sorghum Gene-Targeted Sequencing And Microarray Analysis
- 89 Sheehan, Moira J. Characterization of the phytochrome gene family in Zea mays cv B73
- 90 Song, Rentao Sequence analysis of the chromosomal regions containing all members of the maize alpha zein multigene family
- 91 Vaudin, Mark A comparison of maize gene recovery from genomic sequence obtained from the undermethylated fraction of the maize genome vs. that obtained from whole BAC sequencing.
- 92 Vroh Bi, Irie Single nucleotide polymorphism discovery and mapping in maize.
- 93 Yim, Young-Sun Characterization and physical mapping of maize BAC libraries using high density BAC filter hybridization
- 94 Yim, Young-Sun A BAC pooling strategy: powerful tool for the maize integrated genetic and physical map construction.

- 95 Yuan, Yinan New and Old Approaches to Selective Sequence Analysis of Gene-Containing Regions of the Maize Genome

MOLECULAR GENETICS

- 96 Andaluz, Sandra Analysis of Maize Inflorescent Genes Using Association Studies
- 97 Beckert, Michel Molecular analysis of maize lines involved in in vitro or in vivo double haploids production
- 98 Bodempudi, Vidya Tubulin gene knockouts in maize
- 99 Cahoon, A. Bruce Evaluation of BMS cells as a model system to study organellar gene regulation
- 100 Casacuberta, Josep MARK, A Maize Atypical Receptor-Like Kinase Expressed During Embryogenesis And In The Meristems Of The Adult Plant, Interacts With MIK a New GCK/SPS1 Kinase
- 101 Casati, Paula Gene Expression Profiling after Decreased and Supplemental Ultraviolet Radiation in maize genotypes with varying amounts of flavonoids.
- 102 Clemente, Tom Agrobacterium-mediated transformation of maize
- 103 Dang, Diana Allele frequencies in fall armyworm populations
- 104 Gallagher, Cynthia Characterization of gene families encoding enzymes for carotenoid and isoprenoid biosynthesis in maize.
- 105 Goettel, Wolfgang Paramutagenicity of the p1 locus is correlated with enhancer element copy number
- 106 Gomez, Elisa Identification of compartment specific genes in the maize kernel
- 107 Guo, Mei Epigenetic Silencing and Heterosis in Maize
- 108 Guo, Mei Environmental Stress Influences Allele-Specific Gene Expression and Yield Heterosis in Maize
- 109 Harris, Linda Functional Genomics of the *Zea mays*/Fusarium graminearum Interaction
- 110 Hernandez, Julia
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- 111 Huang, Li-Fen Emerging Spectrum of Mu-Tagged Seed Mutants Derived from UniformMu Maize
- 112 Hueros, Gregorio Precise and Efficient Genetic Improvement of Cereal Seeds (SeedDesign)
- 113 Hueros, Gregorio Searching for the ZmMRP-1 target sequence in the promoter of BETL-1
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- 126 Pryor, Tony *mis1* ñ A Rust Inducible Gene In Maize
- 127 Ream, Thomas Studies on the molecular basis of heterosis
- 128 Reinders, Jon SNP Marker Development using SAGE Unigene Tag Libraries (SUTL) in Maize
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- 182 May, Bruce Maize Targeted Mutagenesis: progress in maize gene knockouts
- 183 Nagel, Alexander Mapping of 225 MITE markers from 5 different families in rice
- 184 Peterson, Thomas Unconventional transposition during DNA replication can generate complex chromosome rearrangements
- 185 Rudenko, George Initiation of silencing of the MuDR/Mu transposon family
- 186 Rudenko, George Towards identification of the transposase responsible for MuDR/Mu element insertion
- 187 Schneider, Bret Efficient Recovery and Sequencing of *RescueMu* Maize Genomic Clones
- 188 Singh, Manjit Generation of allelic diversity at the pink scutellum1 locus through Ac insertional mutagenesis
- 189 Tanurdzic, Milos Retroarrays - Microarray Analysis Of LTR Retrotransposons From The Maize Genome
- 190 Walbot, Virginia RescueMu-tagging grids - what are they and how to make use of them for your own research?
- 191 Yu, Jianhua DNA repair of *Ac/Ds* excision sites in the yeast *Saccharomyces cerevisiae*
- 192 Zhang, Xiaoyu PIF-like elements are widespread and associated with Tourist-like MITEs.

ABSTRACTS – TALKS AND POSTER PRESENTATIONS

PLENARY TALKS

Robert Schmidt	Genes Controlling Floret and Inflorescence Development in Maize
Ingo Potrykus	Towards Nutritional Optimization of a Major Staple, Vitamins, Iron and Essential Amino Acids
Virginia Walbot	<i>Mu</i> Element Transposition: Regulation and Applications in Gene Tagging

TALKS

Fri 8:30 am

Genomics I

- 1 Cone, Karen iMap: The integrated genetic and physical map of maize
- 2 Latshaw, Susan Optimization of MuTAIL-PCR to amplify Mutator-flanking sequences in maize
- 3 Brutnell, Tom Regional Mutagenesis Utilizing Activator (Ac) in Maize
- 4 Ware, Doreen Gramene: A Resource For Comparative Grass Genomics
- 5 Chen, Mingsheng An integrated physical and genetic map of the rice genome

Fri 10:40 am

Genomics II

- 6 Feschotte, Cedric Two new families of transposases are responsible for the origin and amplification of MITEs.
- 7 Braun, David Utility and distribution of conserved noncoding sequences (CNSs) in the grasses
- 8 Wen, Tsui-Jung High-Throughput Mapping Tools For Maize Genomics
- 9 Dias, Anusha Duplication and Functional Divergence of *R2R3 Myb* Regulatory Genes in the Grasses
- 10 Jaenicke, Viviane Investigation of key genes in maize domestication from archaeological samples

Fri 3:00 pm

Maize Transgenics Workshop

- 11 Wang, Kan Agrobacterium-mediated maize transformation using standard binary vectors
- 13 Hannah, Curt Super-Modified Shrunken2 Increases Wheat and Rice Yield 38% and 28%.

- 14 Clough, Richard Recombinant protein expression in seed is increased in high oil and *opaque 2* germplasms.
- 15 Moose, Stephen Gain-of-Function Analyses of *glossy15* and its Role in Regulating Phase Change in the Leaf Epidermis
- Sat 8:30 am** **Reproduction**
- 16 Valdivia, Elene Functions of the Maize Group-1 Pollen Allergen, *Zea m1*
- 17 Pawlowski, Wojtek Genetics of homologous chromosome pairing in maize meiosis.
- 18 Bantin, Joerg Maize and *Tripsacum* as model systems to compare gene expression pattern after fertilization and parthenogenesis
- 19 Bomblies, Kirsten The maize FLORICAULA/LEAFY homologs (ZFL1 and ZFL2) control floral identity and determinacy.
- Sat 10:20 am** **Gene Regulation and Function**
- 20 Weil, Cliff *Ac* transposition and the Mre11/Rad50 complex
- 21 Carey, Charles Cloning of Pale Aleurone Color 1, a WD40 repeat protein regulating the anthocyanin pathway.
- 22 Smith, Harle Selective interaction of plant TALE homeodomain proteins mediates high DNA-binding affinity
- 23 Monde, Rita-Ann *Tha8*: a new factor involved in the delta-pH thylakoid protein targeting pathway.
- 24 Frank, Mary Three Brick Genes Function In A Common Actin-Dependent Pathway
- 25 Hollick, Jay Trans-silencing interactions at the purple plant locus
- Sat 3:00 pm** **Grass Workshop**
- 27 Pring, Daryl Development of AFLP markers and mapping of fertility restoration genes in sorghum
- 28 Chopra, Surinder Analysis of transposon induced mutations in the regulator of 3-deoxyflavonoid pathway and its implication in understanding the phytoalexins synthesis in sorghum
- 29 Ramakrishna, Wusirika Sequence and physical map analyses of the maize and sorghum Rpl complex uncovers numerous sites and unexpected mechanisms of local rearrangement
- 30 Song, Rentao Mosaic Organization of Orthologous Sequences in Grass Genomes
- Sat 9:00 am** **Kernels and Metabolism**
- 32 Gierl, Alfons Inspection of Maize Metabolism by NMR
- 33 Dermastia, Marina Cytometrical Evidence that the Loss of Seed Weight in the *miniature1* Seed Mutant of Maize is Associated with Reduced Mitotic Activity in the Developing Endosperm

- 34 Sakai, Hajime GIANT EMBRYO: A Genomic Approach To Dissect Genetic Circuits Controlling Embryo/Endosperm Size
- 35 Lid, Stein Erik The maize defective kernel 1 (*dek1*) gene encodes a membrane protein of the calpain gene super family
- 36 Gibbon, Bryan Proteomic analysis of modified opaque 2 maize endosperm

POSTER ABSTRACTS

BIOINFORMATICS

- 1 Buckler, Edward TASSEL: a software package for Trait Analysis by aSSociation, Evolutionary, and Linkage.
- 2 Fang, Zhiwei Comparative Bioinformatic Analysis of Genetic Maps From Rice and Maize
- 3 Jiang, Cizhong Phylogenetic and Computational Analysis of Myb Gene Evolution and Regulation
- 4 Moose, Stephen Evaluation of Phylogenetic Footprinting to Identify Promoter Regulatory Elements in Maize and Rice Genes
- 5 Pechanova, Olga Proteomic Analysis of Maize Rachis Tissue in *Aspergillus flavus* Resistant Inbreds
- 6 Polacco, Mary MaizeDB 2002
- 7 Rauh, Bradley Analysis of Diverse Germplasm using Microarrays
- 8 Springer, Nathan Relationships among maize (*Zea mays*) and Arabidopsis SET domain proteins
- 9 Tiffin, Peter Sequence diversity in three defense-related genes in North American inbred lines, land-races, and teosinte
- 10 Vincent, Leszek Controlled Pollinations Of Maize
- 11 Vincent, Leszek The Plant Ontologytm Consortium (POC) And Plant Ontologies

BIOCHEMICAL GENETICS

- 12 Colleoni, Christophe Insight into the roles of debranching enzymes in starch biosynthesis from carbohydrate profiling of an allelic series of *sugary1* mutants.
- 13 Cross, Joanna ADP-glucose pyrophosphorylase activity from maize-potato mosaics
- 14 Dinges, Jason Analysis of a null *zpu1* mutation indicates a role for the pullulanase-type debranching enzyme in both the synthesis and mobilization of maize starch
- 15 Frey, Monika Two glucosyltransferases are involved in detoxification of benzoxazinoids in maize

- 16 Grsic-Rausch, Slobodanka The CIF/VIF-related proteins in maize: Search for invertase inhibitor homologs
- 17 Kozaki, Akiko Identification of putative binding sites for the maize ID1 transcriptional regulator.
- 18 Licciardello, Nicholas Characterization and functional analysis of a maize cDNA encoding betacarotene hydroxylase
- 19 Miguel, Cervantes-Cervantes Characterization of the maize gene family encoding geranylgeranyl pyrophosphate synthase, a key enzyme in biosynthesis of plant isoprenoids
- 20 Park, Woong June Characterization of maize nitrilase genes and enzyme activities: implications for auxin biosynthesis
- 21 Schneerman, Martha Molecular Analysis of Zein Accumulation in the Illinois Protein Strains
- 22 Woodruff, Dana Evaluating the Growth of *Aspergillus flavus* with Flavanoids and their Derivatives

CYTOGENETICS

- 23 Golubovskaya, Inna Discovery and characterization of genes controlling meiosis in maize
- 24 Harper, Lisa A Cytogenetic Map of Maize
- 25 Kaszas, Etienne Centromere structure in intact and rearranged maize chromosomes derived from oat-maize additions lines.
- 26 Koumbaris, George 3D FISH analysis of the structure of maize chromosome 9
- 27 Lee, Michael Increased Recombination After Water-Deficit Stress In Maize
- 28 Mroczek, Rebecca Cytogenetic Analysis Of The Distribution Of Gypsy- And Copia-Like Retroelements In Maize.
- 29 Stack, Stephen Crossing over on maize chromosomes is related to the pattern of synapsis and the distribution of early recombination nodules
- 30 Weber, Gerd Frequency and physical localization of the major repetitive DNA sequences in maize

CYTOPLASMIC INHERITANCE

- 31 Chourey, Prem Gene expression analyses during pollen maturation in S-cytoplasmic male-sterile (S-CMS) and -fertile genotypes in Maize.

DEVELOPMENTAL GENETICS

- 32 Al, Reinoldus H. Maize endosperm development; aleurone organization. Three allelic mutant lines with disorganized aleurone layers.
- 33 Arthur, Kirstin The Rho GTPase RopB and Maize Pollen Function.

- 34 Barrell, Philippa A screen for non-reduction mutants in maize
- 35 Betz, Stephanie Chimeric mitochondrial open reading frames unique to cms-S maize and their hypothetical role in pollen collapse
- 36 Bommert, Peter Molecular cloning and characterization of CLAVATA1-like genes from *Zea mays*
- 37 Cartwright, Heather The Brick and Pangloss Genes Affect the Polarized, Asymmetric Divisions of the Stomatal Complex
- 38 Chuck, George The control of spikelet meristem identity by the branched silkless1 gene, a new member of the maize AP2 gene family
- 39 Colasanti, Joe Loss of indeterminate1 gene function affects leaf cell structure
- 40 Costa, Liliana The globby (glb) mutation affects early kernel development
- 41 Deleu, Wim The maize MADS-box gene ZMM6 distinguishes between the sessile and pedicellate spikelet and affects inflorescence branching.
- 42 Dorweiler, Jane *Mediator of Paramutation2-1* mutation perturbs normal development and disrupts transgene silencing
- 43 Fellner, Martin Tolerance of neighbor proximity in corn may involve light- and auxin-regulated development of leaf angle
- 44 Hay, Angela Characterization of the dominant mutation Wavy Auricle in Blade (Wab)
- 45 Henderson, David ragged seedling*2 affects medio-lateral and dorsiventral patterning in maize leaves
- 46 Hochholdinger, Frank Comparative proteomic analysis of lateral root initiation in maize
- 47 Inada, Noriko Morphological characterization of maize coleoptile mutant lco1-R
- 48 Ji, Jiabing NARROW SHEATH 2 is a NITRILASE 4 ñ like protein required for the recruitment of a lateral domain in the maize leaf
- 49 Juarez, Michelle Adaxial/Abaxial polarity specification in Maize leaf development
- 50 Kaplinsky, Nicholas Combinatorial control of meristem identity in the maize inflorescence.
- 51 Kessler, Sharon Xcl1 causes delayed periclinal cell divisions in developing maize leaves leading to cellular differentiation by lineage instead of position.
- 52 Kladnik, Ales Three-Dimensional Model Of Maize Endosperm
- 53 Lauter, Nick Developmental and genetic analyses of macrohair initiation

- in maize
- 54 Lee, Michael Genetic Analysis of Totipotency in Maize
- 55 Lee, Michael Photoperiod Response and Two Genetic Pathways for Flowering in Maize
- 56 Lisch, Damon Phenotypic effects of the mop1 mutation
- 57 Ma, Zhengrong A mutation in chloroplast ribosomal protein S9 leads to embryo lethality in maize
- 58 Mesteen, Paula Expression profiling of maize inflorescence development
- 59 Nardmann, Judith The maize homeobox gene MOM1 is expressed at the margins of growing coleoptile and leaf
- 60 Osmont, Karen The genetic network controlling leaf development in maize: new and old players
- 61 Pennington, Paul Douglas Imprinting in the Maize Endosperm
- 62 Phelps-Durr, Tara Maintenance of Gene Silencing by the rs2 Gene in Maize
- 63 Satoh, Namiko The SHOOTLESS2 gene is involved in both the initiation and maintenance of the shoot apical meristem in rice.
- 64 Scanlon, Michael empty pericarp2 encodes a negative regulator of the heat shock response and is required for early stages of embryo development.
- 65 Schreiber, Daniela N. The ZmMADS2-Transcription Factor Gene is Involved in Anther and Pollen Maturation
- 66 Suzuki, Masaharu Shoot apical meristem mutants of maize embryo; application of Uniform Mu population.
- 67 Tracy, Bill Altered timing of vegetative phase change and response to Puccinia sorghi
- 68 Turbeville, Christopher Characterization of *Developmental Disaster 1 (Dvd1)*, a dominant mutation with reduced inflorescence branching and a pleiotropic disruption of vegetative development.
- 69 Tyers, Randall A recessive allele of *rs1* suppresses the leaf phenotype of the *rs2* mutant.
- 70 Vollbrecht, Erik Molecular and evolutionary analysis of *ramosa1* in inflorescence architecture
- 71 Whipple, Clinton Conservation of B-class Gene Function Across 120 Million Years of Angiosperm Evolution
- 72 Wierzba, Michael Identification Of Proteins Involved In Zein mRNA Trafficking In *Zea mays*
- 73 Wisniewski, Jean-Pierre Two novel genes specifically expressed in the embryo sac and the basal endosperm transfer layer of maize

74 Yamawaki, Tracy Efforts to Clone Ramosa2

GENOME STRUCTURE/ SYNTENY

- 75 Bowers, John High Throughput Placement Of Overgo Markers On Bac Libraries Of Maize And 3 Other Grass Species
- 76 Carson, Chris Genetic Mapping of Maize Mutants with SSR Markers
- 77 Daviere, Jean-Michel Rp1 -a complex disease resistance locus in maize.
- 78 Davis, Georgia A High-Resolution Maize Map Based on a Randomly Intermated B73 x Mo17 Population.
- 79 Doebley, John The origin and structure of genetic diversity in maize as revealed by multilocus microsatellite genotyping
- 80 Elumalai, Rangasamy cDNA Microarrays for Maize Gene Discovery
- 81 Gardiner, Jack An Integrated Map for Maize
- 82 Ilic, Katica Structural evolution of an orthologous region in maize, sorghum and rice
- 83 Lai, Jinsheng Genomic organization and expression pattern of the duplicate factors Zmfie1 and Zmfie2 in maize
- 84 Ma, Jianxin Genic rearrangements of Wx1 homologous regions in barley, maize, pearl millet, rice, sorghum and diploid wheat revealed by comparative sequence analysis
- 85 Musket, Theresa IBM Maize Community Resources
- 86 Okagaki, Ron Low and Medium Resolution Radiation Hybrid Maps for Maize Chromosomes 2 and 9
- 87 Paterson, Andrew Sorghum and its value to the maize community.
- 88 Rabinowicz, Pablo D. Maize And Sorghum Gene-Targeted Sequencing And Microarray Analysis
- 89 Sheehan, Moira J. Characterization of the phytochrome gene family in Zea mays cv B73
- 90 Song, Rentao Sequence analysis of the chromosomal regions containing all members of the maize alpha zein multigene family
- 91 Vaudin, Mark A comparison of maize gene recovery from genomic sequence obtained from the undermethylated fraction of the maize genome vs. that obtained from whole BAC sequencing.
- 92 Vroh Bi, Irie Single nucleotide polymorphism discovery and mapping in maize.
- 93 Yim, Young-Sun Characterization and physical mapping of maize BAC libraries using high density BAC filter hybridization
- 94 Yim, Young-Sun A BAC pooling strategy: powerful tool for the maize integrated genetic and physical map construction.

- 95 Yuan, Yinan New and Old Approaches to Selective Sequence Analysis of Gene-Containing Regions of the Maize Genome
- MOLECULAR GENETICS**
- 96 Andaluz, Sandra Analysis of Maize Inflorescent Genes Using Association Studies
- 97 Beckert, Michel Molecular analysis of maize lines involved in in vitro or in vivo double haploids production
- 98 Bodempudi, Vidya Tubulin gene knockouts in maize
- 99 Cahoon, A. Bruce Evaluation of BMS cells as a model system to study organellar gene regulation
- 100 Casacuberta, Josep MARK, A Maize Atypical Receptor-Like Kinase Expressed During Embryogenesis And In The Meristems Of The Adult Plant, Interacts With MIK a New GCK/SPS1 Kinase
- 101 Casati, Paula Gene Expression Profiling after Decreased and Supplemental Ultraviolet Radiation in maize genotypes with varying amounts of flavonoids.
- 102 Clemente, Tom Agrobacterium-mediated transformation of maize
- 103 Dang, Diana Allele frequencies in fall armyworm populations
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- 181 Lisch, Damon Epigenetic Regulation of MuDR Activity
- 182 May, Bruce Maize Targeted Mutagenesis: progress in maize gene knockouts
- 183 Nagel, Alexander Mapping of 225 MITE markers from 5 different families in rice
- 184 Peterson, Thomas Unconventional transposition during DNA replication can generate complex chromosome rearrangements
- 185 Rudenko, George Initiation of silencing of the MuDR/Mu transposon family
- 186 Rudenko, George Towards identification of the transposase responsible for MuDR/Mu element insertion
- 187 Schneider, Bret Efficient Recovery and Sequencing of *RescueMu* Maize Genomic Clones
- 188 Singh, Manjit Generation of allelic diversity at the pink scutellum1 locus through Ac insertional mutagenesis
- 189 Tanurdzic, Milos Retroarrays - Microarray Analysis Of LTR Retrotransposons From The Maize Genome
- 190 Walbot, Virginia RescueMu-tagging grids - what are they and how to make use of them for your own research?
- 191 Yu, Jianhua DNA repair of *Ac/Ds* excision sites in the yeast *Saccharomyces cerevisiae*
- 192 Zhang, Xiaoyu PIF-like elements are widespread and associated with Tourist-like MITEs.

PLENARY SESSIONS –

Thursday 7:45 –8:30 pm

Molecular Control of Host-Pathogen Interactions - Parasitizing the Weed

Jeff Dangl

University of North Carolina – Chapel Hill

8:30 –9:15 pm

Genes Controlling floret and inflorescence development in maize

Robert Schmidt

University of California – San Diego

Comparative studies of the molecular-genetic basis of plant development are providing insights into the evolution of plant form and function. As flower development represents one of the most dramatic examples of developmental change in plants, it has been the focus of intense research during the past decade. We and others have been comparing the degree to which the genes orchestrating floral organ and inflorescence development have been conserved in sequence and function between model eudicots and model grass species. To this end we have identified putative maize orthologues of eudicot floral organ and meristem identity genes. Through studies of their expression in developing maize spikelets, analyses of mutant phenotypes, and ectopic expression studies in transgenic rice and Arabidopsis, we have been elucidating the degree to which gene functions have been conserved. In addition, the cloning of maize genes that affect specific meristem fates during inflorescence development now opens the door for reciprocal investigations of the role of the orthologous genes in eudicot floral development. These comparative developmental genetic studies are providing new criteria on which to assess evolutionary relationships between grass and dicot floral organs and the process of floral organ specification. In spite of the morphological differences between grass spikelets and eudicot flowers, our results to date indicate that the genes orchestrating floral organ development and their respective activities have been largely conserved between the grasses and core eudicots.

Friday 7:30 – 8:15 pm

Towards nutritional optimization of a major staple: vitamins, iron, and essential amino acids.

Ingo Potrykus

Institute Plant Sciences/Swiss Federal Institute of Technology

The social and the scientific challenge. Malnutrition disorders are the cause for 24 000 deaths per day. Golden Rice represents a genetic engineering concept for development of nutrient-dense staple crops as contribution to reduction of malnutrition in developing countries. Major micronutrient deficiency disorders concern 1) protein/energy, 2) iron/zinc, 3) vitamin A, and 4) iodine. These deficiencies are especially severe, where rice is the major staple crop. Traditional interventions such as distribution, fortification, dietary diversification, and measures against infectious diseases are helpful in reducing deficiency disorders, but they have not, and probably can not solve the problem. Statistics demonstrate that we are still faced with e.g. 2.4 billion iron-deficient women and children, and 400 million vitamin A-deficient children per year. Nutrient-dense staple crops offer the chance to complement the traditional interventions. Protein deficiency relates to the amount and the quality of dietary protein. Rice provides, with a typical daily diet of 300 g, only 10% of the required essential amino-acids. Asp-1, a synthetic gene, developed by Jesse Jaynes, and coding for an ideal storage protein, offered the opportunity to approach the otherwise difficult task of engineering appropriate amounts of nine amino-acids. The gene was placed under endosperm-specific control, and linked to an appropriate target sequence for the endosperm protein storage vesicles. A series of Asp-1-transgenic rice plants accumulate different amounts of the Asp-1 protein in their endosperm, thus providing the mixture of the essential amino-acids required. Iron deficiency caused by a rice diet is

the consequence of 1) far too low amounts in rice of iron, 2) the presence of an extremely potent inhibitor of iron re-sorption, and 3) lack of any iron re-sorption-enhancing factors in a vegetative diet. Our engineering task for the endosperm was, therefore, to increase iron content, to reduce the inhibitor, and to add re-sorption-enhancing factors. Transgenic ferritin increased, so far, the iron content by two-fold; a transgenic metallothionin led to a seven-fold increase in re-sorption-enhancing cysteine, and a transgene coding for a heat-stable phytase produced high inhibitor-degrading phytase activity. (P. Lucca et al. TAG 102: 392-396, 2001). Vitamin A-deficiency from a rice diet is due to the fact, that endosperm is totally devoid of any provitamin A. The introduction of transgenes for phytoene synthase, a phytoene / x-carotene double-desaturase, and lycopene cyclase completed the biochemical pathway to pro-vitamin A. Biochemical analysis of the polished rice kernels confirmed, that the yellow endosperm colour was due to varying amounts of provitamin A and further terpenoids of dietary interest. The concentration of 1.6mg/g may, according to experienced nutritionalists, be sufficient to prevent vitamin A-deficiency disorders from a daily diet of 200g of Golden Rice alone. Nutritional studies testing this hypothesis are in progress, but will require ca 18 months. (Xudong Ye et al., Science 287: 303-305, 2000). The challenge of free donation to developing countries. Golden Rice can contribute to relieve from malnutrition in developing countries only, if it reaches the poor free of charge and limitations. As the technology used to develop provitamin A-rice alone, made use of 70 IPR's, „freedom-to-operate for humanitarian use“ became a major undertaking. The inventors solved the problem thanks to an alliance with the agbiotech industry. The rights for commercial use were transferred to industry which in turn supports the humanitarian project. “Humanitarian” is defined as income from Golden Rice per farmer or trader below \$ 10 000 p.a. Thanks to this agreement the technology is now available via free licences to public research institutions for breeding and variety development. Transfer was, so far, to IRRI and PhilRice (Philippines), Department of Biotechnology, Delhi and Directorate of Rice Research, Hyderabad (India), Cuu Long Delta Rice Research Institute (Vietnam), Institute of Genetics, Academia Sinica, Beijing and National Key Laboratory of Crop Genetic Improvement, Wuhan (China), and Agency for Agricultural Research and Development, Jakarta (Indonesia). The challenge of safe technology transfer and variety development. To ensure proper handling of the GMO material, a "Humanitarian Board" has been set up, to supervise the choice of partners, to support further improvement, to overlook needs, availability, bio-safety, and socio-economic assessments, to coordinate the activities in the different countries, to support fund raising from public resources, to support deregulation, to facilitate exchange of information, and to mediate information of the public and general support for the humanitarian project. Members of the Board include G.Toenniessen, A.Dubock, W.Padolina, R.M.Russell, H.E.Bouis, G.Khush, K.Jenny, and the inventors P.Beyer and I.Potrykus. Variety development in the partner institutions is via backcrossing into or direct transformation of popular local varieties. Backcrossing requires ca. eight generations (or three years). Direct transformation may be faster and has already been achieved into a series of Indica varieties by the Vietnamese and Philippine partner institutions. Deregulation, of course, requires all the standard biosafety assessments for each single variety, and this again will take at least two years from the completion of the variety. The challenge of a radical GMO opposition and consumer acceptance. Golden Rice has, unfortunately, become a key topic in the fight between proponents and opponents of plant biotechnology in food production. A radical GMO opposition is the last major stumbling block, which might prevent that the poor in developing countries benefit from the project. Greenpeace and numerous other NGO's are determined to prevent success of Golden Rice, which they see as a “Trojan Horse”, opening the road for the technology in developing countries. As the opposition has lost, with the Golden Rice case, all of their standard arguments used so far, and as the public and the media understand the moral dimension of the project, Greenpeace and the NGO's are trying to bypass a moral dilemma, by claiming that Golden Rice is useless anyhow, because children have to eat 9 kg/day. This is definitely wrong, but data to prove our view will, unfortunately, only be available in fall 2003.

Friday 8:15 –9:00 pm

Regulation of MuDR/Mu Transposons of Maize and Use of RescueMu for Gene Tagging and Sequencing

Virginia Walbot

Stanford University

MuDR/Mu elements show a strong preference for insertion into genes, they are mobile late in somatic development and in gametophytes, and they exhibit "cut only" and "cut and paste" transposition in somatic cells but not replicative transposition (no revertant alleles) in pre-germinal, germinal, and haploid cells. These activities require a transcriptionally active MuDR element. MuDR encodes two genes, each of which produces multiple transcript types as a result of alternative splicing. *mudrA* is predicted to encode at least three large transposase proteins, and *mudrB* is predicted to encode at least four "helper" proteins. Progress in matching MURA and MURB proteins to specific components of Mu element transposition will be discussed. MuDR/Mu elements are held in check by a multitude of post-transcriptional mechanisms; several models will be discussed to explain developmental timing and the lack of correspondence between MuDR transcript levels and protein levels. Epigenetic regulation of the family will be briefly mentioned, particularly the discovery of hMuDR elements in the maize genome, nuclear retention of poly(A)- transcripts at the initiation of silencing in somatic tissues, the presence of short 21 - 25 base asymmetric RNAs, the apparent immunity of MuDR to antisense transcript regulation, and the sequence requirements for Mu element methylation in transgenic constructs. The current status of the RescueMu gene discovery project will be summarized: grids G, H and I have yielded ~5000 likely sequenced, germinal insertion mutations, verifying that RescueMu sequencing is an efficient method of gene discovery and mutation. The combination of somatic and germinal RescueMu flanking host sequences match ~18% of the contig EST assemblies at ZmDB; by BLAST analysis the majority of RescueMu insertions are into gene-like DNA. Fewer than 4% of insertion locations are near or within a retrotransposon-like sequence. The methods for accessing RescueMu insertion sites will be demonstrated. Basic research is supported by the NIH. The Maize Gene Discovery, Sequencing and Phenotypic Analysis Project is supported by the NSF.

Friday 8:30-10:10 am Genomics 1

1:180

iMap: The integrated genetic and physical map of maize

Maize Mapping Project Consortium

University of Missouri-Columbia Clemson University

As a resource for future maize genomics research, we have developed the integrated genetic and physical map, which we have designated iMap. The key components of iMap are: a marker-dense high-resolution genetic map, a set of physical map segments (bacterial artificial chromosome [BAC] contigs), anchoring information that ties the genetically mapped markers to the BAC contigs, and an interactive interface for display. The genetic map was constructed using the intermated B73/Mo17 (IBM) recombinant inbred population and a combination of marker types, including RFLPs, SSRs, MITEs, etc. The physical map is being produced by fingerprint analysis of BAC clones from libraries made using three different restriction enzymes. Together, the libraries represent over 20-fold coverage of the genome. Anchors between the genetic map and BAC contigs were made by screening the BAC libraries with RFLP core markers and with overgo probes derived from a unigene set of 10,000 ESTs. A key feature of iMap is the interactive user interface, which displays side-by-side views of the genetic and physical maps for each chromosome. The genetic map shows loci detected by multiple marker types. The physical map shows BAC contigs that are detected by genetically mapped markers. Multiple options are available for viewing the linkages between the genetic and physical maps--including locus and contig queries--and for retrieving probe data, screening images and Genbank entries. We invite you to explore iMap by using the computers at our poster station or by accessing iMap via our website, www.maizemap.org (available March 14, 2002). Your input on what you would like to see as part of the iMap display will be essential as we refine the presentation of this mapping information to meet the needs of the maize genomics community. This project was funded by NSF Plant Genome Research Program Grant DBI 9872655.

2:194

Optimization of MuTAIL-PCR to amplify Mutator-flanking sequences in maize

Latshaw, Susan(1) Tan, Bao-Cai(1) Settles, A. Mark(1) McCarty, Donald R.(1)

(1) University of Florida

The UniformMu population was developed to generate a large number of independent endosperm mutants induced by Robertson's Mutator transposons. TAIL-PCR was adapted to amplify Mu flanking sequences (MuTAIL-PCR) from each mutant for use as probes on endosperm cDNA microarrays and for direct sequencing of Mu amplicons. For accurate identification of transposon insertion sites, high efficiency and fidelity amplification of Mu flanking sequences is essential. We have optimized MuTAIL-PCR using two assays. First, amplification efficiency was estimated from 10 diverse Mu-tagged genes. MuTAIL-PCR products were amplified from total genomic DNA from each mutant gene and analyzed by Southern blotting. Using the original 6 AD arbitrary primers developed for Arabidopsis, we detected 4 of the 10 mutant genes. Fifty-nine new arbitrary primers were designed and tested in the assay. A 12 member subset was assembled that detects all 10 of the mutant genes. Second, comparative microlibrary sequencing of amplicons from W22 and an UniformMu rgh class mutant, 99F-249-02, confirms that the 12 member primer set broadens the range of amplified Mu-flanking sequences and that these primers maintain high fidelity of bona fide Mu-flanking sequences. However, significant numbers of divergent Mu-like elements (MULES) were also detected in these libraries. In order to minimize amplification from MULES relative to known active Mu elements we developed a new secondary TIR specific primer. Statistical analysis of microlibrary sequences derived from this optimized primer set enabled construction of a model for the number and population structure of active Mu elements in the Uniform Mu population. A set of 200 random single pass sequences provided 27% sampling of all Mu ends representing roughly 50% of the total Mu insertions in the genome of a typical Mu-off line derived from UniformMu BC4. Fifty-one unique insertion sites were detected indicating a total population of 90-100 active elements in mutagenic Uniform Mu plants. These elements comprise roughly one third (~30) that are fixed in the W22 inbred parent, one third parental insertions created in previous generations and one third new insertions unique to that line. A steady-state model implies that an average of 30 transpositions per line per generation is required to maintain the stable 6-7% seed mutation frequency observed in UniformMu. Further tests of this model in advanced UniformMu lines are in progress.

3:200

Regional Mutagenesis Utilizing Activator (Ac) in Maize

Brutnell, Tom {1} Conrad, Liza {1} Hardeman, Kristine {2} Lewis, Paul {1} Lebejko, Sara {2} Ahern, Kevin {1} Chomet, Paul {2}

{1} Boyce Thompson Institute {2} Monsanto Co

As part of a recent NSF-funded Plant Genome Research grant, we have been distributing and mapping the transposable element Ac throughout the maize genome. The goal of this project is to create approximately 200 lines, each containing a single active Ac element at a precisely defined location. These near-isogenic W22 lines will be deposited in the Maize Genetics Cooperative Stock center for use without restriction by the maize genetics community. By exploiting the well-characterized genetics of Ac and its propensity for short-range transpositions, these lines will serve as launch pads for regional mutagenesis. Our current progress will be presented detailing the methods used for distributing and mapping Ac elements throughout the genome as well as our progress on the development of a web-based resource for accessing data pertaining to each Ac line. To date, sequences flanking 64 Ac elements have been cloned. Of these, 38 sequences have been verified as linked to an active Ac element and 18 have been mapped using publicly available recombinant inbred lines. Seed stocks for these lines are currently being propagated in our winter nursery and will be genotyped prior to distribution (est. April 2002). A database containing Ac map positions relative to anchor markers on the IBM or BNL populations, frequencies of Ac transposition and flanking primer sequences for tracking individual Ac lines is now under development. Please refer to abstracts by Chomet et al. and Singh et al. that demonstrate the utility of Ac mutagenesis.

4:123

GRAMENE: A RESOURCE FOR COMPARATIVE GRASS GENOMICS

Doreen Ware {1} Pankaj Jaiswal {2} Junjian Ni {2} Xioakang Pan {1} Kenneth Clark {1} Leonid Teytelman {1} Steven Schmidt {1} Wei Zhao {1} Sam Cartinhour {3} Susan McCouch {2} Lincoln Stein {1}

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Gramene (<http://www.gramene.org>) is a comparative genome mapping database for grasses and a community resource for rice that has been funded by grants from the USDA-IFAFS and USDA-ARS. Rice, in addition to being an economically important crop, is also a model monocot for understanding other agronomically important grass genomes. Gramene replaces the existing AceDB database RiceGenes with a relational database based on Oracle. Gramene provides curated and integrative information about maps, sequence, genes, genetic markers, mutants, QTLs, controlled vocabularies and publications. The Gramene project will use the rice genetic, physical and sequence maps as fundamental organizing units to provide a common denominator for moving from one crop grass to another and serve as a portal for interconnecting with other web-based crop grass resources.

5:218

An integrated physical and genetic map of the rice genome

Chen, Mingsheng^{1} **Presting, Gernot**^{1} **Barbazuk, W. Brad**^{2} **Goicoechea, Jose Luis**^{1} **Blackmon, Barbara**^{1} **Fang, Guangchen**^{1} **Soderlund, Carol Soderlund**^{1} **Dean, Ralph A.**^{1} **Wing, Rod A.**^{1}

^{1} Clemson University Genomics Institute ^{2} Monsanto Company

Rice was chosen as a model organism for genome sequencing due to its economic importance, small genome size, and syntenic relationship with other cereal species. We have constructed a BAC fingerprint based physical map of the rice genome (*Oryza sativa* ssp. japonica cv. Nipponbare) to facilitate whole genome sequencing of rice. Approximately 92% of the rice genome was genetically anchored by overgo hybridization, Southern hybridization, and in silico anchoring. Genome sequencing data were also integrated into the rice physical map. Comparison of the genetic and physical maps reveals that recombination is severely suppressed in centromeric regions as well as the short arms of chromosome 4 and 10. This integrated physical and genetic map of the rice genome will greatly facilitate whole genome sequencing by identifying a minimal tiling path of clones to sequence and organizing the efforts of the many sites participating. Furthermore, the high-resolution physical map will aid map-based cloning of agronomically important genes, and will provide an important tool for comparative analysis of grass genomes.

6:162

Two new families of transposases are responsible for the origin and amplification of MITEs.

Feschotte, Cedric^{1} **Zhang, Xiaoyu**^{1} **Jiang, Ning**^{1} **Swamy, Lakshmi**^{1} **Stevenson, Tesheka**^{1} **Wessler, Susan R.**^{1}

^{1} University of Georgia, Department of Botany

A decade ago, the results of computer-assisted searches for repeats demonstrated that miniature inverted-repeat transposable elements (MITEs), predominate in the noncoding regions of grass genes. These elements were structurally reminiscent of active class 2 nonautonomous elements, with their small size (less than 600 bp) and terminal inverted-repeats. However, their high copy number, target site preference (TA or TAA) and the uniformity of related elements served to distinguish them from the previously described DNA transposons. Although descriptions of MITE families have proliferated in a number of plant and animal species, their origin and mechanism of transposition have remained mysterious. This situation has changed dramatically in the last few years as MITE classification and relationships with transposases have become established. First, most of the tens of thousands of MITE elements in plant genomes can be divided into two groups based on similarity of their terminal inverted-repeats and target site duplications: there are Tourist-like MITEs and Stowaway-like MITEs. Second, in silico and experimental approaches have been combined to establish relationships between each group and newly discovered plant transposase families. We present evidence that Tourist-like MITEs are related to a new superfamily of eukaryotic transposases called PIF/Harbinger, while Stowaway-like MITEs were mobilized by transposases of the Tc1/mariner superfamily. Both of these transposase superfamilies are represented by multiple lineages in grass genomes. Based on these results, we propose a model for the origin and amplification of MITEs.

7:163

Utility and distribution of conserved noncoding sequences (CNSs) in the grasses

Braun, David {1} Kaplinsky, Nicholas {1} Penterman, John {1} Goff, Steven {2} Freeling, Michael {1}

{1} University of California-Berkeley {2} TMRI, Syngenta Corp., San Diego, CA

Control of gene expression requires cis acting regulatory DNA sequences. Historically these sequences have been difficult to identify. Conserved noncoding sequences (CNSs) have recently been identified in mammalian genes through cross species genomic DNA comparisons, and some have been shown to be regulatory sequences. Using sequence alignment algorithms, we compared genomic noncoding DNA sequences of the *liguleless1* (*lg1*) genes in two grasses, maize and rice, and found several CNSs in *lg1*. These CNSs are conserved among multiple grass species representing over 200 million years of evolutionary divergence. Seven other maize/rice genes were compared and six contained CNSs. Based on nucleotide substitution rates, these CNSs exist because they have biological functions. Our analysis suggests that grass CNSs are smaller and less frequent than those identified in mammalian genes and that mammalian gene regulation may be more complex than that of grasses. CNSs make excellent pan-grass PCR-based genetic mapping tools. They should be useful as characters in phylogenetic studies and as monitors of gene regulatory complexity.

8:122

HIGH-THROUGHPUT MAPPING TOOLS FOR MAIZE GENOMICS

Wen, Tsui-Jung {1} Qiu, Fang {1} Guo, Ling {1} Lee, Michael {1} Russell, Ken {2} Ashlock, Daniel {1} Schnable, Patrick {1}

{1} Iowa State University {2} University of Nebraska

A normalized B73 cDNA library with a complexity of 1.7×10^6 has been prepared using mRNA from a wide variety of organs, stages of development, and conditions. Over 35,000 3' EST sequences from this and other libraries have been deposited in GenBank. Based on CAP3 analysis 27,000 of these ESTs define 8,206 genes (4,666 singletons and 3,540 clusters). Pairs of PCR primers were designed based on the 3' UTRs of 10,900 unique ESTs. Over 8% of these primer pairs reveal a polymorphism between B73 and Mo17. These InDel Polymorphisms (IDPs) are being used to develop genetic markers suitable for high-throughput mapping experiments. Over 500 have been genetically mapped using a panel of 94 RIs from the IBM population. Approximately one-third of these primers reveal a polymorphism within a collection of 24 inbred lines. These polymorphisms are being used to genetically map 3,000 ESTs in F₁BC populations.

9:50

Duplication and Functional Divergence of *R2R3 Myb* Regulatory Genes in the Grasses

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The R2R3 Myb family of regulatory genes is characterized by an explosive amplification early in the history of land plants (250-450 Mya). A group of recently duplicated maize *R2R3 Myb* genes is characterized by a change Ala 63 to Pro, forming the P-to-A subfamily (Myb^{PtoA}) (Rabinowicz et. al. 1999). This group comprises of at least 10 members, including the well characterized P gene, which controls a subset of flavonoid biosynthetic genes, a regulatory function acquired recently in the evolution of plants. So far, a single rice gene containing the P-to-A substitution has been found, further supporting the ancient origin, yet recent expansion of this group. Therefore study of the Myb^{PtoA} provides a unique opportunity to understand the mechanism by which large gene families increase in size, and the role of amplification and divergence of regulatory genes in the development of novel cellular functions. To establish the function of Myb^{PtoA} gain and loss of function approaches are combined with a novel metabolite profiling strategy to establish whether a general function of R2R3 Myb genes is the control of plant metabolism. Application of metabolite profiling in functional characterization of the maize Myb^{PtoA} will be discussed.

10:36

Investigation of key genes in maize domestication from archaeological samples

Jaenicke, Viviane {1} Buckler, Edward S. {2} Smith, Bruce {3} Doebley, John {4} P▯bo, Svante {1}

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Maize was domesticated in Mexico between 10,000 and 5,000 years ago from its wild ancestor teosinte (*Zea mays* ssp. *parviglumis*) and spread to North America approximately 3,000 years ago. During the process of domestication morphological and biochemical characters were selected that distinguish maize and teosinte today. While changes in cob morphology are observable in archaeological finds, changes in plant morphology and biochemical characters can rarely be deduced from the archaeological record. To better understand the role of the latter type of characters during maize domestication, three genes were chosen for analysis in DNA recovered from archaeological specimens. One gene (*tb1*) controls aspects of plant morphology while the other two (*su1*, *pbf*) are key genes of starch synthesis and storage. All three genes show reduced diversity in maize compared to teosinte. This is thought to be the result of the selection process during domestication. The main goal of this project is to determine the timing and order of selection of these characters. The archaeological samples used range in age from 4,000 to 1,000 years and stem from Mexico and southwestern USA. Preliminary results show only alleles common to modern maize at *tb1* and *pbf*. For *su1*, 1,000-year-old cobs carry two alleles known from modern maize while in a 2,000-year-old cob an allele known in modern teosinte but unknown in modern maize was found.

11:73

Agrobacterium-mediated maize transformation using standard binary vectors

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{1} Iowa State University {2} University of Wisconsin-Madison

A protocol for routinely transforming maize Hi-II immature zygotic embryos using a standard Agrobacterium binary vector system is described. Stable transformation has been achieved at an average efficiency of 5.5% (5.5 independent bialaphos resistant events per 100 embryos infected) by co-cultivating infected embryos for 3 days (20C) in the presence of the anti-oxidant, L-cysteine (400 mg/L). Transgenic events have been successfully recovered using Agrobacterium strains EHA101 and AGL0 harboring constructs in which the selectable marker gene, bar, was driven by either the CaMV 35S or the maize ubiquitin promoter. Most experiments carried out to date have used a pPZP-derived binary vector backbone. Results from molecular and progeny analysis confirm the integration, expression, and inheritance of the transgene in R0, R1, and R2 progeny of transgenic events. To our knowledge, this represents the first report in which fertile, stable transgenic maize has been routinely produced using a standard Agrobacterium binary vector system. We will present results on experiments aimed at further protocol optimization as well as our efforts in extending the protocol to maize inbred genotypes.

13

Super-Modified Shrunken2 Increases Wheat and Rice Yield 38% and 28%

Hannah, C

University of Florida-Gainesville

The enzyme adenosine diphosphate glucose pyrophosphorylase (AGP) plays a key, rate limiting role in starch biosynthesis. While AGP is present in all plant tissues that synthesize starch, the various isoforms differ dramatically in intracellular location, heat stability and response to allosteric effectors. We have focused on modification of this enzyme with the aim of increased starch biosynthesis. Our recent studies point to the importance of two such variants. The Rev6 variant of Sh2 arose via excision of the transposable element Ds. AGP from this allele exhibits reduced inhibition by the allosteric effector, inorganic phosphate. Three doses of Sh2-Rev6 gives a 34% increase in maize seed weight. A second variant, HS33, was isolated by expressing Sh2 in an E. coli expression system and selecting for increased glycogen synthesis at elevated temperatures. Sh2- HS33 increases AGP heat stability by enhancing subunit interactions. We placed a Rev6/HS33 Sh2 gene into wheat and into rice. Yield increases of 38% in wheat and 23% in rice were obtained when the transgene was homozygous. Surprisingly, seed number is increased in both species. One explanation is that the increased sink strength blocks early seed abortion. Transgenic maize containing Rev6/HS33 Sh2 was also synthesized. Transgenic events with only 10% wild type expression levels produced seed weights comparable to wild type.

14:149

Recombinant protein expression in seed is increased in high oil and *opaque 2* germplasms.

Clough, Richard C. (1) Delaney, Donna (1) Bailey, Michele (1) Wiggins, Barry (1) Hood, Elizabeth E. (1) Howard, John (1)

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While standard elite corn lines provide great yields, they may not always provide the best production system for recombinant protein expression in seed. Therefore, we tested germplasms whose grain may serve to provide greater target protein yield or content. Two such germplasms are high oil lines and *opaque 2* mutants. High oil lines are known to contain more oil due to their increased embryo-to-endosperm size ratio, which also results in increased soluble protein content. The second germplasm chosen was an *opaque 2* mutant which contains decreased amounts of alpha zein, a major storage protein of the endosperm. However, *opaque 2* mutants contain increased amounts of soluble protein, making them a good candidate for foreign protein expression. We crossed stable transgenic corn lines expressing either laccase, a fungal redox enzyme, or avidin, a chicken egg white-derived protein, with several high oil lines and *opaque 2* mutants. Soluble protein extracts from seed were analyzed for target protein content among the progeny, and compared with that of transgenic lines in elite backgrounds. Laccase content in high oil lines increased by as much as 40%, while avidin levels doubled in either the high oil or *opaque 2* backgrounds. Avidin content was even higher in opaque seed resulting from crosses with high oil lines.

15:61

Gain-of-Function Analyses of *glossy15* and its Role in Regulating Phase Change in the Leaf Epidermis

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Detailed phenotypic analyses of loss-of-function mutations in the *glossy15* (*gl15*) gene have demonstrated that *gl15* is required to both activate juvenile (e.g., leaf waxes) and suppress adult (e.g. macrohairs) leaf epidermal cell traits during the late juvenile phase. The *gl15* gene encodes an APETALA2-like DNA binding protein that acts cell-autonomously to specifically program leaf epidermal cell identities, but its mRNA expression responds to upstream factors that regulate the overall process of vegetative phase change. These observations suggest that the distribution of juvenile and adult leaf epidermal cell phenotypes depends on antagonistic interactions between *gl15* and factors that promote the transition to adult vegetative shoot development. This hypothesis predicts that specifically increasing GL15 activity will lead to a corresponding increase in the expression of juvenile leaf epidermal traits. We increased GL15 activity in its appropriate developmental context by generating transgenic maize lines with additional copies of a 6-kb *gl15* genomic clone that spans the entire *gl15* transcription unit and 1.5-kb of *gl15* promoter sequence. The *gl15* gene fragment complemented the *gl15* mutant phenotype but had no effect on other plant phenotypes, demonstrating that the 6-kb fragment functions similarly to the *gl15* gene. One transgenic line that contains at least three additional *gl15* gene copies and overexpresses *gl15* mRNA relative to wild-type plants showed an increased number of leaves expressing juvenile epidermal traits and slightly delayed the onset of adult traits. These results are consistent with predictions made from the analysis of *gl15* loss-of-function mutations and support a model where 1) the activation of juvenile and repression of adult leaf epidermal cell identity genes are differentially sensitive to GL15 activity and 2) phase change in the leaf epidermis is regulated primarily by mutually antagonistic interactions between *gl15* and factors that induce adult leaf development.

16:11

Functions of the Maize Group-1 Pollen Allergen, Zea m1

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Zea m1 is specifically expressed in pollen and classified as a -expansin based on its sequence and activity. Expansins are secreted proteins that characteristically exert strong loosening effects on plant cell walls. We propose that the role of Zea m1 is to loosen the cell walls of the silks to aid in the penetration and growth of the pollen tube through the silks. Maize contains three Zea m1 genes, Zm1a, Zm1b, Zm1c, with high sequence similarity. In collaboration with Pioneer HiBred, we have obtained a transposon insertional mutant for Zea m1a. Preliminary observations show an unexpected phenotype associated with disruption of the Zea m1 gene. Pollination with wild type pollen normally results in a rapid cessation of silk growth. In contrast, pollination with pollen bearing the disrupted Zea m1 gene results in continued growth of the silks for many days, even though pollination is successful and seeds are set. The Zea m1 mutation segregates with this silk-response phenotype. We have examined the Zea m1 transcript in this mutant by northern blot analysis, western blot analysis, and RT-PCR, but have not seen a difference in the size or quantity of the message in these plants. We are currently investigating several hypotheses that are consistent with both the co-segregation of the mutation and phenotype, and our failure to detect any change in our gene transcript. Our results suggest that Zea m1 isoforms may play a signaling role in the cessation of silk elongation upon pollination.

17:16

Genetics of homologous chromosome pairing in maize meiosis.

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To dissect the genetic control of meiotic chromosome pairing and synapsis we have collected existing and generated new maize meiotic mutants defective in these processes. Our collection of over 30 mutants has been examined in detail using light, fluorescence, and transmission electron microscopy to characterize the dynamics of chromosome pairing and establishment of the synaptonemal complex between the paired chromosomes. To classify our mutants in regard to the meiotic recombination pathway, we used 3-dimensional immuno-fluorescence microscopy to follow the changes in distribution of the recombination protein RAD51 during meiotic prophase. In wild-type maize plants, RAD51 forms numerous distinct foci on chromosomes during zygotene. In twelve maize meiotic mutants that we studied, we found dramatic changes in the number and shape of the RAD51 foci including: (1) normal number but mis-shaped RAD51 foci, (2) slightly decreased number of RAD51 foci, (3) very few foci, (4) RAD51 foci completely absent. This classification will eventually allow us to determine the temporal order of wild-type function of these genes. Our mutant collection is a valuable source of genes playing key roles in meiotic prophase events. Using a Mutator-tagged allele, we cloned a gene poor homologous synapsis (phs1) that is crucial for proper pairing and synapsis of homologous chromosomes during meiosis. A Mu insertion into phs1 results in a presence of 20 univalents at metaphase I, causing male and female sterility. Extensive promiscuous pairing of non-homologous chromosomes was detected in the mutant plants at zygotene and pachytene after examining the synaptonemal complex spreads with transmission electron microscopy. The phs1-O::Mu1 mutation also results in abnormalities of telomere bouquet formation that precedes chromosome pairing. These defects are accompanied by a dramatic decrease in the number of RAD51 foci at zygotene and pachytene, although the level of the RAD51 protein in the anthers remains unchanged from the wild-type. Phs1 shows meiosis-specific expression that peaks at zygotene, although it is also expressed at a low level in some somatic tissues. It encodes a novel protein that seems to be found exclusively in plants. This observation is interesting since the mechanistic of meiosis and most genes involved in meiotic DNA metabolism are evolutionarily conserved among all sexually reproducing organisms.

18:49

Maize and Tripsacum as model systems to compare gene expression pattern after fertilization and parthenogenesis

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In maize zygotic genome activation (ZGA) occurs soon after fertilization. We have analysed paternal genome activation in fertilized embryo sacs by crossing wild type maize plants with pollen from transgenic lines expressing GUS under control of the actin and the ubiquitin promoter, respectively. After histochemical GUS-staining GUS-activity was detected in the egg and central cell, 18 hap, activated from the paternal genome. At the molecular level we could detect paternal genome activity shortly after fertilization in egg cells using SNP's in endogenous genes of different inbred lines. However, in polyploid lines from *Tripsacum dactyloides*, a close, wild relative of maize, the egg cell develops autonomously without male contribution whereas fertilization of the central cell is needed to form viable endosperm (pseudogamous apomixis). Genes expressed in maize before and after fertilization (i.e. in the embryo sac) will be used to compare gene expression pattern in sexual and apomictic embryo sacs of *Tripsacum* to examine whether the apomictic egg cell expresses parthenogenetic-specific genes or follows the 'normal' sexual pathway of reproduction. A protocol for the isolation of *Tripsacum* egg apparatus for molecular and cytological analysis will be presented.

19:62

The maize FLORICAULA/LEAFY homologs (ZFL1 and ZFL2) control floral identity and determinacy.

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The FLORICAULA/LEAFY gene, known from *Arabidopsis* and *Antirrhinum* respectively, encodes a transcription factor that plays a role in the transition from vegetative to reproductive development and is essential for proper floral meristem identity and determinacy in both species. To better understand the molecular basis of these processes in maize, we have investigated the function of the duplicate maize FLORICAULA/LEAFY homologs, ZFL1 and ZFL2, by mutant analysis. Plants homozygous for Mutator insertions in either ZFL1 or ZFL2 are essentially normal. However, plants homozygous for insertions in both ZFL genes show defects in the transition to reproductive development, as well as in floret organ identity and determinacy. Associated effects on kernel row number and tassel phyllotaxy and branching suggest that the ZFL genes play an additional role in inflorescence development in maize. The mutant phenotype suggests that the redundant ZFL genes control similar developmental processes as their known dicot homologs, implying some conservation of this developmental pathway between monocots and dicots. In addition, the effect of ZFL on inflorescence phyllotaxy supports the candidacy of ZFL2 as a major QTL for kernel row number involved in maize domestication.

20:174

***Ac* transposition and the Mre11/Rad50 complex**

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Host cell factors are required for the repair of DNA breaks associated with transposon excisions. This repair occurs through an end-joining process in plants and in yeast cells (even in the presence of an homologous repair template), prompting us to examine the roles of genes such as *KU70*, *KU80*, *RAD50*, *MRE11* and, in yeast, *XRS2* in the repair of *Ac* excision sites. Cells that are forced to repair double-strand breaks by end-joining, but that cannot because of mutations, typically undergo cell cycle checkpoint arrest. This arrest requires the integrity of the Mre11/Rad50/Xrs2 complex. Interestingly, *rad50* and *xrs2* mutants undergoing transposition escape the checkpoint and do not arrest, as expected. However, cells lacking Mre11 do not escape the checkpoint. Potential roles of Ku70 and Ku80 are also discussed.

21:29

Cloning of Pale Aleurone Color 1, a WD40 repeat protein regulating the anthocyanin pathway.

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The *pac1* locus is required for full expression of RNAs of the anthocyanin biosynthetic pathway (Selinger et. al. 1999). The recessive mutant phenotype is pale aleurone color. A *Mu1* element was found that cosegregated with the mutant phenotype enabling cloning of the *pac1-ref* allele. The *Mu1* insertion interrupts a coding region with high homology to WD40 repeat proteins. An independent allele, *pac1-2* contains a *Mu1* insertion in the same coding region, proving that this is the *pac1* locus. Sequencing of 8.3 Kb of a hybridizing genomic clone from a B73 bacterial artificial chromosome library (CUGI, ZMMBBb library) and 3' RACE products revealed the *pac1-B73* 353 amino acid open reading frame and probable intron/exon structure. In both *Arabidopsis* and *Petunia*, WD40 repeatproteins play a role in regulation of the anthocyanin pathway. To determine if *pac1* is a functional ortholog of these proteins 3 *TTG1* (*Arabidopsis*) and 1 *an11* (*Petunia*) mutant lines were transformed with the CaMV 35S promoter driving the *pac1-B73* ORF. Our initial results show that several *ttg1* mutant phenotypes are complemented. These phenotypes will be discussed. In maize, we have detected *pac1* transcripts in both aleurone and immature tassel. However, the anthocyanin mutant phenotype is restricted to the aleurone. This suggests functional redundancy or no requirement for *pac1* in regulation of the anthocyanin pathway in other tissues. Southern analysis with the full *pac1* ORF reveals at least one other cross hybridizing gene fragment. It is hypothesized that this is a paralogous gene required for expression of the anthocyanin pathway in tissues other than the aleurone.

22:53

Selective interaction of plant TALE homeodomain proteins mediates high DNA-binding affinity

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In animals, TALE homeodomain proteins, MEIS and PBX, form heterodimers that act as functional units to control developmental pathways important for specifying cell fate. Understanding molecular mechanisms that control cell fate in the shoot apical meristem (SAM) is a fundamental question in plant development. Genetic and molecular studies show that the maize TALE homeodomain KNOTTED1 (KN1), which is related to MEIS, is involved in SAM function. We show that KN1 forms heterodimers with the KNOTTED INTERACTING PROTEIN (KIP), which is a BELL-like TALE homeodomain protein. The association of KN1 and KIP occurs through conserved domains in the N-termini of both proteins. The KN1 DNA-binding sequence, TGACAG(G/C)T was biochemically identified and *in vitro* DNA-binding assays show that KN1 and the homeodomain of KIP alone specifically bind to this site with low affinity. However, as a heterodimer, KN1-KIP binds specifically to the KN1/KIP consensus sequence with high affinity, indicating that the association of KN1 and KIP may function as a transcriptional unit. Currently, we are examining the localization of KIP in meristems to determine whether or not KIP localizes to the nucleus in cells that express KN1. Future work is aimed at determining the function of the KN1-KIP complex *in vivo*.

23:60

Tha8: a new factor involved in the delta-pH thylakoid protein targeting pathway.

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The transformation of a proplastid into a chloroplast is a complex process involving the assembly of the abundant and protein-rich thylakoid membrane system. We have used *Mu*-transposons in forward and reverse genetic screens to identify mutants with defects in targeting proteins to the thylakoid membrane. These mutants, together with biochemical assays in other laboratories, defined three distinct targeting machineries: the delta-pH, cpSec, and cpSRP pathways. The delta-pH system was first characterized in chloroplasts, and two components, Hcf106 and Tha4, were initially identified genetically in maize. A homologous system (called Tat) has been shown to exist in bacteria, but the composition and mechanism of the delta-pH machinery is not well understood. We have identified a new gene involved in the delta-pH pathway called *tha8*. Mutations in *tha8* result in a phenotype that is similar to the previously characterized delta-pH mutants, *hcf106* and *tha4*, but *tha8* is not allelic to either of those genes. I have cloned the *tha8* gene by *Mu*-tagging. Analysis of additional alleles obtained from our laboratory's Photosynthetic Mutant Library (<http://PML.uoregon.edu>) confirmed the correct gene was cloned. The cloned *Mu* insertion disrupts an open reading frame encoding a 263 amino acid protein with a high degree of identity to several hypothetical proteins in plants, but with no detectable homology to bacteria proteins. Thus, Tha8 may be a plant-specific factor involved in the delta-pH mechanism. Several targeting prediction programs indicate that Tha8 is a chloroplast-localized protein. One intriguing feature of the delta-pH (Tat) translocase is that soluble factors are *not* required for *in vitro* translocation. Surprisingly, Tha8 is predicted to be a soluble protein. We are now exploring where Tha8 is found in the chloroplast. Is it in the thylakoid lumen or bound extrinsically to the stromal face of the thylakoid membrane? We will also determine whether Tha8 interacts with other delta-pH components (Hcf106, Tha4 and/or TatC) and what role it plays in the translocation process.

24:209

THREE BRICK GENES FUNCTION IN A COMMON ACTIN-DEPENDENT PATHWAY

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Cells of the maize leaf epidermis assume a wide variety of shapes; however, many of the mechanisms governing cell morphogenesis are not well understood. We isolated mutations in three Brick genes, which are required for polarized growth and division in epidermal cells. In wild type leaves, epidermal pavement cells have finger-like projections along the lateral margins, which interlock with those of neighboring cells. In *brk* mutants, epidermal pavement cells expand normally without forming these marginal lobes, resulting in rectangular-shaped cells. Previous studies on the morphogenesis of lobed epidermal and mesophyll cells in a variety of species have attributed lobe formation to the organization of cortical microtubules into bands that direct a non-uniform pattern of cellulose deposition and suggested a role for F-actin in microtubule organization. In expanding *brk* epidermal cells, microtubules rearrange and form bands, but the bands are less distinct than in wild type cells. However, localized enrichments of cortical F-actin seen at the tips of emerging and expanding lobes in wild type cells were never observed at any stage in expanding *brk* cells, suggesting an additional role for actin in lobe formation. Double mutant analysis showed that the phenotypes of *brk1;brk3*, *brk1;brk2* and *brk2;brk3* double mutants are the same as those of single *brk* mutants, indicating that all three *Brk* genes are involved in the same pathway affecting epidermal cell morphogenesis. Mosaic analyses revealed that *Brk1* acts non-cell autonomously while *Brk2* and *Brk3* act cell autonomously in pavement cells. Recently, the *Brk1* gene was cloned and shown to encode a small, novel protein, which is highly conserved in both plants and animals. These observations suggest that all three *BRK* proteins function in the same actin-dependent pathway, which may be conserved among eukaryotes.

25:197

Trans-silencing interactions at the purple plant locus

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Heritable epigenetic changes occurring at the purple plant locus (*pl1*) are reflected by visual differences of anthocyanin pigment levels and tissue-specific pigment patterns. This observation is due to the fact that the *PL1 R2R3-Myb* protein is a transcriptional activator of the genes encoding enzymes required for anthocyanin biosynthesis. The *PL1-Rhoades* allele (*Pl*) can confer relatively high levels of anthocyanin pigment production in many sporophytic tissues. However, this allele is unstable and can spontaneously change to a transcriptionally-repressed, meiotically-heritable, derivative designated *Pl1'-mahogany* (*Pl'*). The *Pl* allele exclusively changes to *Pl'* when carried in heterozygous condition with another *Pl'* allele; a phenomenological hallmark of paramutation (see Chandler et al 2000). In addition, stable maintenance of the *Pl'* state requires the presence of another *Pl* or *Pl'* allele on the homologous chromosome (Hollick and Chandler 1998). Two broad models are considered to explain the trans-silencing interaction between these alleles on homologous chromosomes: 1) physical homologue contact and 2) diffusion of silencing information between unpaired homologues. Based on similar experimental designs used to test chromosome pairing interactions in *Drosophila*, a series of translocation chromosomes have been tested for their ability to attenuate the trans-silencing interaction occurring at *pl1*. Evidence for a chromosome-pairing requirement from these studies has not been obtained. Analysis of mutations in *rmr* loci (required to maintain repression) indicate that both transcriptional and post-transcriptional regulatory mechanisms operate to maintain meiotically heritable repression of *Pl'* in *Pl/Pl'* plants (Hollick and Chandler 2001, Hollick et al. 2000; J Hollick, unpublished). These results raise the possibility that trans-silencing interactions at the *pl1* locus involve an RNA intermediate. Support of this model comes from loss-of-function alleles derived from *Pl* that simultaneously lose the ability to produce detectable *pl1* RNA and the ability to cause trans-silencing. Hollick, J.B. and Chandler, V.L. (2001) *Genetics* 157: 369-378. Hollick, J.B., Patterson, G.P., Asmundsson, I.M., Chandler, V.L. (2000) *Genetics* 154: 1827-1838. Chandler, V.L., Eggleston, W.B., and Dorweiler, J.E. (2000) *Plant Mol Biol* 43: 121-145. Hollick, J.B. and Chandler, V.L. (1998) *Genetics* 150: 891-897.

27:137

Development of AFLP markers and mapping of fertility restoration genes in sorghum

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Cytoplasmic male sterility (CMS) conferred by the sorghum IS1112C cytoplasm is an unusual two-gene gametophytic system requiring complementary action of two restoring alleles, designated Rf3 and Rf4, for individual pollen viability. Action of Rf3 is associated with enhanced transcript processing internal to mitochondrial orf107, a chimeric orf suspected as causal of CMS. Rf3 is tightly linked to the dominant allele Mmt1, which similarly is associated with enhanced transcript processing, 5' to mitochondrial urf209, the sorghum counterpart of maize orf25. No information is currently available regarding a possible mode of action for Rf4. Markers for the rf4 locus were identified through BSA-AFLP, conducted with a segregating BC3F1 generation, and subsequent conversion to STS/CAPS markers. Three such markers flanking the rf4 locus were identified, mapping to 5.31, 3.18, and 0.79 cM from the locus, as determined by scoring nearly 400 BC1F1 progeny. Two flanking markers were used in screening BAC libraries to identify the genomic location carrying the rf4 locus. Exon capture was utilized to recover and identify polymorphisms suitable for use in an RIL population, and the data were appended to the high-density genetic map of sorghum. A series of BAC clones representing a region of linkage group E were identified by the markers and assigned to coordinates 60.2-82 cM of LG E. We are using cDNA capture in an attempt to align the genomic region flanking rf4 with the syntenic region of rice.

28:148

Analysis of transposon induced mutations in the regulator of 3-deoxyflavonoid pathway and its implication in understanding the phytoalexins synthesis in sorghum

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Sorghum phytoalexins are one of the well-characterized compounds for their biochemical structures and fungi-toxicity to the anthracnose fungus, *Colletotrichum sublineolum*. However, biochemical route through which these sorghum anti-fungal compounds are synthesized has been speculative. One of the reasons for this gap has been the non-availability of genetically well-defined and related (in genetic background) mutant and wild type stocks. Molecular characterization of the sorghum candystripe phenotype (Chopra et al., 1999, PNAS) has allowed us to genetically dissect the pathway of flavonoids and phytoalexins. We will present results demonstrating the importance of sorghum as a genetic system to understand the complexity of the 3-deoxyanthocyanidin biosynthetic pathway in maize.

29:77

Sequence and physical map analyses of the maize and sorghum Rp1 complex uncovers numerous sites and unexpected mechanisms of local rearrangement

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Rp1 is a complex disease resistance locus in maize that provides race-specific resistance to the leaf rust disease caused by the fungus *Puccinia sorghi*. The Rp1 locus is exceptional in both allelic variability and meiotic instability. Genomic sequence analysis of three maize and two sorghum BACs from the Rp1 region revealed several interesting features. In one maize BAC of 95 kb, we found two Rp1 homologues and twelve other gene-homologous sequences, of which at least ten genes are truncated. Eight gene-homologous segments were found in 99 kb of a contig comprised of two overlapping maize BACs, of which two were Rp1-related and the other six were truncated. Thirteen of the truncated genes are found in three clusters, suggesting that they arose from multiple illegitimate break repairs at the same three sites or from dramatic repairs of each of these three sites with multiple unlinked DNA templates. A 43 kb region that contains an Rp1 homologue, six truncated genes and three Opie retrotransposons was found to be duplicated in this region. This duplication is relatively recent, occurring after the insertion of the three Opie elements. The break points of the duplication are outside of any genes or identified repeat sequence, suggesting a duplication mechanism that did not involve unequal recombination. Comparison of Rp1-homologous sequences in the region demonstrates a history of unequal recombination and diversifying selection within the leucine-rich repeat 2 region, resulting in chimeric gene structures. In the orthologous region of sorghum, a sequenced segment of 268 kb contains a cluster of five Rp1 homologues, of which two are truncated with N-terminal deletions. Only one of the Rp1 homologue appears to be potentially functional. The Rp1-homologous region in sorghum has several genes that are either duplicated, inverted, or both. Two Xa1-like genes are present downstream of the Rp1 cluster. A physical map of the Rp1 complex indicates the presence of eleven Rp1 homologues in a region of about 400 kb in the maize B73 inbred compared to six in 50 kb in the sorghum BTx623 inbred. Molecular evolutionary analysis suggests that either a single ancestral Rp1 gene gave rise to the known Rp1 genes (by unequal crossing over) in their respective lineages after the divergence of maize and sorghum or ectopic conversion has homogenized the Rp1 gene family within each species.

30:33

Mosaic Organization of Orthologous Sequences in Grass Genomes

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All grasses belong to a single family Gramineae that contains approximately 10,000 species. Family members include the important cereal crops rice, corn (maize) and wheat. Despite their vast diversity, comparative genetic mapping has revealed that grass genomes exhibit extensive conservation. Grasses have therefore been considered a single genetic system. The current consensus grass genome map was drawn based on low copy DNA markers anchored to the genetic map of each species. However, the question arises how chromosomal regions are organized between those markers at the DNA sequence level. Although BAC libraries of several cereal genomes have been constructed, single BAC clones are not large enough to allow comparisons of orthologous blocks of genes between smaller genomes like rice (0.43 Gb) and larger genomes like maize (2.7 Gb). Therefore, we used a minimum tiling path in maize and sorghum (0.77 Gb) to select chromosome regions that are orthologous to single BAC clones of two rice subspecies. This four-way comparison suggests that sequence divergence started from hotspots in chromosomes and expanded into non-conserved regions by accumulating micro-scale changes that are marked by gene amplification and translocation. Differential initiation and divergence of non-conserved regions during speciation resulted a mosaic organization of orthologous sequences. As a result, although an ancestral orthologous set of genes remains, each pair-wise comparison between species becomes unique. The prominent gene plasticity may also explain why there appears to be more gene sequences in plants.

32:17

Inspection of Maize Metabolism by NMR

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Information on metabolic networks and metabolic flux is the basis for metabolic engineering. The metabolism in developing maize kernels was analysed by quantitative nuclear magnetic resonance spectroscopy after ¹³C-labelling. Highly specific labelling patterns were obtained from the primary metabolites analysed and used to characterize the pathways leading to amino acids. The biosyntheses of key metabolic intermediates and of auxin was reconstructed from these data that revealed quantitative information about carbon flux. The metabolic fate of hexoses prior to incorporation into starch was also determined. In conclusion, this method can be used to study metabolic flux under various conditions, such as optimal versus stress conditions, for the comparison of different elite lines and the analysis of effects caused by expressing metabolic genes in transgenic plants.

33:23

Cytometrical Evidence that the Loss of Seed Weight in the *miniature1* Seed Mutant of Maize is Associated with Reduced Mitotic Activity in the Developing Endosperm

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The *miniature1* (*mn1*) seed, first described by Janet Lowe and Oliver Nelson (1946), is the most drastic nonlethal single gene seed mutation wherein the mutants lose >70% of the seed weight relative to the wild type. The causal basis of it is the loss of the *Mn1*-encoded cell wall invertase, seen specifically in basal transfer cells in developing endosperm (Plant Cell 4:297-305 and 8:971-83). We report here results from our newly developed three-dimensional model of endosperm, constructed from the longitudinal sections of normal (*Mn1*) and mutant (*mn1*) 16 DAP kernels. We used quantitative image analysis to determine spatial distribution of cells in relation to their size, nuclear DNA content (C-values) and the total number of cells in each endosperm. Several observations are noteworthy: (1) The total endosperm volume, was 31 mm³ with 740K cells in the wild type, and 8 mm³ (25%) with 410K cells (55%) in the mutant. (2) There was no detectable change in the level of endoreduplication in the two genotypes; except, the number of cells in each C-value class in the mutant was in the range of 40-60 % of the wild type. (3) A positive correlation between cell volume and C-values in both genotypes suggested that cell volume and endoreduplication were interdependent processes. Collectively, these data suggest that invertase-deficiency in the *mn1* mutant leads to, among other developmental and metabolic effects, reduced mitotic activity presumably due to the impaired in vivo release of hexose sugars, an essential signal for cell division in developing seeds.

34:26

GIANT EMBRYO: A Genomic Approach To Dissect Genetic Circuits Controlling Embryo/Endosperm Size

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In many crop plants including maize, the seed is composed of two compartments, the embryo and the endosperm, which are originated from the double fertilization event. These two structures are formed through complex genetic patterning and proliferation after fertilization. Genes involved in the pathways are in the process of being characterized. However, our knowledge still remains very limited. As part of our efforts to gain insights into the genetic circuits, we focused on the interaction between the embryo and endosperm during seed development. Especially, in order to learn how the embryo/endosperm size is controlled, we took a phenotype-to-gene approach using several new genomic strategies. We started analyzing the rice GIANT EMBRYO gene, whose loss-of-function mutations caused an enlarged embryo structure. We cloned the gene by defining its map position. The GE gene encodes a protein that belongs to the cytochrome P450 family. GE expression is detected in a spatially and temporally distinct pattern predominant on the epidermal layer of the scutellum that faces to the endosperm. Based on the expression pattern and the mutant phenotype, GE function is thought to control proliferation of the scutellum epidermis in coordination with the endosperm growth. Possibly, the function is mediated through the molecule modified by the prospective enzymatic activity of the GE protein. Such a hormonal molecule produced by GE is being analyzed through the use of newly developed metabolic profiling technologies. The knowledge obtained from GE in rice was further applied to understanding embryo size control in maize, where large embryo size is strongly correlated with the high oil trait. By using several genomic tools, we identified three maize genes very homologous to GE. The genomic sequence of BACs containing these genes revealed a syntenic relationship between rice and maize as well as an intrinsic problem of the ancient tetraploid structure of the maize genome. Potential functions of these genes were further analyzed by mapping, linkage analysis to high oil QTL and expression studies using the Lynx MPSS (Massively Parallel Signature Sequence) technology, which allowed simultaneous samplings of more than one million cDNA sequence from one cDNA library. We present these recent findings in our seed development research, implementing a forward genetic approach with new genomic tools.

35:89

The maize defective kernel 1 (dek1) gene encodes a membrane protein of the calpain gene super family

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Homozygous dek1 mutant grains carry endosperm without an aleurone layer and embryos that sometimes contain a root primordium, but lack shoot structures. We have used a novel dek1 allele, dek1-mum1 isolated in a microscopy screen of Pioneer Hi-Bred Internationals TUSC collection to clone the Dek1 gene. The predicted maize DEK1 protein of 2159 amino acid residues is identified as a member of the calpain (calcium requiring cysteine proteinases) gene super-family by the presence of a conserved cysteine proteinase domain shared by all calpains. In addition, maize DEK1 shares 70 % overall identity with its Arabidopsis orthologue, and contains both a novel and extensive N-terminus with five distinguishable domains, including 21 membrane spanning segments and a predicted extracytoplasmic loop region. That DEK1 appears to be the only representative of the calpain super-family in plants is central to our model for DEK1 function in aleurone cell development.

Proteomic analysis of modified opaque 2 maize endosperm**Gibbon, Bryan C. {1} Wang, Xuelu {1} Larkins, Brian A. {1}**

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The opaque2 mutation (o2) results in kernels with soft, floury endosperm, reduced zein content and increased levels of essential amino acids, such as lysine. Although the increased nutritional quality of o2 mutants is highly desirable, the soft kernels associated with this mutation are prone to damage and are not suitable for agricultural production. These deficiencies led to the development of o2 lines with hard endosperms that retain the nutritional quality of o2 mutants. However, the mechanism for the conversion of soft endosperm to hard endosperm is not known. We have begun a proteomic analysis of wild type, o2 and modified o2 (Mo2) lines to better understand the basis of the hard endosperm phenotype. Comparison of 2-D SDS-PAGE gels revealed changes in several abundant proteins associated with hard kernels. The most prominent change is a 56 kDa polypeptide, which was identified as the Waxy1 gene product (granule bound starch synthase) by mass fingerprinting. Measurements of starch amylose content and Waxy1 protein content demonstrated no relationship with kernel hardness. Further analysis showed that the starch grains of the Mo2 lines swell to approximately twice the volume of those in wild-type and o2. Analysis of the distribution of amylopectin branch lengths revealed that starch from modified lines has a lower degree of polymerization than wild-type or o2 starches. Together, these data suggest the increased extractability of Waxy1 protein from Mo2 endosperm starch is due to altered starch granule structure. Therefore, differences in starch composition resulting from the activity of another starch synthesis enzyme may contribute to modification of endosperm texture in o2 backgrounds.

Bioinformatics

1:67

TASSEL: a software package for Trait Analysis by aSSociation, Evolutionary, and Linkage.

Buckler, Edward

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TASSEL is a software package that can analyze diversity among groups of sequences, SNPs, or SSRs. It can then relate the genotypic variation to phenotypic variation. Diversity and linkage disequilibrium statistics can be easily calculated and then graphed. Trait associations can be carried out using ANOVA or by controlling for structured populations using the logistic regression ratio test. Key features of the software include: written in Java so it can be run on multiple platforms, extensive use of the standard PAL library, ability to interact with databases, analyses insertions/deletions, good graphical interface, and easy integration of phenotypic and genotypic data. The software will be demonstrated, and it can be downloaded at <http://statgen.ncsu.edu/buckler/bioinformatics.html>.

2:228

Comparative Bioinformatic Analysis of Genetic Maps From Rice and Maize

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Comparative genomics research facilitates the identification and manipulation of agronomically important genes and traits among crops by utilizing information from various genome databases, sequence depositories, and other information systems. For the integration and comparison of information, bioinformatics provides and uses the tools to analyze and visualize these genomic data. In collaboration with the Japanese Rice Genome Research Program (RGP), we have developed software cMap, a tool for graphical genome mapping comparison with text lists of shared locus information within or across species. By using cMap, genomic information from various maize genetic maps and rice RGP2000 map has been compared and analyzed. Intra genomic comparisons reveal duplicated regions in the maize genome. Comparing several maize maps where different SSRs have been resolved facilitates marker selection, as there are only 0.4% of SSRs among SSR maps that have two or more loci whereas other maps such as UMC98 and BNL96 at least have 20% of markers that hit two or more loci. Therefore, SSR can be used as a specific marker for the identification and manipulation of genes and traits in comparative genomic research. Inter genomic comparisons between rice RGP2000 map and maize UMC98 or Pioneer99 composite map confirm that maize has more duplicated regions of the genome than rice and show that syntenic relationships are revealed in some regions of chromosomes between rice and maize and need more underlying probe data in support. Application of cMap is accessible at <http://www.agron.missouri.edu/cMapDB/cMap.html>. Supported by NSF Plant Genome Grant DBI 9872655.

3:140

Phylogenetic and Computational Analysis of Myb Gene Evolution and Regulation

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Myb domain proteins contain a conserved DNA-binding domain comprised of one to three conserved repeat motifs. In animals, Myb proteins are encoded by a small gene family and commonly contain 3 repeat motifs (R1R2R3); whereas, plant Myb proteins are encoded by a very large and diverse gene family in which a motif containing 2 repeats (R2R3) is most common. One goal of our project is to clarify the evolutionary history of the Myb gene family in plants, especially maize and rice. As a pilot study, we analyzed the entire set of Myb genes from Arabidopsis and Oryza sativa (genomic sequences obtained from TIGR and Monsanto Rice Research Org., respectively). Generally, the plant R2R3 Myb genes encode proteins with a conserved N-terminal Myb domain, and a highly variable C-terminal region. In Arabidopsis, the 107 Myb genes are distributed randomly among the five chromosomes. Sequence comparisons suggest that most extant Myb genes were generated during several recent episodes of gene duplications. Interestingly, the pattern and timing of Myb gene duplications appears different among the five Arabidopsis chromosomes. A second goal is to identify, with the assistance of computational techniques, conserved functional motifs that regulate gene expression in specific tissues. A highly conserved scheme of TATA-box and transcription start site sequences was found in a group of p-like orthologous Myb genes. A CA-box was also detected in the 5'UTR region of these Myb genes. The functional significance of these motifs is being tested in transgenic assays.

4:56

Evaluation of Phylogenetic Footprinting to Identify Promoter Regulatory Elements in Maize and Rice Genes

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Maize and rice are grasses that are separated by approximately 50 million years of evolution, yet their genomes still exhibit a high degree of gene conservation. Comparison of promoter sequences from orthologous genes among related species, also known as phylogenetic footprinting, is a powerful approach to identifying sequence elements that have been conserved throughout evolution, presumably because of their functional importance in regulating gene expression. Although comparative studies of noncoding DNA sequences have been done for genes from bacteria, yeast, nematodes, Drosophila, and mammals, relatively few examples exist for maize and rice genes. Sixty genomic sequences from putative orthologous pairs of maize and rice genes have been compiled from publicly available databases. The promoter DNA sequences for these gene pairs represent a sequence space of nearly 120 kb. A variety of existing analysis tools were used to identify conserved sequence blocks among orthologous maize and rice gene promoter sequences. These tools include VISTA, DNA Block Align (DBA), pairwise BLASTN alignments (Bl2seq), Dialign, and dot matrix plots. The different software tools were evaluated for their ability to predict conserved regulatory promoter sequence elements that are supported by biochemical studies of DNA-protein interactions and functional promoter activity assays. Among these tools, VISTA performs the best at predicting known regulatory motifs. To confirm the functional significance of sequence blocks that are conserved between maize and rice promoters, we are using these conserved sequences as substrates in DNA-protein interaction assays. The results from our initial evaluation of promoter sequences from the orthologous maize and rice Waxy1 genes will be presented.

5:44

Proteomic Analysis of Maize Rachis Tissue in *Aspergillus flavus* Resistant Inbreds

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Several maize inbred lines resistant to *A. flavus* infection and aflatoxin contamination have been developed by ARS scientists at Mississippi State University. Our goal is to identify proteins in these lines that may be involved in resistance to fungal infection by comparing proteins separated by 2-D gel electrophoresis (2-DE) from the rachis (cob) tissue of resistant and susceptible inbreds. Rachis was selected because studies with GFP-tagged *A. flavus* indicated that fungal growth was blocked in this tissue in resistant lines (see poster Magbanua et al.). Inbreds were tagged at silk emergence and rachis samples were collected at various intervals during ear development. The 2-D gels shown here represent rachis samples collected 21 days after silking from the resistant line Mp313E and susceptible line Sc212M. Analysis of rachis 2-D gels with PDQuest software (BioRad) revealed 300 Coomassie blue-stained proteins in each genotype. There were 25 proteins specific to Mp313E and 20 proteins specific to Sc212M. MALDI-TOF MS analysis of one "spot" that was shifted in position between the two inbreds indicated that it was a member of the RP1-D family of transcription factors. MALDI-TOF MS will be used to identify the proteins that differ between these two inbreds and other proteins that may be specific to the rachis.

6:3

MaizeDB 2002

Polacco, Mary {1,2} Coe, Ed {1,2} Hancock, Denis {1} , Fang, Zhiewei {2}, Schroeder, Steve {2}, Sanchez-Villeda, Hector {2}

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MaizeDB, www.agron.missouri.edu focuses on documentation of genome maps and associated gene functions and expression. Functionality includes gene products, both empirically determined or inferred by sequence similarity, phenotypic variation and agronomic traits. Documentation includes data sources, published literature, researchers with updated addresses, available genetic stocks, mutant images, available DNA clones, screening images, map scores and recombination data and in addition, relevant records in external databases. In this way, MaizeDB both credits sources and facilitates evaluation and reproducibility of data.

What good are data if you can't access them? The computer demonstration and poster will highlight new accesses and their potential to facilitate discovery and to drive hypothesis-based research:

- simplified form queries which return tables, rather than lists of symbols;
- a BLAST server that supplies map data and links to relevant databases in addition to sequence alignments and scores; external database include ZmDB, CUGI, TIGR and GenBank.
- hypertext, dynamic map viewers for genetic/physical map and for comparison of genetic maps, both inter- and intra species. We thank the [Rice Genome Program, Tsukuba, JP](#) for source code to the GIOT map display, the NSF Plant Genome Program DBI 9872655 for funding of the US [Maize Mapping Project, www.maizemap.org](#), the USDA for funding of MaizeDB.

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Analysis of Diverse Germplasm using Microarrays

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Diverse maize germplasm has varying levels of protein, starch, and oil. Upwards of 100 genes are involved in the protein biosynthesis pathways alone. Two of many possible transcriptional factors responsible for coordinately regulating protein accumulation during the development of the maize kernel, opaque 2 (o2) and prolamin binding box factor (pbf), have been sequenced and analyzed previously. Both proteins are only expressed in the developing endosperm. Using a sliding window of diversity across the gene o2 has over 6% diversity in some regions of the gene and pbf has a maximum of 0.1% diversity with up to 11 fold decrease in diversity relative to *Z.m. ssp. parviglumis*. To investigate expression levels of these transcription factors and to identify additional candidate genes of energy biosynthesis in the maize kernel we will use microarrays produced by the Maize Gene Discovery group that are specific to the genes expressed during maize endosperm development. Ears were harvested from 45 diverse inbred lines and genetic stocks 14 days after pollination throughout the summer in North Carolina. Total RNA was isolated, labeled and hybridized to arrays in a loop design. We present the results of expression profiles of diverse lines and association testing with kernel phenotypes that have been observed.

8:85

Relationships among maize (*Zea mays*) and Arabidopsis SET domain proteins

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Histone proteins play a central role in chromatin packaging, and modification of histones is associated with chromatin accessibility. SET domain (Su(var)3-9, Enhancer-of-zeste, Trithorax) proteins are one class of proteins that have been implicated in regulating gene expression through histone methylation. The relationships of twenty-two novel maize SET domain proteins and thirty-two Arabidopsis SET domain proteins were evaluated by phylogenetic analysis and domain organization. We provide evidence for five classes of SET domain proteins in plants that can be further divided into seventeen orthology groups. In some cases, such as the Enhancer of zeste-like and trithorax-like proteins, plants and animals contain proteins with a similar domain organization. However, the majority of plant SET domain proteins do not have an animal homolog with similar domain organization. The domains unique to plant SET domain proteins have generally been implicated in protein-protein interactions, indicating that most SET domain proteins work in complexes. The structural diversity among plant and animal SET domain proteins suggests that these complexes are different in plants and animals. Combined analysis of the maize and Arabidopsis SET domain proteins reveals that duplication of SET domain proteins in plants is extensive and has occurred via multiple mechanisms. Plants contain a large family of SET domain proteins that may play specific roles establishment of a histone code that controls developmental and epigenetic regulation of gene expression.

9:117

Sequence diversity in three defense-related genes in North American inbred lines, land-races, and teosinte

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In order to explore the diversity and evolutionary history of plant defense genes in natural and domesticated *Zea* species I surveyed nucleotide diversity at three defense related genes in North American inbred lines, maize land-races, and the teosinte *Zea mays* ssp. *parviglumis*. These genes (*wip1*, *mpi*, and *cpi2*) code for protease inhibitors, a central function of which is thought to be plant defense against herbivores and pathogens. For each of these genes sequence diversity in the land-races was similar to diversity in teosinte. However, for *mpi* and *wip1*, diversity in the inbred lines was considerable lower than diversity in either land races or teosinte. Linkage disequilibrium between polymorphic sites in the inbred lines extended much further than linkage disequilibrium in the land-races or teosintes. Linkage disequilibrium in the inbred lines also extends much further than in most other genes for which linkage disequilibrium in maize has been examined. These results may reflect differences in the population-level diversity of defense and neutral genes in the populations from which the North American inbred lines were derived.

10:224

CONTROLLED POLLINATIONS OF MAIZE

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A website has been created that covers the various methodologies that are widely used by many researchers, at the University of Missouri-Columbia, USA, who perform controlled pollinations of maize/corn. The website (<http://www.agron.missouri.edu/IMP/WEB/pollen.htm>) provides detailed descriptions of several relevant topics as well as digital video clips which illustrate various aspects of the methodology of controlled pollination. Topics covered are: Ear shoot bagging, Cutting back, Pollen production, Pollination (collecting pollen, tassel bagging, performing multiple pollinations), Some precautions (contamination, pollen viability), Dating tassel bags, Supplies and equipment and Useful references. Each digital video clip is in AVI and Quicktime formats. Each video format is provided in three levels of quality (high, medium, low), corresponding to relative file size. The different qualities are intended to facilitate access by those accessing the internet via a range of access speeds. This is a good facility for new assistants (and seasoned researchers) to consult prior to and during the maize growing season. *Funded by NSF Plant Genome Grant DBI 9872655. [Poster]*

11:225

THE PLANT ONTOLOGY™ CONSORTIUM (POC) AND PLANT ONTOLOGIES

Vincent, Leszek {1} Coe, Edward {1} Polacco, Mary {1}

{1} University of Missouri-Columbia

Plant genomic databases need to accurately and consistently document features (e.g. gene products, functions, phenotypes, traits, developmental stages, anatomy, morphology), using a syntax that facilitates inter-database searches. This interoperability will enable comparative genomic strategies to elucidate plant functions. The Plant Ontology Consortium (POC) is applying and extending the Gene Ontology (GO) paradigm to knowledge domains pertinent to plant taxa. The GO paradigm is making it possible to annotate homologous gene and protein sequences in many databases for multiple organisms using a common vocabulary (<http://www.geneontology.org>). The POC aims at providing ontologies and controlled vocabularies for monocot and dicot plant taxa - initially *Zea mays*, *Oryza sativa* and *Arabidopsis thaliana*, but extending to other important taxa in due course. The POC aims to facilitate the communications, productivity and collaborations amongst the core participants of the POC involved in developing ontologies and controlled vocabularies for some monocot and dicot taxa. Further aims: numerical growth of participants, extended collaboration with the research of the GO Consortium, provision of educational opportunities in this area of bioinformatics research. It is anticipated that the POC will impact the bioinformatics research of other national and international plant-based research groups/researchers (e.g. soybean, sugarcane, cassava, potato, tomato, trees, grains etc.), via the provision of ontology products, community resources and educational inputs. A sample of ontology and controlled vocabulary for leaf morphology of maize, will be presented. *Funded by NSF Plant Genome Grant DBI 9872655.* [Poster]

Biochemical Genetics

12: Insight into the roles of debranching enzymes in starch biosynthesis from carbohydrate profiling of an allelic series of *sugary1* mutants.

Colleoni, Christophe {1} Myers, Alan M. {1} James, Martha G. {1} {1} Iowa State University

Two conserved forms of starch debranching enzymes are present in plants, termed isoamylase- or pullulanase-type DBE. Isoamylase-type DBE is required for normal starch metabolism, although its precise function is unknown. Investigation of this problem is complicated by the fact that mutations in the maize gene that codes for the isoamylase-type DBE, *su1*-, also affect activity of the pullulanase-type enzyme. In this study the enzymatic activities of the two DBE isoforms were determined in a series of *su1*- mutants that condition kernel phenotypes of different severity. The content of specific carbohydrates and the structure of starch was also determined in the mutant kernels, with the goal of correlating certain compositional and structural defects with specific changes in enzyme activity. *su1-Ref* and the null allele *su1-4582* condition a severe phenotype with translucent, shrunken and glassy kernels; less severe intermediate phenotypes result from *su1-Bn* and *su1-st*; weak kernel phenotypes typically indistinguishable from wild type result from *su1-P*, *su1-am*, and *su1-66*. The levels of glucose, fructose, sucrose, and phytylglycogen typically are very low in nonmutant kernels and increase in accordance with the phenotypic strength of the *su1*- mutation. Higher than normal amylose:amylopectin ratios also are observed in the mutants. Characterization of the chain length distributions in amylopectin from plants homozygous for the severe alleles showed enrichment of short chains containing 4-13 glucosyl residues, and corresponding decreases of chains from 33-70 residues in length. These structural modifications are consistent but less extreme in plants homozygous for alleles that condition the weak or intermediate phenotype. In contrast to these distinct differences in the carbohydrate chemotype, the activities of isoamylase- and pullulanase-type DBE activity conditioned by the various *su1*- alleles all were fairly similar. One- or two-dimensional zymogram analyses revealed that isoamylase-type DBE activity is not detectable in *su1-Ref*, *su1-4582*, or *su1-st* kernels, and is greatly reduced in kernels bearing the weak mutations *su1-P* or *su1-am*. A specific assay for pullulanase-type DBE showed that this activity is reduced by no more than 50% in any of the mutants, and there are not great differences among the alleles. Therefore, there was no obvious correlation between the amount of isoamylase- or pullulanase-type DBE activity and the severity of the starch metabolism defect. We conclude that minimal isoamylase-type DBE activity is sufficient to confer relatively normal starch production. If this DBE is the critical factor, the threshold of required activity is very low. Alternatively, as yet uncharacterized pleiotropic deficiencies may be involved in the explanation of why DBE mutations can drastically affect starch biosynthesis.

13:74

ADP-glucose pyrophosphorylase activity from maize-potato mosaics

Cross, Joanna M.F. {1} Greene, Thomas W. {2} Shaw, Janine R. {1} Clancy, Maureen {1} Okita, Thomas W. {3} Schmidt, Robert R. {1} Hannah, L. Curtis {1}

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The allosteric enzyme adenosine diphosphate glucose pyrophosphorylase (AGPase) catalyses the synthesis of ADP-glucose, a rate limiting step in starch synthesis. Plant AGPases are heterotetramers activated by 3PGA and inhibited by Pi. The objective of these studies is to identify regions in the subunits important in regulation. We exploit an E.coli expression system and mosaic AGPases composed of potato tuber and maize endosperm subunit fragments to unravel this question. While potato and maize subunits have long been separated by speciation and evolution, they are sufficiently similar to form active mosaic enzymes. Since potato tuber and maize endosperm AGPases exhibit different regulatory properties, comparing the kinetic properties of the mosaics to those of the maize endosperm and potato tuber AGPases has enabled us to identify regions important in regulation. The data presented here conclusively show that both subunits are involved in the allosteric regulation of AGPase. In addition, extent of 3PGA activation and 3PGA affinity were found to be separate entities which mapped to different regions in both subunits.

14:120

Analysis of a null *zpu1* mutation indicates a role for the pullulanase-type debranching enzyme in both the synthesis and mobilization of maize starch

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Plants contain two distinct isoforms of alpha-(1,6) glucan hydrolase, both of which are very highly conserved. Genetic analysis of *sugary1* (*su1*) mutations indicates that the isoamylase-type DBE isoform is required for normal starch biosynthesis. To date, however, no mutation affecting a pullulanase-type DBE has been described. Here we report the first such mutation, a TUSC-generated null allele of *Zpu1* (*zpu1-204*). This mutation is caused by insertion of a *Mutator* transposable element in the first exon of the gene. Mutant plants and kernels do not exhibit obvious morphological defects, but are impaired in their ability to degrade transient starch in leaves and storage starch in germinating seeds. Thus, the hydrolytic activity of the pullulanase-type DBE functions in starch turnover. Starch abundance and structure in mutant *zpu1-204* kernels at mid-development are essentially the same as for wild-type kernels. However, the mutants accumulate a small, branched water-soluble polysaccharide (WSP) that is not present in wild type, and are deficient in small, linear WSP. These data are consistent with a role for ZPU1 in glucan hydrolysis during starch formation in kernels. Plants were generated that are doubly mutant for *zpu1-204* and *su1-st*. That latter mutation conditions a weak kernel and starch phenotype, but is largely deficient in isoamylase-type DBE activity. These plants had a markedly more severe kernel phenotype and starch production abnormality than did either single mutant. We conclude that the pullulanase-type DBE functionally compensates for the isoamylase-type DBE, and thus that it has a function in starch biosynthesis as well as starch degradation. Zymogram analysis of starch metabolic activities in *zpu1-204* kernel extracts showed that, in addition to completely eliminating ZPU1 activity, the mutation negatively affects the enzymatic activity of starch branching enzyme IIa (BEIIa). The effect on BEIIa activity is post-translational, because the BEIIa polypeptide is present at near-normal levels. The same pleiotropic effect on BEIIa results from the *su1-st* mutation, and *su1-* mutations in general also indirectly affect ZPU1 activity. Such prevalent pleiotropic effects indicate complex relationships between these enzymes of starch metabolism, which remain to be defined at the molecular level.

15:6

Two glucosyltransferases are involved in detoxification of benzoxazinoids in maize

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Benzoxazinoids are major compounds of the chemical defense in grasses. These toxins are stored in the vacuole as glucosides. Two glucosyltransferases, BX8 and BX9, that catalyze this last step of benzoxazinoid biosynthesis have been isolated via functional cloning. No close relative of these maize genes was found among the known glucosyltransferases. The enzymes display a very high degree of substrate specificity. DIMBOA, the major benzoxazinoid in young maize, is the preferred substrate. Both genes are highly expressed in the young maize seedling, the developmental stage with the highest activity of benzoxazinoid biosynthesis. Bx8 is included in the cluster of DIMBOA biosynthesis genes that is located on the short arm of chromosome 4. Hence, the gene cluster comprises three different enzymatic functions and a complete set of genes for the biosynthesis of DIMBOA glucoside. Bx9 mapped to chromosome 1. Expression of Bx8 and Bx9 in *Arabidopsis* corroborated the potency of the enzymes to detoxify their substrates. This capacity might have implications on allelopathic interactions.

16:20

The CIF/VIF-related proteins in maize: Search for invertase inhibitor homologs

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For the regulation of apoplastic and vacuolar invertases, post-translational control of their activities via direct interaction with inhibitory/regulatory proteins has emerged as a central mechanism. Via specific protein-protein-complexes with their target enzymes, these inhibitors may cause the rapid and efficient silencing of enzyme activity. We have previously cloned the first invertase inhibitor cDNAs from tobacco, namely Nt-CIF1 and Nt-VIF1 (cell wall/vacuolar inhibitor of α -fructosidase). Starting from the maize EST database, we have now cloned cDNAs encoding 4 CIF/VIF-like proteins. These putative invertase inhibitor homologs were named Zm-CIF/VIF-RP(related proteins)1-4, as their *in vivo* functions have not yet been determined. Regarding the limited sequence conservation, it cannot be predicted whether all CIF/VIF-RPs are bona fide invertase inhibitors. In fact, protein sequences of Zm-CIF/VIF-RPs were equally similar to Nt-CIF1/VIF1 and to a recently described pectin methylesterase inhibitor from kiwi fruit. All predicted Zm-CIF/VIF-RP ORFs contain signal peptides for the secretory pathway. Furthermore, in all Zm-CIF/VIF-RPs 4 cysteine residues are found at conserved positions. Zm-CIF/VIF-RP1-4 transcripts were most abundant in anthers and/or pollen; only for isoform 3 were transcripts detected in other tissues. Zm-CIF/VIF-RP1-4 were expressed as recombinant proteins in *E. coli* for further functional studies. Recombinant Zm-CIF/VIF-RP1 affected tomato vacuolar invertase *in vitro*. When fusions of Zm-CIF/VIF-RP1 with GFP were delivered to maize suspension culture cells via particle bombardment, the fusion protein was detected in the vacuole(s) (CLSM), indicating that the vacuolar invertases IVR1 and IVR2 may be the *in vivo* targets of Zm-CIF/VIF-RP1. Localization studies for Zm-CIF/VIF-RP2-4 are under way.

17:134

Identification of putative binding sites for the maize ID1 transcriptional regulator.

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The indeterminate gene (*id1*) is an important regulator of the transition to flowering in maize. Maize plants that are homozygous for the recessive *id1* mutation have an extended vegetative growth phase, where mutant plants make many more leaves than normal plants and often form aberrant flowers with vegetative characteristics. The *id1* gene was cloned and found to encode a zinc-finger protein that is similar to a class of transcription regulators found in other species. Searches of available genome databases revealed that *id1* is the founding member of a highly conserved family of zinc finger proteins found in all higher plants. The similarity of the deduced ID1 protein to transcription factors suggests that it acts by controlling the expression of genes that participate in signaling the transition to reproductive growth in maize. Expression studies show that *id1* mRNA and ID1 protein are confined to immature leaf tissue, suggesting that *id1* acts by regulating transcription of genes in leaves. To understand the role played by *id1* in controlling the transition to flowering, we are trying to identify potential targets recognized by the ID1 protein. Preliminary experiments are focused on identifying the DNA sequence that is recognized by the ID1 protein. Using ID1 protein expressed in *E. coli* and in vitro binding assays, a possible consensus sequence for ID1 binding was identified. Determination of ID1 binding sites may help to distinguish the potential direct targets of *id1* from other genes that are selected in microarray and other differential display experiments aimed at identifying genes controlled by *id1*.

18:188

Characterization and functional analysis of a maize cDNA encoding betacarotene hydroxylase

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Carotenoids represent a class of over 600 compounds with a 40-carbon backbone derived from 5-carbon isoprenoid units. In plants, they are essential in photosynthesis and serve as precursors to abscisic acid. This undergraduate research project focuses on a cDNA encoding the enzyme betacarotene hydroxylase, which catalyzes the addition of hydroxyl groups at position three on the cyclic rings of betacarotene to form zeaxanthin, a xanthophyll. We identified and fully sequenced a maize (*Zea mays*) EST encoding a protein with 56% identity to a homolog from the dicot *Arabidopsis thaliana*. The 1.38-kb maize cDNA, cloned into *XhoI/EcoRI* sites of pBluescript SK(-), encodes a 461-amino acid polypeptide. Since sequence homology implies, but does not always indicate enzyme specificity and activity, we decided to use a heterologous bacterial system to demonstrate function of the putative maize betacarotene hydroxylase. However, the predicted protein encoded by the maize cDNA was not in-frame with the upstream sequences encoding beta-galactosidase, preventing direct use of the cloned cDNA to test in vivo function of the encoded product. Therefore, we identified a *BamHI* site just upstream of the cloned cDNA for directed modification that would result in expression of an in-frame fusion protein. The re-engineered cDNA can then be tested by co-transformation with a second plasmid conferring accumulation, in *E. coli*, of the betacarotene hydroxylase substrate, betacarotene. Function of the maize gene product is expected to result in accumulation of zeaxanthin and, possibly small amounts of the monohydroxy carotenoid betacryptoxanthin, altering the colony pigmentation and which will be further confirmed by HPLC analysis. Our progress to date will be reported. This undergraduate research project is funded by the National Institutes of Health.

19:182

Characterization of the maize gene family encoding geranylgeranyl pyrophosphate synthase, a key enzyme in biosynthesis of plant isoprenoids

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Isoprenoid compounds, including carotenoids, abscisic acid, gibberellins, sterols, phytoalexins, etc., are derived from geranylgeranyl pyrophosphate (GGPP), a C₂₀ compound that is synthesized in different subcellular compartments by sequential addition of three isopentenyl units to dimethylallyl pyrophosphate. In plants, this reaction is catalyzed by the enzyme geranylgeranyl pyrophosphate synthase (GGPPS). Previously we cloned maize cDNAs encoding the putative GGPPS by a functional approach using *Escherichia coli* cells expressing genes for carotenoid biosynthesis absent a functional GGPPS. Given that GGPPS shares structural features with other prenyl transferases, it was not surprising that the sequence was misidentified in the literature as an FPPS (farnesyl pyrophosphate synthase). For additional evidence that the isolated maize cDNAs encoded GGPPS and not FPPS, we tested whether the cloned GGPPS cDNA could complement a yeast *erg* mutant defective in FPPS activity. A 1.425-kb *XhoI-EcoRI* insert from pGGPPS1030 containing a putative *Ggps* cDNA was subcloned in both orientations into pDD62, a yeast expression vector. Since neither subclone complemented the *erg* mutation, as was seen with an FPPS cDNA from *Arabidopsis*, we concluded that the cloned maize cDNAs did indeed encode GGPPS and not FPPS. To further characterize genes encoding maize GGPPS, we used the GGPPS cDNAs to screen a maize B73 BAC library. Ten clones were isolated and are being grouped into gene family members by Southern analysis and DNA sequencing. Twenty-two *HincII*-derived fragments from the ten maize B73 BAC clones were found to hybridize with the 1.425-kb *XhoI-EcoRI* fragment from pGGPPS1030. Additionally, some of these fragments also hybridized to a 35-mer oligonucleotide designed to the 5'-end of the *Ggps* insert. The *HincII* fragments, some of which are shared among the BAC clones, range from 1.3 to 8 kb. Thirteen unique clones, out of the 22 original *HincII* fragments, have been subcloned in the *HincII* site of pBluescript II SK(). Currently, Southern blots are being produced to test for true positive clones, by using both the entire 1.4-kb *XhoI-EcoRI* insert from pGGPPS1030 and the 5' oligonucleotide.

20:9

Characterization of maize nitrilase genes and enzyme activities: implications for auxin biosynthesis

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We isolated two nitrilase genes, NIT1 and NIT2. The deduced amino acid sequences show 68% identity and 76% similarity. NIT2 displays especially high identity/similarity (75%/83%) to *Arabidopsis* NIT4 which is involved in detoxification of cyanide. However, in vitro enzyme activity tests with heterologously expressed nitrilases revealed that maize NIT2 has no substrate specificity for the detoxification intermediate (beta-cyanoalanine), but shows high activity towards indole-3-acetonitrile, the immediate precursor of IAA in Trp-dependent biosynthetic pathways. Western analysis indicated the existence of nitrilases in maize kernels where Trp-dependent pathways play a major role in IAA biosynthesis. These results suggested the involvement of maize nitrilases in auxin biosynthesis. To confirm this hypothesis, we isolated *nit1* and a *nit2* mutants by using the Mu element. However, there were no clear phenotypical changes in the single mutants. Currently the preparation of the double mutant is in the progress.

21:131

Molecular Analysis of Zein Accumulation in the Illinois Protein Strains

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The University of Illinois has been conducting a long term selection experiment for changes in the relative concentrations of grain protein. Initiated in 1896, this selection experiment is now in its 100th generation and has resulted in the development of 4 strains, all derived from the same source population, that span the extremes for grain protein composition. These Illinois Chemical Strains represent a unique genetic resource to investigate questions related to the response of quantitative traits to selection and the physiological and molecular mechanisms that influence the ability of corn plants to assimilate, translocate, partition and store carbon and nitrogen, especially within the seed. We have begun to associate changes in gene expression with the divergent grain protein phenotype in the Illinois Protein Strains: Illinois Low Protein (ILP), Illinois High Protein (IHP), Illinois Reverse Low Protein (IRLP), and Illinois Reverse High Protein (IRHP). Our initial focus is on zeins because the changes in protein content among the strains are largely due to differential accumulation of these proteins. It is also known that zein accumulation responds to soil nitrogen. To investigate whether zein responses to the Illinois Protein Strain genotypes and the environmental effects of soil N have a common molecular basis, we have grown the Illinois Protein Strains and their hybrids under different rates of soil N. The F1 plants and grain are being analyzed for differences in protein levels by NIR, zein by SDS-PAGE, and zein mRNA expression.

22:75

Evaluating the Growth of *Aspergillus flavus* with Flavanoids and their Derivatives

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Aflatoxin is a naturally occurring mycotoxin produced by *Aspergillus flavus* and *A. parviticus*. It is the most potent natural carcinogen found. The U.S. Food and Drug Administration prohibits the sale of grain with aflatoxin levels exceeding 20 parts per billion (ppb). Once maize is found to be contaminated with aflatoxins, very few detoxification and utilization options are available. Some flavanoid compounds and derivatives can alter the growth rate of fungi including *A. flavus* or the amount of toxin produced. In maize a defect in chalcone synthase (*c2*), a gene controlling the rate-limiting step in anthocyanin biosynthesis, results in a 7-fold increase in toxin production. Our objective is to determine the effect of hesperetin, quercetin, rutin, and naringenin on *A. flavus* growth and aflatoxin production. *A. flavus* strain NRRL3357 was grown on Czepak's media with 10g/L NaCl. The media was supplemented with hesperetin, rutin, naringenin, and quercetin at concentrations of 50 M, 100 M, 150 M, 200 M, and 250 M. Fungal growth was measured from digitalized images at 2, 4, 6, 8, 10, 12, and 14 days after inoculation with a 1 cm square of mycelia. Aflatoxin levels were assayed on fungal sample at day 14. All treatments reached maximal growth by 10 days after inoculation. At concentrations of 250 M rutin, 250 M quercetin, and > 100 M of hesperetin fungal growth is slower. Naringenin had no apparent affect on growth. Additional flavanoids and their derivatives will be analyzed in future experiments. With this information we hope to identify which enzymatic reactions are critical to reducing aflatoxin production and use this information to identify naturally occurring maize alleles that can be used to produce commercially acceptable maize varieties that inhibit toxin production.

Cytogenetics

23:58

Discovery and characterization of genes controlling meiosis in maize

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We are using an integrated strategy of forward and reverse genetics combined with cytology to identify, characterize and clone new meiotic genes in maize. We are focusing on meiotic prophase, especially on problems of initiation of meiosis, homology chromosome search and synapsis, and meiotic recombination. There were 38 meiotic mutants in 1999, and they represented mutations of 19 meiotic genes. The collection included well known meiotic mutants that have been discovered since the 1930s, Inna Gs original mutants induced by N-nitroso-N-methylurea in 1973, and some mutants that have been isolated in 1991 and 1993 from active Mu stocks in collaboration with W. F. Sheridan (UND, North Dakota). Since 1999 we have isolated thirteen new meiotic mutants using Mu transposon mutagenesis as well as EMS treatment (UC Berkeley). Now we have a collection of over 50 meiotic mutants in maize. Preliminary results of allelism tests with ten of the new mutants indicate that they each represent new genes. Further genetic analysis of the new mutants is in progress. We currently have mapped a few meiotic genes. The *pam1* and *Mei025* genes have been localized at chromosomes 1 and 5, correspondingly, using crosses with T-waxy series. Three other meiotic genes *dsy2*, *mms25*, and *afd1*, have been fine mapped at chromosomes 5, 4, and 6, respectively, using SSR's molecular markers. The *psh1* (poor homologous synapsis 1) gene encoding a new protein is likely cloned. We are working toward a comprehensive characterization of the well-known and new mutants using fluorescence in situ hybridization (FISH) coupled with 3-D microscopy to study bouquet formation, chromosome homologous synapsis as well as synapsis of specific 5S rDNA loci (distal region of 2 L) and their relationship. We quantitatively described the pattern of chromosome pairing in nine new mutants, and monitored the installation of the recombination machinery in maize mutants by studying the distribution pattern of recombination protein Rad51. The project is supported by grants from NIH and TMRI/PMB.

24:145

A Cytogenetic Map of Maize

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We present our current cytogenetic map of maize. This map is based on real images of 3-D pachytene chromosomes, overlaid with genetically mapped breakpoint data collected from the maize community, and with our 3-D FISH spots which correspond to a few genetically mapped loci. The maize community has generated a wealth of genetically mapped chromosome breakpoints. Much of these data has been collated by Ed Coe, and integrated by David Hoisington. The last maize cytogenetic map was presented in the MNL in 1993. We are updating this classic cytogenetic breakpoint map and overlaying our data obtained by using repetitive and single/low copy sequences as probes to pachytene chromosomes. We also present a large table of references with all the breakpoint data used. If you know of data we have overlooked that should be incorporated into this map, please bring this to our attention.

25:161

Centromere structure in intact and rearranged maize chromosomes derived from oat-maize additions lines.

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Although centromeric sequences have been studied in many organisms, there is no clear picture that emerges on the functional requirements to make a centromere. In multicellular eukaryotes the centromere consists of repetitive DNA embedded into large regions of heterochromatin, and there is little sequence conservation even between related species. In maize several families of repetitive DNA have been characterized that are localized exclusively to the centromere. They include the tandem repeat CentC and the retrotransposon centA. The organization of both sequences for individual maize centromeres in oat-maize addition lines was determined. It was found that the centromere size varied between 300 to more than 2000 kb, and that there was no obvious similar organization between chromosomes at a large scale level. Maize chromosomal arm deletions were generated in lines with one copy of chromosome 9. Several derivatives exhibited deletions within the CentC cluster, and the most reduced had only 340 kb of centromeric DNA left. No significant reduction in transmission was observed for all three derivatives. The majority of deletions showed no change in CentC organization suggesting that breaks occurred in other regions of the centromere.

26:70

3D FISH analysis of the structure of maize chromosome 9

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We are developing new technologies to cytogenetically map single loci onto meiotic prophase chromosomes. Using a maize chromosome addition line of oat, OMA_d9.2, we hope to accelerate the construction of a more detailed cytogenetic map of maize. This study should also shed light on meiotic chromosome structure and function. We designed a direct-labeled oligonucleotide FISH probe, MCCY, based on the junction between two CentC repeats (GenBank AF078918). The MCCY probe hybridizes to a small discrete region of maize 9 (Koumbaris and Bass, 2002, MNL v76). Using 3D deconvolution microscopy and computationally-straightened chromosomes, we measured the arm ratio (long/short) for maize 9 in the oat genome background. The average arm ratio was about 1.7 even though the total chromosome length varied from 23-35 microns. Previously published arm ratios for maize 9 range from 1.6 to 2.1. This suggests that the pachytene maize 9 chromosome in OMA_d9.2 is structurally conserved and thus suitable for cytogenetic mapping. We are currently attempting to localize by FISH several sorghum BAC clones that correspond to maize 9 Core Bin markers.

27:66

INCREASED RECOMBINATION AFTER WATER-DEFICIT STRESS IN MAIZE

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In maize, effects on recombination have been documented for sex, genotype, macro-environment, transposons, chromosome rearrangements and supernumerary chromosomes. The effect of water stress, first reported by our group in 2001, is investigated in greater detail and with larger sample sizes. Recombination was estimated in F1 plants (B73 x Mo17) grown in either water-deficit or non-stress conditions. 6 populations (3 F1 plants from each treatment) were created by crossing each F1 plant as male to B73. 93 progeny of each population were genotyped at microsatellite loci to create genetic maps for chromosomes 1 and 10. The genetic maps of chromosomes 1 and 10 were larger for stressed plants (172 vs 149 cM for chr 1 and 83 vs 72 cM for chr 10). The long arms of chromosomes 1 and 10 were the regions of additional recombination in the stressed plants. Over all populations, crossovers were not detected in 19% of the gametes for chromosome 1 and in 44% of the gametes for chromosome 10. Analyses of other genotypes and treatments are being conducted.

28:88

CYTOGENETIC ANALYSIS OF THE DISTRIBUTION OF GYPSY- AND COPIA-LIKE RETROELEMENTS IN MAIZE.

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In maize, retrotransposons make up approximately 50% of the genome. These retrotransposons are classified as belonging to either the Ty1/copia or Ty3/gypsy-like based upon the organization of the functional regions of their pol genes. In an effort to examine the distribution of particular families and classes of retrotransposons in the maize genome, we are using the fluorescently labeled LTRs of a number of gypsy- and copia-like elements as hybridization probes on maize pachytene chromosomes. The two type of transposons localize to different parts of the chromosomes: the gypsy-type elements are localized towards pericentric heterchromatin and the copia-type elements primarily in the chromosome arms. The data also clearly indicate the presence of retrotransposon islands which tend to be found within the weakly-staining (non-chromomere) regions of the chromosomes.

29:118

Crossing over on maize chromosomes is related to the pattern of synapsis and the distribution of early recombination nodules

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Recombination nodules (RNs) are proteinaceous ellipsoids found in association with the synaptonemal complex (SC) during prophase I of meiosis. Numerous early RNs (ENs) are observed during zygotene, and they may be involved in homologous synapsis and early recombination events. Fewer late RNs (LNs) are observed during pachytene, and they occur at crossover sites. Here we describe the pattern of synapsis and the distribution of ENs and LNs in maize. Synapsis starts almost exclusively at chromosome ends, although later in zygotene there are many additional sites of interstitial synaptic initiation. ENs are not evenly distributed on maize zygotene bivalents: There are more ENs per unit length of SC than per unit length of paired axial elements. The frequency of ENs is higher on distal compared to medial SC segments, and the highest concentration of ENs occurs at synaptic forks. The number of ENs on an SC segment does not change during zygotene. ENs do not show interference, except possibly at distances less than or equal to 0.2 m. LNs, like ENs, are more concentrated distally on bivalents, but unlike ENs, LNs show interference. The distal concentration of both ENs and LNs on maize SCs and possibly the early distal initiation of synapsis may be related to the tendency for genes to be concentrated distally on the chromosomes of cereals with large genomes.

30:24

Frequency and physical localization of the major repetitive DNA sequences in maize

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The genome of maize like in many higher plants is comprised to a large extent of repetitive DNA sequences. This work shows the distribution of repetitive DNA sequences throughout the maize genome. The frequency and physical localization of the major repetitive DNA sequences of maize genotype KYS were analyzed by fluorescence in situ hybridization (FISH) using as probes repetitive DNA fractions defined by their reassociation kinetics. The most highly repeated DNA sequences were identified to be those of the knob regions, whereas centromere specific sequences were repeated to a lesser degree. It was shown that knobs of maize consisted of more repetitive sequences than the previously described 180 bp tandem repeats. From our data other knob specific sequences could be predicted which were also highly repetitive. Furthermore, sequences specific for rRNA genes were not as highly repeated as centromere specific sequences. Combining reassociation kinetics and FISH offered a simple yet powerful approach for investigating frequency and physical localization of repetitive DNA on chromosomes. Its potential may be applied for analyzing the complexity and physical distribution of repetitive DNA classes in genomes of other species.

Cytoplasmic Inheritance

31:43 Gene expression analyses during pollen maturation in S-cytoplasmic male-sterile (S-CMS) and -fertile genotypes in Maize.

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We report gene expression profiles in immature pollen/microspores in S-CMS and two related male-fertile genotypes at two developmental stages: early marked by the lack of starch prior to or during pollen mitosis I (PM-I), and late, an active starch-filling post-PM-I phase. Starch biosynthesis in these binuclear (or bicellular) cells that are symplastically discontinuous from the mother plant is critical not only because starch is a reserve source of energy for pollen germination but it also serves as a checkpoint of pollen maturity. The male-fertile starch-positive, but not the CMS starch-deficient metabolically alive, samples showed global changes in the expression patterns of a large number of genes during this metabolic transition. In addition to a usual battery of housekeeping genes of carbohydrate metabolism, we observed changes in the levels of gene products of ZmMADS1, hexose transporter, plasma membrane H⁺-ATPase and Grf1-encoded 14-3-3 proteins. Reduction or deficiency in 14-3-3 protein levels in amyloplasts (starch), mitochondria and cytosol, in male-sterile relative to male-fertile genotypes are of particular interest because of potential for inter-organellar communication in this nucleo-cytoplasmic interacting CMS system. Further, the levels of hexose sugars were significantly reduced in male-sterile relative to male-fertile samples, not only at early and late stages but also at an earlier point, during meiosis. We suggest that these global changes in gene expressions, and ultimately pollen sterility, result from the combined effects of reduced sugars and their reduced flux in starch biosynthesis (i.e., metabolic signaling) along with a strong possibility for altered redox passage in immature pollen.

Developmental Genetics

32:113 Maize endosperm development; aleurone organization. Three allelic mutant lines with disorganized aleurone layers

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Following double fertilization, the triploid primary endosperm nucleus starts dividing in the absence of cytokinesis, leading to a multinucleated syncytium that occupies the periphery of the central cell. Subsequent cellularization results in formation of the four major endosperm cell types; starchy endosperm, aleurone, transfer cells and embryo surrounding region. At first, anticlinal cell walls form between nuclei, forming alveoli with their open ends towards the central vacuole. The nuclei within alveoli then divide synchronously in the periclinal plane, resulting in the formation of cell walls between the daughter cells, parallel to the outer wall of the central cell. This results in an outer layer of cells, and an inner layer of alveoli. The peripheral cells assume aleurone cell fate, while the alveoli continue proliferation, cellularization and become the starchy endosperm. Subsequent reorientation of cell division planes in the peripheral layer to predominantly anticlinal is essential for the surface area of the aleurone to expand, while the strict cell division plane is lost in starchy endosperm cells. Anticlinal cell division of the peripheral cells results in a single layer of cuboidal aleurone cells similar in size and shape. In short, strict control of the division plane is essential for the characteristic and functional organization of the aleurone layer. At developmental stages prior to 20 DAP, occasional periclinal division of aleurone cells leads to one peripheral aleurone cell and an inner daughter cell that redifferentiates to become a starchy endosperm cell. In order to identify genes involved in cell division control and/or (re)differentiation of cereal endosperm cells, we have performed a microscopy based mutant screen of the Pioneer TUSC-Collection, which consists of 43,000 maize lines with active transposable elements belonging to the Mutator family. From this screen we have identified several lines showing abnormal aleurone development. These include mutant lines in which the cell division plane control is perturbed, leading to an unorganized aleurone layer. Three such mutants are *disorgal1*, *disorgal2*, and *defectal*. Preliminary analyses indicate that these mutations are allelic. The first two mutant lines, *disorgal1* and *disorgal2*, have similar phenotypes. A flat defective kernel, in which the aleurone cells are not limited to one layer, and cell shape and size, are variable. Using DNA blot analyses we have identified an element which hybridizes to a specific Mu probe, that is linked to the *disorgal1* and *disorgal2* alleles. Efforts are underway to clone the genomic sequence flanking these Mu-element insertions. The third mutant (*defectal*) has a different phenotype. A moderately defective kernel in which aleurone cell morphology and organization is disturbed. No Mu-element co-segregating with the mutant phenotype has so far been identified.

33:153

The Rho GTPase RopB and Maize Pollen Function.

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Rho family GTPases, a plant-specific Rho subfamily, have been implicated in the regulation of plant development and cell morphogenesis processes. However, the mechanisms through which Rho proteins affect cell morphogenesis in plants are not clearly understood. In maize, nine *rop* genes have been identified: *rop A, B, C, D, 5, 6, 7, 8, and 9*. We are using a collection of mutant *rop* alleles, isolated in collaboration with Pioneer Hi-Bred, Intl, Inc., to explore ROP function in maize. We have determined the specific lesions associated with seventeen distinct mutations in five of the *rop* genes (*rop B, C, D, 6, and 7*). RT-PCR analysis indicates that maize *rops* show a high degree of overlap in their expression patterns. The exception is *ropB*: of seven *rops* analyzed, it is the only *rop* that is highly expressed in pollen. Moreover, in genetic crosses three *ropB* mutant alleles transmit at a lower frequency through the pollen than the wild type allele. Transmission through the female gametophyte is not affected by *ropB* mutation. Similar crosses with *rop D, 6 and 7* mutants indicate no significant abnormalities in transmission of these mutant alleles through either the male or female. The pollen expression data and male-specific transmission defect both suggest that *ropB* is important for pollen function. Pollen function could be affected by the *ropB* mutation during several phases; i) germination, ii) pollen tube growth and guidance, or iii) fertilization. Experiments investigating pollen germination have indicated that both the pollen tube growth rate and pattern of pollen tubes grown *in vitro* were unaffected by the *ropB* mutation. *In vivo* growth patterns of pollen from wild type and *ropB* mutant plants were observed using aniline blue, a fluorescent stain for callose in the cell wall of the pollen tube. To date, these experiments have not shown any significant growth difference of wild type from mutant pollen. Current experiments are focused on the further investigation of pollen tube growth patterns as well as analysis of ROPB-mRNA in *ropB* mutants.

34:104

A screen for non-reduction mutants in maize

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Normal kernel development in maize strictly requires a 2m:1p ratio of maternal to paternal genomes in the endosperm. 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. This ploidy barrier can be utilised to screen for mutants that produce functional, non-reduced gametes. Fertilisation of an unreduced embryo sac with pollen from a tetraploid results in a 4m:2p ratio ensuring normal endosperm development. As anthocyanin pigmentation can easily be scored visually, a tetraploid stock tetraplex for the R-Navajo (R-nj/R-nj/R-nj/R-nj) marker that pigments both the crown of the aleurone and the embryo, was developed. The R-nj tetraploid stock was used as the pollen parent in order to distinguish progeny derived from contaminating pollen. Families lacking anthocyanin pigmentation (r-g/r-g) derived from individuals with high Mu activity were used as female parents and scored for segregating plants that produce plumb kernels when pollinated with a 4n pollen-donor. Over 1000 families (about 15 plants each) were crossed to the R-nj stock and screened for segregating plants producing plumb kernels that are presumably derived from non-reduced functional embryo sacs. Two putative non-reduction mutants were identified. The mutants were out-crossed to inbred lines. Their phenotypes were confirmed in reciprocal test-crosses to diploid and tetraploid plants. These crosses have shown that the resulting embryo was tetraploid. Thus, not only the central cell but also the egg cell was unreduced, demonstrating that the mutants affect meiosis and not a later step in gametophyte or seed development. Confocal microscopy and genetic tests will be used to try to identify whether meiosis I or meiosis II is altered in the mutants. Additionally, transposon display will be used to identify the mutated genes.

35:57

Chimeric mitochondrial open reading frames unique to cms-S maize and their hypothetical role in pollen collapse

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In maize plants exhibiting S-type cytoplasmic male sterility, pollen cells collapse during the early starch-filling stage. Based on their morphology, cytochrome c levels, transcript degradation and protease activity, cms-S collapsed pollen appear to undergo a mitochondria-regulated programmed cell death cascade (PCD). Analysis of mitochondrial transcripts from sterile and restored-to-fertile lines shows a 1.6 kb transcript unique to sterile pollen. This transcript contains two open reading frames, orf355 and orf77. In this work we analyzed the editing status of the orf355/77 transcript and found that orf355 is largely unedited or contains silent edits while in 80% of transcripts orf77 is edited to code for a 17 amino acid truncated peptide (ORF17) homologous to the C-terminal transmembrane helix of atp9. A 40 kDa protein corresponding to the predicted size of ORF355 accumulates in sterile pollen but is absent from sporophytic tissue and restored-to-fertile pollen; however, no proteins of the appropriate sizes for ORF77 or ORF17 could be detected by SDS PAGE. We suggest that the 40 kDa protein product of orf355 is the initial instigator of a PCD cascade in cms-S maize collapsed pollen. Attempts to express full-length ORF355 protein in *E. coli* were unsuccessful, which could indicate a role for ORF355 in mitochondrial dysfunction.

36:103

Molecular cloning and characterization of CLAVATA1-like genes from *Zea mays*

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Plant development requires the activity of shoot apical meristems (SAM) that serve as a source of pluripotent stem cells. In the *Arabidopsis* SAM, the balance between stem cells and cells destined for subsequent developmental steps depends on the activity of a molecular signaling pathway involving the CLAVATA 1, 2 and 3 proteins. The recent characterization of the LRR receptor-like protein FASCIATED EAR2, a maize orthologue of CLAVATA2, provides evidence that the CLAVATA signaling pathway is at least in part functionally conserved among angiosperms. We have isolated maize cDNAs with high sequence homology to the LRR receptor like kinase CLAVATA1. Phylogenetic analysis indicates the presence of a CLV1-like sub-family of closely related receptor-like kinases in maize and *Arabidopsis*. Analysis of the expression patterns of maize CLV1-related genes is in progress. In *Arabidopsis*, CLV1 is expressed in a small domain in the centre of shoot and floral meristems. Preliminary results of in-situ hybridisations indicates, that the cDNA most closely related to CLV1, designated as ZmLRK1, is expressed within male and female inflorescences. Mapping of genes corresponding to the isolated cDNAs may associate these to fasciation mutants.

37:206

The Brick and Pangloss Genes Affect the Polarized, Asymmetric Divisions of the Stomatal Complex

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One of the most important events in leaf development is the formation of stomata, which control the exchange of gases and moisture between the plant and its environment. These four-cell complexes are the result of a series of asymmetric cell divisions. Among these are the asymmetric divisions of the subsidiary mother cells (SMCs), which produce lens-shaped subsidiary cells that flank the guard cell pair. Brick genes are required for the polarization of a variety of epidermal cells, including the SMCs. Mosaic analysis of the three Brk genes revealed each acts non-cell autonomously in SMC divisions. Additionally, we have isolated mutations in two other genes that affect polarized divisions of SMCs. In pangloss1 and pangloss2 (pan1 and pan2) mutants, SMCs often fail to polarize correctly, and frequently invade neighboring cell files. We are currently investigating how the pangloss genes function alone and in combination with each other, as well as with the brick genes to promote subsidiary cell division.

38:52

The control of spikelet meristem identity by the branched silkless1 gene, a new member of the maize AP2 gene family

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Most of the world's food supply is derived from cereal grains such as rice, wheat and maize. The seeds of these crops are born in a unique structure called the spikelet, which is the fundamental floral unit of all grasses. The spikelet is a compact floral branch that consists of one or two glumes that enclose a variable number of florets. The spikelet is made by a specialized meristem called the spikelet meristem, which is one of several meristems of the maize inflorescence that undergoes branching. In the branched silkless1 (bd1) mutation the identity of the spikelet meristem is altered. In the bd1 ear, the mutant spikelet meristem resembles a branch meristem, while in the tassel it has characteristics of both a branch meristem and spikelet pair meristem. We cloned the bd1 gene by transposon tagging and found that the DNA sequence is similar to the EREBP class of AP2 transcription factors. Northern gel blot analysis showed that expression of BD1 occurs only within inflorescence tissue. In situ hybridization experiments localized BD1 transcript to a circular domain at the base of the spikelet meristem. Double mutant analysis of bd1 with another spikelet meristem mutant called indeterminate spikelet1 showed that the two genes act in the same genetic pathway. We propose two models for how BD1 specifies spikelet meristem identity based on whether it functions as an activator or a repressor.

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Loss of indeterminate1 gene function affects leaf cell structure

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The indeterminate gene, *id1*, plays a key role in controlling the transition from vegetative to reproductive growth in maize. Homozygous *id1* mutant plants produce many more leaves than normal plants and, consequently, they flower much later. The *id1* gene encodes a putative zinc finger protein similar to transcription factors found in diverse species, suggesting that *id1* regulates the expression of other genes (see poster by Kozaki et al). The *id1* mRNA and ID1 protein is restricted to developing leaves of maize, and it is not detected at the shoot apex or in leaf primordia. This finding suggests that the function of *id1* is to regulate the production or transmission of floral-inducing signals that are produced in leaves and then transported to the shoot apex. Physiological studies support the existence of such leaf-generated long distance signals, yet the nature of these signals remains a mystery. Even though *id1* is expressed in developing leaves, loss of *id1* function has no obvious effect on leaf growth and development. However, when we examine *id1* leaves at the cellular level we find that there are some differences in the structure of the leaf cells. Most conspicuously, chloroplasts in the mesophyll cells of *id1* mutant leaves appear to be smaller and appressed against the walls of the cell. This preliminary observation suggests that loss of *id1* function, either directly or indirectly, may affect chloroplast development. The significance of these findings with respect to floral inductive signals will be considered.

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The globby (*glb*) mutation affects early kernel development

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In a bid to understand the complex processes underlying development of the maize diploid embryo and triploid endosperm, our group is analysing a group of mutants with discernable seed phenotypes. A novel defective class of kernel mutant, named *globby* (*glb*) due to its characteristic globular shaped kernel, has been identified from an *Ac* mutagenised population. The recessive mutant kernel phenotype consists of a partially collapsed pericarp overlying a small, defective endosperm and aberrant embryo. Two candidate *Ac*-induced alleles (*glb-1* and *glb-2*) have been isolated and mapped to chromosome 1L. EM and light microscopy analysis reveal how *glb-1* affects correct development of mid-apical aleurone and central starchy endosperm structures, while basal transfer tissue remains intact. In addition, further analysis of *glb-1* indicates that development of the endosperm is affected by 4 days after pollination (*dap*), while embryo development is arrested shortly after the pro-embryo stage. This evidence suggests that *GLB* is required for early kernel development. Molecular genetic approaches are currently being used to further understand the effects of the *glb* mutation in maize seed development.

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The maize MADS-box gene ZMM6 distinguishes between the sessile and pedicellate spikelet and affects inflorescence branching.

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The gene ZMM6 is a member of the subfamily of AGL2-like MADS-box genes. Maize AGL2-like genes have extraordinary expression patterns, suggesting they have been recruited to establish novel positional information within the inflorescences that are not found in eudicots. ZMM6 is strongly expressed in both the male and female inflorescence, the tassel and ear. The expression is low in the spikelet pair primordia. After transition of the spikelet pair meristem into the spikelet meristems, it becomes restricted to only one of a pair of spikelet primordia. Later, the gene is expressed in both spikelet and floret primordia. Although ZMM6 (1S131) maps near the branching mutant *bif2* (1S122), a transgenic approach in maize revealed a different branching function. In total 24 independent lines were obtained. The phenotypes of the tassel correlated well with those observed in the ear. Transgenic plants showed a higher degree of inflorescence branching. Instead of the wild-type paired arrangement of spikelets, the spikelets were clustered per three or four, indicating that ZMM6 specifies the level of determinacy of the spikelet pair meristem. Also the spikelet meristem was affected producing three to four florets enclosed by extra glumes. The floret meristem was partially rendered indeterminate forming a higher number of floral organs. Additionally, the floral organ identity was partially lost, showing lodicules converted into paleas.

42:78

Mediator of Paramutation2-1 mutation perturbs normal development and disrupts transgene silencing

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Paramutation is an allelic interaction where one allele causes a meiotically heritable reduction in expression of the other allele. At the *b1* locus, the *B'* allele, which specifies light purple plant color, and the *B-I* allele, which specifies intense purple plant color, participate in paramutation. In a *B'/B-I* heterozygote, the *B'* allele heritably reduces the transcription of the *B-I* allele, such that it becomes a *B'* allele, fully capable of altering other *B-I* alleles in subsequent crosses.

We are dissecting the mechanism of paramutation through the isolation and characterization of trans-acting mutations defective in paramutation. *Mop2-1* is a dominant mutation that disrupts the establishment of *b1* and *p11* paramutation. When *Mop2-1* is homozygous, pleiotropic developmental effects such as late flowering and tasselseed are often seen. Furthermore, *Mop2-1* behaves as a recessive mutation with respect to maintenance of the reduced expression state associated with paramutation. Nevertheless, this expression phenotype can be subtle and has a late developmental onset, becoming most prominent post-anthesis. Additionally, homozygous *Mop2-1* can reactivate a transcriptionally silenced transgene. Experiments are underway to determine whether this re-activation is heritable. Preliminary data suggest that *Mop2-1* is able to inhibit the establishment of *p1* paramutation. We are currently pursuing a map-based strategy for cloning *mop2*.

43:65

Tolerance of neighbor proximity in corn may involve light- and auxin-regulated development of leaf angle

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Modern corn varieties have been selected for their ability to maintain productivity in dense planting. Previous studies indicate that plant-plant interaction may be mediated by perception of changes in R:FR light ratio which affects the auxin signaling pathways. We have tested the possibility that the physiological consequence of the selection of the modern hybrid, 3394, involves changes in responsiveness to light and auxin. We found that, relative to an older line (307), etiolated 3394 seedlings are less responsive to light (W, R, FR, B) which was associated with resistance to inhibitory effects of exogenous auxin (NAA), inhibitors of polar auxin transport (NPA, TIBA), and antiauxin (PCIB). Similarly, elongation of leaf sheath in young 3394 seedlings was inhibited by light (W, R, FR) and by NPA less than in 307. A characteristic feature of 3394 plants is erected leaves. In both hybrids, light (W, R, B) and auxin promote growth of the leaf auricle and thus enhances leaf angle. In contrast, NPA blocks auricle growth and reduces leaf angle development in 307 but not in 3394 plants. Our results support findings showing that auxin or its polar distribution is involved in growth of corn seedlings. We further show that light interacts with auxin or polar auxin transport in regulation of leaf angle development. We hypothesize that, in comparison with the old line, 307, 3394 plants are able to maintain productivity in dense planting, i.e. to tolerate neighbor proximity, since they develop erected leaves as a consequence of the reduced sensitivity to light and auxin. The mechanism of how light and auxin regulate leaf angle is discussed.

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Characterization of the dominant mutation Wavy Auricle in Blade (Wab)

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The maize leaf is polarized along the proximo-distal axis with the proximal sheath encircling the culm and the distal blade extending out ward. The boundary between these tissue types is defined by a fringe of epidermal tissue, the ligule, and two wedges of auricle tissue. Dominant *knox* mutations have been identified by shifts in this leaf pattern such that proximal tissue types such as sheath and ligule are found in distal positions in the blade. This defect in proximo-distal patterning is correlated with ectopic *knox* expression in the maize leaf [1] [2] [3]. Here we describe a dominant mutation where auricle, ligule and sheath tissue are found in distal positions in the blade. We have mapped this mutation to a novel position on chromosome 2L and, moreover, we have shown that this phenotype is not associated with ectopic *knox* expression in the leaf. Here we present data suggesting a link between proximo-distal and medio-lateral patterning in the maize leaf. The *Wab* blade is conspicuously narrow and contains less than half the number of lateral veins of a wild type blade. Double mutants between *Wab* and both *liguleless1* (*lg1*) and *lg2* have extremely narrow leaves with very little blade tissue. We suggest that a signal produced at the blade/sheath boundary (perhaps *LG1/LG2*) is interpreted by the products of genes such as *Wab* in order to elaborate both medio-lateral and proximo-distal pattern in the maize leaf. 1. Vollbrecht, E., et al., The developmental gene *Knotted-1* is a member of a maize homeobox gene family. *Nature* (London), 1991. 350(6315): p. 241-243. 2. Schneeberger, R.G., et al., Ectopic expression of the *knox* homeobox gene *rough sheath1* alters cell fate in the maize leaf. *Genes and Development*, 1995. 9(18): p. 2292-304. 3. Foster, T., B. Veit, and S. Hake, *Gnarley* is a dominant mutation in the *knox4* homeobox gene affecting cell shape and identity. *Plant Cell*, 1999. 11: p. 1239-1252.

45:141

ragged seedling*2 affects medio-lateral and dorsiventral patterning in maize leaves

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ragged seedling*2 (*rgd*2*) is a recessive mutation that arose in an active Mutator transposon line. *rgd*2* plants exhibit various developmental defects. The lamina of mildly affected leaves display patches of epidermal tissue that have an inverted dorsiventral identity. The midrib is often split with some abaxial tissue on the adaxial side of the midrib. Some of these leaves also exhibit deletion of one or both margins. Severely affected leaves lack lateral expansion and are radial but retain adaxial and abaxial identity. Other defects include shortened internodes, and in some cases, a switch from alternate to opposite phyllotaxy. Immunohistolocalization analyses reveal altered patterns of KNOX accumulation in *rgd*2* apices. Although *rgd*2* bears some phenotypic similarities with *leafbladeless1* (*lb11*), *rgd*2* is morphologically distinct and genetic analysis shows that *lb11* and *rgd*2* are non-allelic mutations. Inheritance pattern of *rgd*2* is less than 3:1, perhaps due to gametophytic defects. TB mapping indicate that the *rgd*2* locus is proximal to the TB break points; SSR mapping is currently underway. The phenotypic data suggest that *RGD*2* acts after *LB11* which has radial leaves that are marked by a loss of adaxial tissue, and before *NARROW SHEATH* which has a loss of marginal domains.

46:35

Comperative proteomic analysis of lateral root initiation in maize

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Proteomics is a high throughput tool to study gene expression on the protein level. It combines the resolution of 2-D protein gels with the analytic power of mass spectrometry. Proteomics complements high throughput transcriptome studies like EST microarray analyses by adding the opportunity to study post-transcriptional modifications of proteins, protein turnover or proteolytic activity. The analysis of protein accumulation under various conditions is also of particular interest to see the extent to which RNA and protein accumulation can be correlated. The focus of this study is the mutant *lrt1*, which is impaired in lateral root initiation. The proteome of the embryonic seedling roots of *lrt1* has been compared with near-isogenic wild-type plants. An overview of proteins identified via mass spectrometry will be given as well as a summary of up- and down regulated proteins in the analyzed roots of the mutant *lrt1* and its wild-type siblings. Finally, experiments showing the analysis of different stages of development and of sub-cellular fractions as well as the use of overlapping pH gradients in an effort to improve the still limited resolution of Coomassie stained 2-D protein gels will be presented and discussed.

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Morphological characterization of maize coleoptile mutant lco1-R

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The maize coleoptile originates as a ring arising from the embryonic scutellum. Cells in the coleoptile are derived from non-meristematic tissue, and the organogenesis completes during embryogenesis. Although leaflike, its structure is distinctive. It is a fused cone-like structure with two veins, and split longitudinally with growth of other leaves. Homozygotes of lco1-R, which arose following EMS treatment, the coleoptile is transformed into a normal leaf-like shape; it shows blade, sheath and ligule structures with multiple veins. To characterize the detailed morphological features of lco1-R, we carried out sectioning analyses on seedlings of this mutant. The lco1-R allele acts primarily in the marginal region outside of the two veins. Here we detected metaxylem and differentiation of bundle sheath cells, which are never found in normal coleoptiles. Chloroplasts around those veins fully developed as expected in leaves. All data indicate that the margin of the lco1-R coleoptile is completely transformed into normal leaf like structure. A defect in roots system was also found in lco1-R. More recently, we have found other recessive mutants that resemble lco1-R in families which are active for Mu transposon insertions. Some of them showed much more severe phenotype than lco1-R do, transforming not only the marginal region but entire coleoptile into leaf. Alleleism tests and comparative morphological studies are in progress. The origin of coleoptile is discussed.

48:100

NARROW SHEATH 2 is a NITRILASE 4 like protein required for the recruitment of a lateral domain in the maize leaf

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Two genes narrow sheath 1 (ns1) and narrow sheath 2 (ns2) are essential to the development of a lateral domain in the maize leaf, which includes the margins of the stem, leaf sheath and lower blade. Plants homozygous for recessive mutations in both ns1 and ns2 fail to recruit founder cells that normally contribute to this lateral domain. The ns2 gene was cloned by Mutator transposon tagging; allelic polymorphisms present in independently derived ns2 mutations demonstrate that ns2 is cloned. A nearly full-length cDNA clone of ns2 is identified in the maize EST collection (Gai et al., 2000). Northern analyses reveal that NS2 expression is down-regulated in whole seedling and apices of ns2-R mutant. Database searches, Northern analyses and RT-PCR reveal that an 884 bp intron , which is retained in the ns2 EST, is inefficiently spliced in from NS2 transcripts. NS2 is predicted to encode a 351-amino acid protein with a high homology to NITRILASE4 in rice, tobacco, and Arabidopsis thaliana. NITRILASES are implicated in the production of IAA in plant shoots, although we have no direct evidence to link the NS2-NITRILASE to IAA biosynthesis. Southern analyses using ns2-derived probes suggest that ns2 is a single copy gene.. Surprisingly, database analyses and RNA/DNA gel blots indicate that ns2 and ns1 are non-homologous sequences . A single non-mutant copy of either ns gene is sufficient for normal leaf development suggesting that NS1 and NS2 perform redundant functions in parallel pathways. Preliminary investigations of the maize gene aldehyde oxidase2 (ZmA02; also implicated in IAA biosynthesis) reveal DNA polymorphisms linked to the ns1 alleles ns1-R, ns1-Mu78 and ns1-Mu61 that are not found in their non-mutant siblings. Also, AO2 transcripts are up-regulated in ns-R mutant plants. Further analysis will determine whether NS1 is allelic to or acts upstream of ZmA02. Moreover , the ns mutant phenotype of maize is very similar to that of tobacco and petunia plants that overexpress IAA-biosynthetic genes (Klee et al., 1987 ; Sitborn et al., 1992). These data implicate existing models indicating a prominent role for the hormone auxin during founder cell recruitment and leaf pattern formation (Reinhardt et al., 2000; Berleth and Sachs, 2001). Subsequent analysis will test whether auxin synthesis , transport and / or metabolism are disrupted in ns mutant.

49:14

Adaxial/Abaxial polarity specification in Maize leaf development

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We have identified several mutant loci that affect adaxial/abaxial patterning in the developing maize leaf. All mutant loci have been introgressed into several different inbred lines. Dramatic differences in the severity of the mutant phenotypes was observed in the various inbred backgrounds. Interestingly, each inbred line affects the severity of the set of mutations in a similar way. The leafbladeless (*lbl1*) mutation causes a loss of the adaxial domain, resulting in radially symmetric, abaxialized leaves. The weakest phenotype of *lbl1* results in male sterility. The most severe phenotype of *lbl1* results in embryo lethality. The *lbl1* mutant embryo fails to form any apical structures, however root development appears normal. SEM analysis and *knotted1* expression in the early embryo illustrates a smaller apical region and absence of meristematic cells in the apex. Perhaps the loss of the adaxial cell identity in the early embryo results in a meristem maintenance defect. The Rolled1-0 (*Rld1-0*) mutant causes a partial inversion of the polarity of the leaf. *lbl1 Rld1-0* double mutants results in suppression of each of the two single mutant phenotypes. Using SEM analysis we are looking at the patterns of epidermal macrohairs in both the single and double mutants. In the *Rld1-0* mutant, the inversion of leaf polarity results in the presence of macrohairs on the abaxial surface. In the weak *lbl1* mutant, patterning of the macrohairs on the reduced adaxial surface is normal. In the double mutant plants the defect in the pattern of macrohairs is alleviated in most leaves. We are currently characterizing the macrohair distribution in the regions of the leaf with dual adaxial polarity and of the boundaries between regions with inverted and normal polarity.

50:129

Combinatorial control of meristem identity in the maize inflorescence.

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The architecture of maize inflorescences, the tassel and the ear, is defined by a series of reiterative branching events. The inflorescence meristem initiates spikelet pair meristems. These in turn initiate spikelet meristems which finally produce the floret meristems. *reversed germ orientation 1* (*rgo1*; chr. 9) and *indeterminate spikelet 1* (*ids1*; chr. 1) mutants both exhibit an increased number of floret meristems produced by each spikelet meristem. The visible phenotypes include increased numbers of anthers in the tassel and kernels with embryos facing the base of the ear. Both *rgo1* and *ids1* behave as single recessive mutations. However, plants heterozygous for *rgo1* and *ids1* exhibit non-allelic non-complementation. Plants homozygous for both mutants have more severe phenotypes than either of the single mutants. In the double mutants the extra branching observed in the spikelets of the single mutants is also seen in spikelet pair meristems, an earlier developmental stage. These data suggest that control of meristem identity at the spikelet is mediated by a dosage sensitive pathway consisting of at least two genes that are functionally interchangeable. Furthermore, *rgo1* and *ids1* are also involved in regulating spikelet pair identity. The reiterative nature of maize inflorescence development suggests that interactions of several genes determines meristem identity, and that some of these genes are involved in specifying more than one type of meristem.

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Xcl1 causes delayed periclinal cell divisions in developing maize leaves leading to cellular differentiation by lineage instead of position.

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Plant cells have been shown to differentiate by position dependent mechanisms rather than lineage. Molecular mechanisms controlling cell division and differentiation patterns have proven difficult to elucidate in plant model systems. However, the predictable patterns of cell division and differentiation in the maize leaf have allowed for the identification of mutations which affect these processes. The maize Extra cell layers1 (Xcl1) mutation affects both cell division and differentiation. Oblique, periclinal divisions occur in the leaf protoderm, producing an extra layer of large, highly vacuolate cells between the mesophyll and epidermal cell layers. Mutant kernels also have several aleurone layers instead of one, suggesting that Xcl1 plays a role in controlling cell division orientation in cells that divide predominantly in the anticlinal plane. The extra cell layers produced in leaves and kernels differentiate according to lineage and not position. In developing maize leaves, cell divisions occur mainly at the base of the primordium, while differentiation occurs in a basipetal gradient from the tip of the leaf down. Dosage analysis of Xcl1 revealed that the mutant phenotype is caused by the overproduction of a normal gene product which shifts the division gradient upward and allows cells that have already received differentiation signals to continue to divide in aberrant planes. Xcl1 also displays interesting interactions with the dominant KNOX mutants Kn1 and Rs1, suggesting that XCL1 may play a role in downregulating homeobox gene expression.

52:21

THREE-DIMENSIONAL MODEL OF MAIZE ENDOSPERM

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In order to quantify cytological parameters related to developmental processes in the maize endosperm, we constructed an image-analysis based model, which allowed for extrapolation of data from the 2-D median longitudinal section to the 3-D structure of the whole endosperm. We outlined the cell walls on the longitudinal section to obtain a grayscale coded image of the longitudinal section, in which gray values represented the measured cell sizes. We then in essence rotated this image along its longitudinal axis. Because of the irregular 3-D shape of endosperm, we used rotation *per partes* - image was rotated as a series of 1 pixel thick slices, thus creating virtual cross-sections. Stacked virtual cross-sections represented an estimate of the spatial distribution of cell sizes in the whole endosperm. The total number of cells in the endosperm was estimated from this model. Instead of cell size, other independently measured parameters (e.g. ploidy level) can be used for grayscale coding in the image of the 2-D longitudinal section. We thus used the model to examine the relationship between cell size and endoreduplication level in the wild type endosperm and in the *miniature1* mutant. The 3-D model can be applied to evaluate any cytological or histological parameters measured on longitudinal sections.

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Developmental and genetic analyses of macrohair initiation in maize

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Macrohairs are the most prominent of 3 types of trichomes that occur on the adaxial blades of adult maize leaves. On mature adult leaves, they are found exclusively within fields of bulliform cells and their distribution within these fields is non-random. We have used light and electron microscopy to perform a developmental analysis of macrohair initiation in inbred W64A. There have been two significant findings. First, at the time of macrohair initiation, the developing epidermis has already differentiated into costal and intercostal regions and the intercostal regions have further differentiated into bulliform fields and short cell/long cell fields. Second, a spacing mechanism operates to prevent macrohairs from initiating in adjacent cells. It is extremely uncommon for fewer than 3 cells to exist between any 2 macrohair initials on the developing blade. On the genetic side, we have identified differences between Mo17 and B73 both for the density of macrohairs within bulliform fields and for the density of bulliform fields across the width of the mature blade. We are pursuing QTL mapping approaches to elucidate loci responsible for these differences and thus, for the regulation of macrohair initiation in general. In addition, we have phenotypically characterized the macrohairless1 mutant, which shows a specific defect in macrohair initiation without affecting bulliform cells. Our targeted Mutator-tagging of the macrohairless1 locus recovered two more mhl1 alleles that have tentatively been designated as mhl1-mum1 and mhl1-mum2. The cloning of mhl1 will provide a molecular entry point into this complex cellular differentiation pathway.

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Genetic Analysis of Totipotency in Maize

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The most widely used method for producing transgenic maize targets the totipotent callus tissue derived from the scutellum of immature embryos. This method is limited to a few genotypes capable of forming totipotent callus from that source. Improved methods could be developed if we acquired a basic understanding of totipotency in maize. Our objectives are: 1) determine the genetic positions of genes controlling totipotency, 2) use differential libraries to isolate genes expressed in highly totipotent (e.g. H99, A188, HiIIA and HiIIB) and recalcitrant genotypes (e.g. Mo17 and B73), 3) identify genes and processes involved in callus initiation, 4) identify genes and mechanisms that control callus initiation and 5) develop approaches to improve methods of maize transformation. A population of 185 recombinant inbred lines (RILs) of Mo17 x H99 has been surveyed for callus initiation in two years. Genes associated with callus initiation in that population have been located to chromosomes 3, 5, 8 and 9. The response of several RILs equaled or exceeded that of H99 in both years. A subset of the responsive RILs has been transformed by particle bombardment.

55:55

Photoperiod Response and Two Genetic Pathways for Flowering in Maize

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The number of days from planting to anthesis (AD) and photoperiod response (PPR; the difference between AD in long and short-days, divided by the AD in short-days), were studied in a population of 236 F3 lines derived from a cross between photoperiod-sensitive inbred CML9 and photoperiod-insensitive A632Ht. The F3 lines were evaluated under long-day and short-day environments. Genetic mapping of QTL was conducted for each daylength using combined and individual environments. A different set of QTL for AD was detected in each daylength. QTL on chromosome 2 had similar positions in both daylengths. QTL on chromosomes 3 (npi108a), 8, 9 (umc39d) and 10 were associated with AD under long-days. In short-day environments, QTL were detected on chromosomes 1, 3 (umc102), 4, 5 and 9 (umc81). QTL for PPR were on chromosomes 8, 9 and 10 at similar map positions of QTL for AD in long-days. CML9 alleles increased AD and PPR at most QTL. Dominance deviations were usually for lower values of AD (i.e. early flowering) and higher values of PPR. Epistatic interactions were observed between chromosomes 3 and 4 for AD in long-days and between chromosomes 5 and 7 for PPR. Candidate genes were linked to some QTL for AD.

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Phenotypic effects of the *mop1* mutation

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Mutations that affect paramutation can also have severe effects on plant development. The most common aberrations are tassel feminization and/or barrenness, reduced stature and lack of ears. These effects tend to be more severe following multiple generations in a mutant background. In order to further explore the effect of one such mutant, *mop1-2*, on development, double mutant plants were derived that were either homozygous for *mop1-2* and heterozygous for *Hsf1-0*, or homozygous for both *mop1-2* and *rs2*. *Hsf1-0* is a dominant systemic heterochronic mutant whose expressivity shows strong background effects. The *rs2* gene encodes a myb-domain protein that regulates the KNOX gene *rs1*. Mutations in *rs2* result in ectopic expression of *rs1*, the result of which is ectopic growth of sheath tissue. Here we present preliminary evidence that both of these mutations interact synergistically with *mop1-2*. *Hsf1-0/mop1-2* double mutants exhibited a very severe *Hsf1-0* phenotype. In contrast, all of the *Hsf1-0 mop1-2* heterozygous siblings exhibited the most mild *Hsf1-0* phenotype. The variation was as great as that observed as a consequence of genetic background. The *rs2/mop1-2* double mutations showed a more variable but even more extreme synergism. In a single family derived from the self fertilization of a double heterozygote, both the *rs2* and *mop1-2* single mutant plants had typical mutant phenotypes. However, three putative *rs2/mop1-2* double mutants were severely compromised in growth and development. Interestingly, these plants looked very much like *rs2;ns1;ns2* triple mutants. These data suggest that chromatin state (as influenced by *mop1-2*) can alter expression of a subset of genes involved in the regulation of important developmental processes.

57:110

A mutation in chloroplast ribosomal protein S9 leads to embryo lethality in maize

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A recessive lethal embryo (lem) mutant was identified in a collection of maize lines carrying the transposon Ac at different locations in the genome. Mutant embryos abort earlier than 10 DAP, but the seed develop relatively normal endosperms. Genetic analysis strongly suggests that lem is tagged by Ac. Southern blots using Ac as a probe detected a unique band which cosegregated with the mutant phenotype. DNA flanking Ac was cloned and mapped to chromosome 1L with recombinant inbred lines. A full length cDNA was obtained by cDNA library screening and 5'RACE. A size-fractionated genomic library was constructed and screened to get the wild-type allele. The gene has very high homology to the rice chloroplast 30S ribosomal protein S9 gene and is present in single copy in the maize genome. Transient expression assays with lem/GFP fusions show that the protein localizes both to the chloroplasts and to the nucleus, suggesting a possible dual function for this protein. This could help to explain how a mutation in a single-copy gene encoding a plastid ribosomal protein can cause abortion of the embryo, but not of the endosperm.

58:212

Expression profiling of maize inflorescence development

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The development of the tassel and ear is determined by the activity of five types of meristem. 1) The inflorescence meristem which produces the inflorescence stem, 2) branch meristems which produce the long lateral branches at the base of the tassel, 3) spikelet pair meristems which produce two spikelet meristems, 4) spikelet meristems which produce two glumes and two floral meristems and 5) floral meristems which produce floral organs. Identification of genes differentially expressed in each of these meristems will provide the raw material for future studies to determine the genes involved in the development and evolution of the inflorescence. We are using cDNA microarrays provided by Maize Gene Discovery to identify genes differentially expressed in each of the meristems of the inflorescence. We are utilizing the many inflorescence mutants of maize, which are arrested at various stages, to identify genes specific to these different meristem types. Furthermore, the relatively large size of the maize inflorescence allows isolation of RNA at different stages of development. Our results show no differential expression of ear genes in *ramosa1*, which controls branch number in the inflorescence, and *branched silkless1*, which controls floral meristem formation. However, hundreds of genes are down-regulated in *barren inflorescence2*, which controls the production of branches, spikelets and flowers. Experiments to evaluate the sensitivity and reproducibility of microarrays for developmental studies will be presented. Funded by NSF Plant Genome #DBI-0110189

59:47

The maize homeobox gene MOM1 is expressed at the margins of growing coleoptile and leaf

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The maize leaf develops along its dorsoventral, proximodistal and centrolateral axes. There is a long-standing discussion whether the identity of the coleoptile in monocots represents the second cotyledon or a leaf with a twisted growing axis. We have isolated a putative homeodomain transcription factor MOM1 (marker of margins) that is expressed in marginal cells of both the developing maize leaf and coleoptile. In the maize embryo, the gene is first expressed at the notch above the shoot meristem where the coleoptile starts to develop. Later, the expression can be detected in one to two cell layers at the margin of the coleoptilar ring, which then starts to enclose the plumule to protect it during germination. The expansion of the MOM1 domain at the tip of the coleoptile corresponds to the progressive enclosure of the shoot meristem. In leaf primordia, MOM1 is expressed in a distinct band of cells along the lateral margins (the marginal meristem) shortly after it emerges from the shoot meristem. In the post-primordial leaf, the MOM1 expression is restricted to the margins of the auricle/ligule region. The strong correlation between the MOM1 expression pattern and outgrowth of the coleoptile and leaf indicates that the gene may mark marginal stem cells. Based on MOM1 activity, growth of the coleoptile and lateral leaf development share significant similarity.

60:193

The genetic network controlling leaf development in maize: new and old players

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The understanding of ligule and auricle development stems from analysis of structures in wildtype plants as well as mutant plants that show disruptions in the ligule region. Aberrations in the auricle are associated with a disrupted ligule, implying that their development is closely linked. Mutations that affect the auricle and/or the blade/sheath boundary include: lg1, lg2, rs2, Rs1, Lg3, Lg4a, Lg4b, Kn1, Gn1, Hsf and Wab. These mutants can be divided into two distinct groups. The first group shows altered ligule and auricle development as a result of absence of essential proteins during leaf primordia development. The second group alters regional leaf identities. We have identified a novel EMS-induced recessive mutant called extended auricle-R (eta-R). Homozygotes of eta-R have a wavy overgrowth of auricle tissue and the auricle/blade boundary is diffuse. However, when eta-R is used to make double mutants with mutants of any of the genes listed previously, exacerbated, non-additive phenotypes occur. Further, eta-R shows dosage effects with lg2-R, indicating that the ETA product works in the same network as transcription factors LG1 and LG2 to specify an exact sheath-blade transition and ligule line.

61:142

Imprinting in the Maize Endosperm

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Imprinting involves differential transcription of a locus dependent on the parent of origin, in contrast to conventional Mendelian inheritance where parental genomes are equivalently expressed. Evidence is accumulating that a wide spectrum of genes is imprinted in a range of plant and animal species. Interestingly it occurs most frequently in organisms in which the female retains and nurtures a number of developing embryos leading to the proposal that imprinting has evolved as a result of differential parental interest in offspring. We have identified, using a range of techniques, a number of imprinted genes expressed during endosperm development and demonstrated that by performing interploidy crosses the contribution of either male or female parentally imprinted loci can be distorted. While most of these crosses result in seed abortion, isolation at an early stage allows us to monitor the timing and location of imprinted gene expression. A genomic approach is being used to understand the totality of the effect of changing the genomic balance, in order to discover how many genes are affected. A close focus is being maintained on the expression of chromatin-remodelling genes during these interploidy crosses, for these sequences are assumed to have an important role in maintaining transcriptional status at particular developmental stages. To provide a wider perspective to these experiments we have been exploring the effect of interploidy crosses on the development of the main cellular domains of the endosperm and embryo.

62:109

Maintenance of Gene Silencing by the rs2 Gene in Maize

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Development in plants is dependent on a balance between the maintenance of stem cells in the shoot apical meristem (SAM) and the recruitment of daughter cells from the SAM to form lateral organs. A family of homeobox genes, the class I knox genes, function to distinguish organ founder cells from the meristematic cells in the SAM. These knox genes are expressed in the SAM but are down regulated in founder cells at the time of leaf initiation. The rough sheath2 (rs2) gene from maize is required to repress knox genes during leaf development. Mutations in the maize rs2 gene phenotypically mimic gain of function mutations in the knox genes and expression studies have shown that at least three knox genes, knotted1 (kn1), rough sheath1 (rs1) and liguleless3 (lg3), are misexpressed in leaves of rs2 mutants. The rs2 gene encodes a unique myb domain protein with high homology to the ASYMMETRIC LEAVES1 (AS1) and PHANTASTICA (PHAN) genes from Arabidopsis and Antirrhinum, respectively. Mutations in either as1 or phan also cause leaf and floral defects due to the misexpression of one or more knox genes. The fact that these proteins are so highly conserved structurally and functionally suggests that meristem function and leaf development involve a conserved pathway in dicots and monocots. Using the yeast two hybrid system and several biochemical approaches, seven RS2 interacting proteins have been identified. Based on their homology with known proteins from other organisms, these interacting proteins can be reconciled with a model in which rs2 is required to maintain the silenced chromatin state of knox genes in leaf primordia.

63:90

The SHOOTLESS2 gene is involved in both the initiation and maintenance of the shoot apical meristem in rice.

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To address whether the rice SHL2 gene functions in the maintenance of the shoot meristem or not. We have analyzed three weak alleles of SHL2, shl2-6, shl2-7 and shl2-8. In these three mutants, a shoot meristem was often formed during embryogenesis. The meristem, however, was incomplete in that it was ill-shaped, the number of indeterminate cells was reduced, and was defective in maintaining shoot meristem identity. In shl2-6 and shl2-7, the meristem was lost after producing a few leaves during embryo development. Only shl2-8 mutant could survive after germination, but showed abnormal initiation pattern and morphology of leaves and died before 2 months after germination. In the weak shl2 alleles, the shoot meristem was composed of a small number of indeterminate cells, as visualized by the expression of the class1-homeobox gene, OSH1, and ultimately the meristem was converted into leaf primordium. Thus the SHL2 gene is involved in both the initiation and maintenance of the shoot apical meristem. Interestingly, in shl2 alleles, there was a positive correlation between the size of the expression domain of OSH1 (which indicates the number of indeterminate cells), the frequency of shoot meristem initiation, and the duration of meristem survival.

64:187

empty pericarp2 encodes a negative regulator of the heat shock response and is required for early stages of embryo development.

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empty pericarp2 (emp2) is a recessive, lethal mutant defective in embryo development. Development of the mutant embryo is severely retarded, although it does proceed to the coleoptile stage/stage1 whereupon embryo abortion and kernel decomposition ensue. Northern gel-blots reveal that transcripts of the major HEAT SHOCK PROTEINs (HSP), especially Zmhspl01, are upregulated in developing mutant kernels. The timing of both the HSP transcript elevation and embryo abortion is correlated with the developmental stage at which maize kernels are first competent to initiate a heat shock response. These data suggests that emp2 mutant kernels die due to an un-attenuated heat shock response. The emp2 mutation maps to the long arm of chromosome 2 and was cloned by Mu1 tagging. Genomic sequencing of this locus shows a Mu1 insertion at the first intron of emp2. EMP2 is predicted to encode a 78 aa protein that is highly homologous to the HEAT SHOCK BINDING PROTEIN, which functions during negative regulation of the heat shock response in *C. elegans*. TUSC reverse genetic analyses have identified several independent Mutator alleles at this locus, all of which contain insertions in the same 5' intron as the reference allele emp2-R. Curiously, although emp2 mutations are recessive genetic nulls, the emp2 transcript is over-expressed in the mutant kernel. S1 nuclease protection assays indicate that the abundant emp2 mutant transcript is truncated in the 5' non-coding region. We are currently testing a model, which predicts that the mutant transcripts are untranslatable, and confer a null EMP2 gene product. We speculate that Mu insertions in the 5' intron activate an alternative promoter, which initiates transcription downstream of the promoter utilized in non-mutant kernels.

65:144

The ZmMADS2-Transcription Factor Gene is Involved in Anther and Pollen Maturation

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MADS-box transcription factor genes have mainly been shown to control identity and development of flower organs, but also of vegetative tissues such as roots, leaves, tubers or the embryo. It is unclear whether they also play a role in regulation of male and female gametophyte development, fertilization and endosperm development. We are investigating the function of the ZmMADS2-transcription factor gene of Zea mays which has been shown to be expressed in mature pollen (the male gametophyte) and during pollen tube growth (Heuer et al., Sex. Plant Reprod. 13, 21-27, 2000). Transient transformation assays with germinated pollen of tobacco and maize confirm this result: expression of the luciferase marker gene under the control of ZmMADS2-promoter deletion constructs was observed in germinated pollen, but not in vegetative tissues such as leaves. Here we further show that ZmMADS2 is also involved in anther development and maturation. The anther wall consists of four cell layers: epidermis, endothecium, transient middle layer and tapetum. The latter three are also referred to as the outer, middle and inner layer of the tapetum. The innermost tapetum layer plays an active role in microsporogenesis prior to undergoing programmed cell death. Transgenic plants expressing the ZmMADS2-cDNA in antisense orientation under the control of the ubiquitin promoter displayed an arrest of anther development one day before anthesis. Pollen development is also arrested at this stage: the pollen grains are almost filled with starch granules and the shape of nuclei is sickled instead of round. Transgenic plants expressing the ZmMADS2::GFP fusion-protein under the control of the ZmMADS2-promoter show the localisation of the protein in the nuclei of a single tapetum cell layer.

66:202

Shoot apical meristem mutants of maize embryo; application of Uniform Mu population.

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The Uniform Mu population is designed to isolate seed mutants efficiently toward the cloning of the causative genes. Significant number of embryo mutants such as germless, viviparous, and embryo defect have been raised within the population, some of which would be directly associated with shoot apical meristem development and its activity. In order to expand the isolation of embryo mutants, we have adapted the Uniform Mu population for seedling screening. In the course of the development of the population we have isolated a number of non-seed mutants, suggesting that the population is fairly mutagenic for screening of new non-seed mutants. These mutants are likely to include the embryo mutants that do not have distinguishable seed phenotypes. We expect to isolate a class of embryo mutants that have altered pattern of shoot apical meristem development. We have initiated the genetic analyses of some candidates including viviparous and defective embryo.

Maize embryo differentiates several leaf initials during seed embryogenesis like other cereals such as rice and wheat, indicating that development of vegetative organs is partly integrated into embryonic stage. We have isolated a new maize embryo mutant designated as *big embryo 1*. The mutant produces the greater size of embryo than wild type. The mutant showed pleiotropic phenotypes including increased number of leaves and early flowering. The phenotypes suggest that the prolific activity of the shoot apical meristem is increased throughout plant vegetative development. The increased activity may also occur during seed embryogenesis, resulting in the enlarged embryo. The enlarged size of the mutant embryo might contain higher oil, suggesting the possible application of the mutant for higher oil production.

67:45

Altered timing of vegetative phase change and response to *Puccinia sorghi*

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Vegetative development in corn can be divided into juvenile and adult phases, each with distinct morphology and physiology. The timing of phase change is highly heritable and in some populations has been associated with resistance to common rust (*Puccinia sorghi*). We have used divergent full sib recurrent selection to develop early and late phase change populations. Divergent selection was successful for last leaf with juvenile wax exhibiting a strong linear response. Average last leaf with juvenile wax in C0 was 9.7, 11.7 in C3 late, and 8.2 in C3. We then initiated a planting dating date study in which all seven cycles were planted at three dates. They then were inoculated on the same day with one planting at the v5 stage, another at v10 and the third at v15. For the v5 group there were no linear trends detected. Inoculation at v10 resulted in a significant linear trend over cycles. While C3E had more rust than C0, C3L and much higher levels. In the case of v15 there was a significant linear trend for the late direction with the late plants having more rust than C0. There was no linear trend in the early direction. To get a better idea of what was happening with rust and development, we rated rust damage on individual leaves below the ear. For C3L and C0 the lower leaves had more rust while there was no difference among C3E leaves. We found that degree of rust damage closely followed the relative developmental stage of the leaf. Close examination of the leaves revealed that within leaves, rust pustules were more abundant on juvenile tissues as indicated by the presence of epicuticular wax.

68:130

Characterization of *Developmental Disaster 1 (Dvd1)*, a dominant mutation with reduced inflorescence branching and a pleiotropic disruption of vegetative development.

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Phenotypic screening of selfed Maize Targeted Mutagenesis (MTM) ears identified several ears with kernels whose embryos were facing the base of the ear. This reversed kernel orientation phenotype is indicative of spikelet branching defects, also seen in *rgo1*, *ids1*, and several other mutants. Seeds of one of these ears ([MTM 44119](#)) segregated a dominant developmental disaster phenotype. In the mutants, the internodes do not elongate, the culm zig-zags (similar to *ns1*, *ns2* double homozygote), leaves are crinkly, and the inflorescence exhibits reduced branching similar to unbranched or barren inflorescence mutants. This mutant does not map close to any known similar mutants, and so we have called this mutant *Developmental Disaster 1 (Dvd1-R)* because of its pleiotropic effects. Introgression into B73 and Mo17 inbreds has revealed that the vegetative effects are subject to modifiers. In a B73 background, *Dvd1-R* mutants appear nearly wild type vegetatively, whereas in Mo17 mutants have severe vegetative developmental defects. Reduced inflorescence branching is seen in both backgrounds. The similarity of the *Dvd1-R* phenotypes to *rs2*, *mop1* double mutants and *rs2*, *ns1*, *ns2* triple mutants suggests a possible role in regulation of KNOX genes and/or chromatin regulation.

A recessive allele of *rs1* suppresses the leaf phenotype of the *rs2* mutant.**Tyers, Randall Theodoris, George Shtivelman, Sarit Freeling, Michael**

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Recessive mutations in the gene *rough sheath2* (*rs2*) have a phenotype similar to but more severe than dominant *rough sheath1* (*rs1*) alleles. Dominant *rs1* alleles disturb the blade-sheath boundary and cause proliferation of sheath like tissue within the auricle and at the base of the blade. In addition to these phenotypes, *rs2* mutants are reduced in stature and have abnormalities in both the ear and tassel. In the ear extra silks (the elongated styles of maize flowers) form and the kernels have a reversed orientation; in the tassel there is excessive branching and aberrant development of male flowers may also occur. In *rs2* mutant alleles, expression of *knox* gene products in leaves has been demonstrated and is convincingly shown to occur for *rs1*. A decrease in auxin polar transport occurs as well. We have determined that ectopic expression from *rs1* is responsible for almost all of the *rs2* mutant leaf phenotype by using a putative knockout allele of *rs1* (*rs1-872D 1*). This allele has a *Mu1* element insert into the second exon followed by a 48 bp deletion. The progenitor (*rs1-872::Mu1* allele) was derived from material generously provided by Bob Meeley and Pioneers Trait Utility System for Corn and only has an apparent homozygous phenotype in a *rs2* mutant background. We are investigating whether the residual leaf phenotype seen in *rs2; rs1-872D 1* mutant plants is due to gene product from the *rs1-872D 1* allele or to ectopic expression from other *knox* genes.

70:222 Molecular and evolutionary analysis of *ramosa1* in inflorescence architecture**Vollbrecht, Erik {1} Martienssen, Rob {1} {1}** Cold Spring Harbor Laboratory

Regulated branching patterns are a key element of plant architecture. In the maize inflorescences, stereotyped branching events generate the highly similar architectures of the ear and the tassel. A critical distinction between ear and tassel is that long branches occur only in the base of the tassel. The ear, the upper portion of the tassel and its long branches may each be considered architecturally as spikes because their main axes bear short branches (spikelet pairs). In strong *ramosa1* (*ra1*) mutants these main axes instead bear long branches leading to a fully branched panicle architecture, implying that the function of *ra1* is to suppress a long branch fate in second order meristems. *ra1* encodes a small protein (predicted 19 kDa) with a single EPF-type zinc finger motif. To better define the role of *ra1*, we examined its expression pattern by in situ hybridization. In B73, *ra1* expression is first detected near the inflorescence apex, in discrete patches marking the initiating, second order meristems that are destined to bear spikelet pairs. Expression persists as third order meristems initiate, and becomes adaxial and strongest between the two third order (spikelet) meristems. In strong *ra1-R* point mutants, as the second order meristem instead forms a long branch, expression remains in an adaxial patch at the base of the branch, therefor marking the junction of first and second order axes and not the second order meristem per se. *ra1* is also expressed slightly later, in the abaxial base of elongating spikelet pedicels. These expression data suggest that *ra1* regulates meristem fate by enforcing a boundary between the main axis and lower order meristems. *ramosa2* (*ra2*) mutants are phenotypically similar to weak *ra1* mutants with respect to second order branching. In *ra2-R* mutants the *ra1* expression domain decreases to a speck at the base of initiating and expanding second order branches. These results and earlier genetic analyses indicate that *ra1* and *ra2* act together in a pathway to imposes determinacy on second order inflorescence meristems. The fully branched panicles of *ra1* and *ra2* mutants resemble the normal architecture of some other grasses. To test the hypothesis that modulations of *ra1* function correlate with evolutionary changes in inflorescence architecture, we have initiated phylogenetic and comparative analyses. We present *ra1* gene phylogenies including the teosintes, *Tripsacum* and other panicoid grasses. *Sorghum bicolor*, a species with a fully branched panicle, contains a single *ra1* ortholog (*ra1-Sorghum*) whose expression in the inflorescence is barely detectable by RT-PCR. Consistent with its greatly reduced expression relative to *ra1*-maize, the *ra1-Sorghum* locus is rearranged relative to maize, which we will further characterize by comparative genomics.

71:210

Conservation of B-class Gene Function Across 120 Million Years of Angiosperm Evolution

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We have undertaken a functional analysis of maize orthologs of the Arabidopsis B-class organ identity genes, APETALA3 (AP3) and PISTILLATA (PI). In model eudicots, B-class gene activity, in concert with C-class gene activity, specifies stamens, whereas B-class and A-class gene activities specify petals. We previously demonstrated that the Silky1 (S1) gene of maize is orthologous to AP3 and is required for the specification of stamens and lodicules rather than stamens and petals (Ambrose et al. 2000). We have also cloned the putative maize orthologue of PI. This gene is duplicated in maize and has been designated *Zea* PistillataA and B (ZpiA and ZpiB, aka, ZMM16 and 29; Munster et al., 2001). Its pattern of expression in developing spikelets mimics that of Silky1, suggesting a similar role in B-function organ identity. However, there are no known ZpiA/B mutants from which to deduce function. To test the capacity of ZpiA to function as a B-class organ identity gene, we have assayed the ability of ZPIA to interact in vitro and in vivo with S11 and the AP3 proteins. We find that ZPIA and S11 together bind specifically to DNA in vitro in a manner similar to that of AP3 and PI. Additionally, S11 and ZPIA are able to interact with the Arabidopsis PI and AP3 proteins, respectively, to bind DNA. Finally, we show that this activity is present in vivo, as both S11 and ZPIA are sufficient to rescue their corresponding Arabidopsis mutants in spite of sharing only about 50% amino acid sequence identity. These results provide compelling evidence that B-class gene function is conserved between eudicots and monocots, and further supports the proposal that the grass lodicule and the dicot petal are homologous structures.

72:7

IDENTIFICATION OF PROTEINS INVOLVED IN ZEIN mRNA TRAFFICKING IN *Zea mays*

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The endosperm provides the primary source of nutrition for maize seedlings following germination and is also a major food source for humans and livestock. During endosperm development protein bodies, composed of zeins, form within the endosperm. Zeins serve as the primary source of protein for the seedling during germination. Given that endosperm cells are large (>100 μ m), it is hypothesized that zein mRNAs are actively transported to their site of translation, possibly along actin microfilaments. The translation factor EF1A and microfilaments have been previously shown to surround endoplasmic reticulum regions at sites where protein bodies are forming. Work has begun to isolate and identify proteins that bind to the 3' untranslated region (UTR) of the 27-kDa γ -zein mRNA. Extracts of developing maize kernels were subjected to several types of chromatographic separation and the fractions were tested for their ability to bind and alter the mobility of a 299 base RNA probe derived from the 3' UTR. Additional experiments were performed that identified proteins transferred to nitrocellulose that bind to this probe. At least 5 polypeptides have been shown to bind to the 3' UTR probe in vitro. One of these proteins was shown by MALDI-TOF mass fingerprinting and tandem mass spectrometry (LC/MS/MS) to be eukaryotic initiation factor 5A (eIF-5A). A γ -zein mRNA-binding protein of approximately 21-kDa (p21) has been shown to bind to and alter the mobility of the 3' UTR probe in gel shifting. p21 has been purified to ~50% purity by reversed-phase HPLC and is tentatively assigned to nascent polypeptide associated complex beta chain (b-NAC) by LC/MS/MS. In addition, a protein of approximately 28-kDa (p28), that does not show binding activity, has been purified to ~90% purity by reversed-phase HPLC and has been shown by LC/MS/MS to be nascent associated-polypeptide complex alpha chain (a-NAC). Recombinant eIF-5A, b-NAC and a-NAC are being developed and work will be done to determine their RNA binding specificity and interactions with other proteins.

73:27

Two novel genes specifically expressed in the embryo sac and the basal endosperm transfer layer of maize

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Two novel genes expressed specifically in the embryo sac and the basal tissue layers of the endosperm were characterised. Gene DD1-b was isolated by a differential display between the upper and the lower half of maize endosperms at 7 DAP. The closely related gene DD1-a was detected during the screen of a genomic library with DD1-b. Sequence analysis of complete cDNA and genomic clones revealed ORFs coding for proteins of 304 aa and 286 aa, respectively. This size difference was due to alternative splicing. A sequence of 54 bp was present in both genomic clones but was found only in the cDNA of DD1-a and not in the cDNA of DD1-b. The sequence identity in the remaining 4 exons was 94% on the nucleotide level and 90% on the protein level. Both protein sequences showed significant homology to the DUF239 family of Arabidopsis, a group of 22 proteins of unknown function, a small number of which are putative peptidases. The expression of both genes was restricted to the maize kernel. It started before pollination and lasted up to 15 DAP with a peak at 7 DAP. Interestingly, the expression of DD1-a was 10 times lower than the one of DD1-b. This may be related to the fact that DD1-a expression was subject to paternal imprinting in certain crosses, while no imprinting was detected in the case of DD1-b. In situ hybridisation with a probe detecting both genes showed that the expression in the kernel was restricted to the embryo sac prior to pollination and to the basal cell layers of the endosperm after pollination. Sequence analysis of upstream regions revealed a potential cis element of 33 bp sequence repeated 6 times between positions -900 and -100 of both genes. A fusion of a 2.6 kb upstream sequence of DD1-b to a Gus reporter gene was trans-activated in tobacco protoplasts by ZmMRP1, a transcription factor with a temporal and spatial expression pattern very similar to DD1-b. Therefore DD1-b is a potential target gene of ZmMRP1.

74:63

Efforts to Clone *Ramosa2*

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The maize *ramosa2* (*ra2*) mutation, which displays more long branches in the tassel, was first described by R.A. Brink. We are currently working to clone the *ra2* gene and characterize the mutant phenotype in detail. From a *Mutator* (*Mu*) tagging screen we have generated four putatively tagged alleles. We also have a putative *ra2* allele obtained from the Don McCarty *Mu* lines. We have mapped the mutation to 8 cM (6/69) from the *umc92* restriction fragment linked polymorphism marker on chromosome 3. Additional mapping data has been generated using simple sequence repeat polymorphisms. Scanning electron microscopy analysis of *ra2* tassels in different backgrounds will also be presented.

Genome Structure/Syteny

75:155

HIGH THROUGHPUT PLACEMENT OF OVERGO MARKERS ON BAC LIBRARIES OF MAIZE AND 3 OTHER GRASS SPECIES

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Overgo probes based on a 2509-locus sorghum RFLP linkage map were used to anchor and construct BAC-based physical maps of maize and 3 other grass species. Synthetic overgo probes were designed by determining the most highly conserved 40 basepair region of each mapped RFLP probe based on cross-species hits to GENBANK (excluding repetitive DNA) and SUCEST. Overgo probes were hybridized in multiplex arrays with up to 24 probes per pool, and the results deconvoluted to provide results for individual probes. Six different BAC libraries, consisting of rice (cv IR64, 4.6 X coverage), rice (cv Nipponbare, 20 X coverage), Sorghum bicolor (cv BTX623, 11 X coverage), Sorghum propinquum (9 X coverage), sugarcane (cv R570 4.5 X coverage) and maize (cv B73, 12.7 X coverage), totaling 29 22.5x22.5 cm filters with 18,432 BACs per filter, were tested. Hybridization and BAC fingerprint data are being used to construct physical maps using the program FPC. A sample physical map will be shown. To date, over 1600 probes have been hybridized to all six libraries. The resulting physical maps will be used to better understand syteny between the grasses and to assist with eventual genome sequencing.

76:223 Genetic Mapping of Maize Mutants with SSR Markers

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Mapping of mutants in the Maize Mapping Project seeks to increase the value of the mutant resource with map information. Because the number of mutants is enormous, and is ever growing, we have worked to increase the rate and resolution by which mutants can be mapped, with molecular markers. By production of F2 families large numbers of mutants can be processed each season. Most of the mutants studied to date are derived from diverse and relatively unpredictable backgrounds, so four inbred parents, A619, A632, B73, and Mo17, are selected for crosses to increase the chances for polymorphism generally, and sometimes predictably. For our purposes the mutants can be divided into two groups: 1) placed mutants that are known to be located on a particular chromosome or chromosome arm/segment, and 2) unplaced mutants, which account for 75% of more than 4500 mutants known. We further divide the mutants into three categories: kernel, seedling, and adult plant. Map information is obtained using SSR markers detected by PCR and agarose gel electrophoresis. Of 596 placed (largely by B-A translocations) EMS-(Neuffer) mutants and another 234 classic mutants (Maize Genetics Cooperation - Stock Center) F2 family sets (from 1-4 different families per mutant), 343 seedling and adult and 133 kernel mutants have been grown for collection through 2001. Predicted mutants appear in more than 80% of the families. These were analyzed using bulk-segregant analysis (BSA) of pools (homozygous pools vs. segregating pools). As many as 19% were either not on the arm tested, or the homozygotes were too difficult to discern accurately. Of 225 placed mutants confirmed, over 40% have been assessed with individual segregational analysis and greater than 95% were verified. Another 200 placed seedling mutants are currently in process. In addition, we have now established a system for mapping unplaced mutants using BSA. Whole genome BSA has placed more than 52 unplaced mutants through 2001. Many new mutants are also found and mapped from EMS-mutant families (e.g. numerous new small seedling mutants appear in sandbench cultures). The method also produces false positives, unlinked markers that appear to be linked as a result of chance; although, cases of multiple unlinked gene interactions have also been identified. Funded by NSF Plant Genome Grant DBI 9872655.

77:102 **Rp1 -a complex disease resistance locus in maize.**

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Several rust diseases are commonly found in maize. One of these, the common rust is caused by the Basidiomycete *Puccinia sorghi*. In maize, the major genes, which encode resistance to this pathogen are called Rp genes and are known to reside mostly on the short arm of the chromosome 10. Several independent resistance specificities have been mapped, suggesting that the locus consists of a gene cluster with several non allelic genes: Rp5; RpG; Rp1-A to Rp1-N. Recently, the Rp1-D resistance specificity has been demonstrated using two independent gene-tagging approaches with the transposons Mutator and dissociation. Molecular characterization has shown that Rp1-D encodes a 1292-amino acid protein belonging to the Nucleotide Binding Site-Leucine Rich Repeats (NBS-LRR) plant resistance gene family (Collins et al. (1999), *Plant Cell* 11(7): 1365-1376). In the Rp1-D genetic background, approximately nine paralogues of Rp1-D (*rpp*) reside in the same locus. Our aim is to investigate the physical order and spacing of these *rpps* and to study variation in the physical organisation among highly diverged genotypes, using large insert libraries providing full coverage of maize genome. With this view, a portion (1.5kb, including 5' region and some coding region of Rp1) has been used to screen a YAC library from the susceptible genotype LH82 (con belt dent line) and a BAC library from F2 (European flint line). Each genotype is thought to harbour 6-7 copies of *rpp* clustered in a single locus. In LH82 line, using fingerprinting technology and YAC end sequencing we have organised the 13 YACs into one contig covering about 600 kb. This characterisation has confirmed that the locus is extremely complex in so much as numerous sequences appear to be duplicated in several regions of the contig. With the view to analyse the level of sequence divergence between *rpp* from a single genotype, we have also carried out sequencing of the different *rpp* copies present on most of the YACs. Moreover, we have compared these *rpp* sequences from LH82 to the other *rp1-D* sequences kindly provided by Dr. S. Hulbert, and with other sequences present in the databases. Interestingly, we did not find any strong link between sequence divergence and intergenic distance within the contig. The results show an important contrast in term of sequence homology between all the *rp1-D* paralogues from LH82 genotype, in comparison with HRP1-D haplotype (respectively 41-99% and 91-97% of DNA identity) (Sun et al. (2001) *Genetics* 158:423-438). The analysis of sequence polymorphism between the *rp1-D* paralogues from these two genetic backgrounds allowed us to obtain an unrooted phylogenetic tree. Different monophyletic groups were found in common for both lines. It seems that the gene family predates the separation of the lines. So, despite differences in the rate of sequence polymorphism in the two lines, some characteristics (specific deletion or insertion) that identify the monophyletic groups are maintained in the both lines. All these information raises questions about the possible mechanisms of evolution for this locus in different genetic backgrounds. The contigs will also provide material for large-scale sequencing of the entire regions spanning the different Rp1-D copies, and this will tell us the nature of the genes which co-inhabit. Locus-specific markers derived from sequence analysis of the LH82 clones are currently being used to align the F2 and LH82 contigs, giving us a preliminary view of the comparative structure of the locus in lines representative of both distinct flint and dent heterotic groups. This comparison of physical maps raise the question about the possibility to deduce ancient events (like insertions /deletions /inversion or unequal crossing over) giving us evidence regarding the mechanisms which could have contributed to the past evolution of the locus. Also, the intra- and inter-genotype spatial combined with sequence relationships between the *rpp* should provide an insight into the level of instability of the locus during its evolution.

78:93 **A High-Resolution Maize Map Based on a Randomly Intermated B73 x Mo17 Population.**

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A high-resolution genetic map of *Zea mays* L. was constructed using 302 lines derived from a cross of B73 x Mo17 followed by four generations of random intermating. The resolution of the Tx303 x CO159 Immortalized F2 population is 16.8-fold less than the 0.05 cM resolution of this mapping population. The Intermated B73 x Mo17 (IBM) map contains both RFLP and SSR markers. 1006 RFLP and 804 SSR loci were used to construct the map. A portion of the RFLP and SSR loci will serve as reference points to the prior public genetic maps. The novel RFLP markers are derived from two sources, a new *Pst* I genomic library (*mmp* loci) and a group of *Mu*-tagged clones for genes with seed development (*ufg* loci). Novel SSR markers were derived by mining public sequence data and from enriched libraries (*umc's* ≥ 1000). 73% of the SSR loci were developed as part of the Maize Mapping Project. The map includes 129 loci probed by non-maize clones that will aid in alignments between different grass maps. The 2.9-fold increase in recombinants per line closely approximates the 3-fold theoretical expectation. This map is being aligned with the maize physical map through both hybridization-based and PCR assays. A subset of 94 lines have been identified as a public mapping resource for individual investigators. A subset of marker data for QTL analysis is also being developed.

79:15

The origin and structure of genetic diversity in maize as revealed by multilocus microsatellite genotyping

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There exists extraordinary morphological and genetic diversity among the maize landraces that were developed by pre-Columbian cultivators. To explain this high level of diversity in maize, several authors have proposed that maize landraces were the products of multiple independent domestications from their wild relative (teosinte). We present phylogenetic analyses based on 264 individual plants, each genotyped at 99 microsatellites, that challenge this multiple origins hypothesis. Instead, our results indicate that all maize arose from a single domestication event in southern Mexico about 9,000 years ago. Our analyses also indicate that the oldest surviving maize types are those of the Mexican highlands with maize spreading from this region over the Americas along two major paths. Our phylogenetic work is consistent with a model based on the archaeological record suggesting that maize diversified in the highlands of Mexico before spreading to the lowlands. This analysis also indicated that the maize exotic germplasm pool can be divided into three supragroups: North American Maize (maize of the Indian tribes of the US and Canada), tropical maize (most maize of Mexico, Central and lowland South America), and Andean maize (maize of the Andes Mountains growing above 2000 meter elevation). We found only modest evidence for post-domestication gene flow from teosinte into maize.

80:12

cDNA Microarrays for Maize Gene Discovery

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One component of the NSF-funded Maize Gene Discovery project headed by Virginia Walbot at Stanford University (DBI- 9872657) is to produce cDNA microarrays containing maize genes, and distribute them to the research community at a reasonable cost. We will present a poster summarizing the available arrays and future plans. The currently available arrays include: 605 Endosperm microarrays with 8064 cDNAs from developing endosperm (14 days post pollination) , representing ~2000 unique genes. 606 Immature Ear microarrays with 5065 cDNAs from developing ear (0.5-2 cm long) representing ~2000 genes. 614 Root microarrays with 5065 cDNAs from developing roots (3-4 days old), representing ~2500 unique genes. 486 Immature Leaf microarrays with 4454 cDNAs from developing leaves (P4/6-P10/11 leaf primordial stage), representing ~2000 unique genes. Unigene I-1-01 microarrays , the first component of our Unigene microarray set, contains 5280 unique genes from three different cDNA libraries (707&945: mixed adult tissues; 687: developing embryo, and 603: salt-stressed root). We will present quality control data and describe experiments using the arrays. Our priority for the coming year is to complete the printing of Unigene I, which will contain 15, 000 unique maize genes. For the latest information and more details, please see the project website at <http://gremlin3.zool.iastate.edu/zmdb/microarray/> .

81:150

An Integrated Map for Maize

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The Maize Mapping Project (MMP) is focused on developing genetic, physical, and database resources for the maize genome. A key resource being developed by the MMP is a well integrated genetic and physical map that will expedite the identification of DNA sequences underlying key traits that have been genetically mapped. Towards this end, a high-resolution genetic map has been developed that contains over 1800 RFLP and SSR markers. HindIII Fingerprinting of 468,000 BACs from EcoRI (162 kb), MboI (165 kb), and Hind III (137 kb) libraries is at the halfway point with 8400 BAC contigs and 7500 BAC singletons. To complete the task of integrating the two maps, several complementary approaches are being taken. AFLPs and MITEs are being used on both the high-resolution genetic map and on BAC pools to link genetically mapped AFLPs and MITEs to BACs on the physical map. Additionally, mapped SSRs are also being localized to BAC contigs using BAC pools. 7700 EST unigene clusters have been anchored to 165,000 BACs using 40 bp overgo sequences designed to hybridize to single or low copy regions. Approximately 3700 EST unigene overgoes localize to BACs contained within a single contig. A portion of these EST unigene clusters contain previously mapped SSRs that will allow BAC contigs from the physical map to be linked to the genetic map. A portion of the BAC contigs with EST unigene anchors that contain no genetic mapping information will be localized to the genetic map by the development of single nucleotide polymorphism markers and their subsequent mapping on the high-resolution genetic map. The MMP is supported by NSF DBI 9872655.

82:203

Structural evolution of an orthologous region in maize, sorghum and rice

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Sequence comparisons of orthologous regions in multiple grass species can provide key insights into the timing and modes of local sequence evolution over the last 50 million years. Orthologous *adh* regions in maize and sorghum were sequenced and analyzed in our lab (Tikhonov et al., PNAS 96:7409-7414, 1999), revealing general conservation of gene content and order. However, the sequence of the rice *adh1/adh2* region (Tarchini et al., Plant Cell, 12: 381-391, 2000) indicated a complete lack of colinearity with the *adh1* regions of maize and sorghum, suggesting that the *adh1* gene moved to its current location in an ancestral *Andropogoneae*. We have now identified and sequenced a rice BAC that contains most of the genes flanking *adh1* gene in sorghum and maize. Comparison between sorghum and rice revealed 12 genes that are shared in order and orientation, however, two gene insertion events in sorghum and a tandem gene duplication in rice differentiates these regions. We have also sequenced the "homoeologous" segment of maize. The BAC clone was identified using sorghum genes (missing from the maize *adh1* region) as probes. The gene order is conserved in two maize regions, but numerous genic deletions differentiate the segments. As a result, only three genes (out of 8) are still present in both homoeologous segments. We will present evidence regarding whether our results support the model that one of the two homoeologous segments is more closely related to sorghum than to the other maize segment (Gaut and Doebley, PNAS 94:6809-6814, 1997). In general, our data indicate that the maize genome exhibits extreme instability over the last 25 million years. Most of the changes involved deletions of more than 50% of the duplicated genes present in the ancestral allotetraploid.

83:115

Genomic organization and expression pattern of the duplicate factors Zmfie1 and Zmfie2 in maize

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Fertilization independent endosperm (fie) null mutations of Arabidopsis cause precocious endosperm development before fertilization, when the mutant is maternally transmitted. The Fie gene encodes a polycomb group-related protein with WD-repeats in their amino-terminus and is believed to act as a suppressor of endosperm development. In maize there are two homologous gene sequences reflecting the fact that many genes in maize exist as duplicate factors because of the allotetraploid origin of maize. We have mapped those two sequences and found that they are closely linked to orp1 at 66.3 map units on chromosome 4 and orp2 at 61 map units on chromosome 10, confirming that they are contained within orthologous regions of the maize genome. We called the sequence linked to orp1 Zmfie2 and to orp2 Zmfie1. The expression of both genes was investigated in two inbreds, B73 and Mo17. Using a polymorphism of the coding regions between these two lines and RT-PCR of mRNAs from reciprocal crosses we could show that Zmfie1 has a parent-of-origin dependent allelic expression, but Zmfie2 not. Zmfie1 is expressed in embryo, endosperm, as well as in many vegetative tissues, while the Zmfie2 is only expressed in endosperm and embryo but not in vegetative tissue. Expression of Zmfie1 can be detected in ovules long before fertilization occurs and may play a similar role like the Fie in Arabidopsis. However, like in other cases maize provides interesting examples of divergent regulation of duplicate factors. To gain a better understanding of the divergence of the two maize genes, we used their cDNAs to screen the public B73 BAC library for clones containing both genes. Sequencing these clones will not only provide us with the regulatory regions of the two genes but also the sequences of other linked genes and their divergence.

84:127

Genic rearrangements of Wx1 homologous regions in barley, maize, pearl millet, rice, sorghum and diploid wheat revealed by comparative sequence analysis

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Single BACs containing Wx1 homologous genomic regions in six grasses (barley, maize, pearl millet, rice, sorghum, and diploid wheat) were sequenced and analyzed. We found that these regions contain several alterations in gene content, order and/or orientation. Some of the rearrangements appear to mark specific lineages. For instance, a cluster of five genes that are 5' to Wx1 appear to be in the same relative order in the lineage that gave rise to maize, sorghum and pearl millet, but are in the inverted orientation in rice. None of the genes around the Wx1 homologues in barley or wheat are homologues of any of the genes in the other four grass species studied. Just 5' to Wx1 in maize, a triplication of an ~10kb segment containing 2 predicted genes per repeat was observed in maize, with the central repeat in inverted orientation relative to the other two. These genes are not seen in the Wx1 BACs for any of the other species investigated, and are probably misannotated transposable elements. Our data provide the first molecular description of a pearl millet genomic segment, and we found that retrotransposons account for approximately 70% of this region. Despite abundant retrotransposons in the maize, barley, pearl millet and wheat genomes in this region, all exhibited a higher gene density than predicted by their overall genome sizes and a random model for gene distribution.

IBM Maize Community Resources

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The Maize Mapping Project is funded to develop a physical map of maize that will be anchored to the genetic map by molecular and trait markers, to develop data management resources allowing for analysis and curation, and to provide accessibility to the public of all the resources developed through this project. A high-resolution genetic map of maize has been created using the Intermated B73 X Mo17 (IBM) mapping population. The population underwent random-mating for 4 generations resulting in a theoretical 3-fold increase in recombination events. 1006 RFLP and 804 SSR loci, both previously mapped markers and novel markers, were placed on 302 IBM lines to construct this map. RFLP and SSR markers previously mapped will allow a bridge for comparison of other genetic maps to the IBM map. Novel single-copy RFLP markers were developed from a new *PstI* genomic library (*mmp* loci) and from a group of Mu-tagged clones for genes associated with seed development (*ufg* loci). Novel SSR markers were derived by mining public sequence data and from enriched libraries (*umc* \geq 1000 loci). Illustrations and text files of the IBM genetic map are available through the project web site at: <http://www.maizemap.org>

Screening images of both the RFLP and the SSR markers are also available on-line as well as the SSR primer sequences. To facilitate public use of this mapping population, a sub-set of 94 lines has been selected and is available to those researchers interested in adding additional markers. Researchers can obtain seed of this 94 line sub-set to grow themselves, seed of the entire 302 lines, or DNA of the 94 line sub-set for PCR based mapping. New data collected from the sub-set of 94 lines can be submitted to an on-line mapping tool so researchers can analyze data themselves. A sub-set of map scores will also be available specifically for QTL mapping. Funded by NSF Plant Genome Grant DBI 9872655.

86:128 Low and Medium Resolution Radiation Hybrid Maps for Maize Chromosomes 2 and 9

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The goal of the radiation hybrid mapping project is to produce chromosome specific high resolution radiation hybrid maps for the maize chromosomes. Maps are being developed from oat plants that carry single maize chromosome segments generated from monosomic oat-maize chromosome addition lines by radiation. Our efforts have been focused on maize chromosomes 2 and 9 with an interim objective of producing low and medium resolution mapping panels. These panels are intended to be used for high throughput mapping of sequences to chromosome segments. We now have a low resolution map for chromosome 9 that divides chromosome 9 into six major segments. We are developing a medium resolution map that divides chromosome 9 into approximately 20 segments. Low and medium resolution maps also are being developed for chromosome 2. We are using these materials to map duplicated genes. Small gene families are identified by grouping EST contigs and singletons into clusters based on BLAST searches. PCR primers are designed against representative sequences from EST clusters. Sequences are first mapped to chromosome using plus/minus PCR assays on chromosome addition lines. Self-fertile disomic addition lines currently are available for all maize chromosomes except chromosome 10. We have a haploid addition of chromosome 10 with efforts to recover a disomic version to complete the set. Sequences located on chromosomes 2 and 9 are then mapped to segment using radiation hybrid mapping panels. DNA is available for all 10 addition lines and the chromosome 9 low resolution mapping lines. This material is based upon work supported by the National Science Foundation under Grant No. 0110134.

87:229

Sorghum and its value to the maize community.

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Sorghum, in addition to its importance as one of the worlds leading grain crops and most noxious weeds (Johnson grass), offers many benefits to maize researchers engaged in genome analysis. These are primarily a result of its small genome size (680-760 Mbp), recent divergence from maize (ca. 20 MYBP versus 50 MYBP for rice), and more extensive diploidization than maize resulting in simpler genetic control of many phenotypes. A detailed molecular map of >2,600 RFLP markers provides for fine-scale alignment of the sorghum and maize genomes to one another and to many other Poaceae. High-coverage BAC libraries for sorghum, maize, and rice that have been completely fingerprinted are being anchored to common DNA probes in parallel, to empower a host of comparative, functional, and evolutionary genomic studies. The resulting genetically-anchored physical maps will be useful to advance dissection of many individual genomic regions, to better understand grass genome evolution, and to assist with eventual assembly of an ordered sequence.

88:22

MAIZE AND SORGHUM GENE-TARGETED SEQUENCING AND MICROARRAY ANALYSIS

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The genomes of important crop species are often very large and repetitive posing a major challenge to approach genome-wide gene discovery by full genomic sequencing. It has been proposed that all diploid higher plant genomes share essentially the same set of genes, called the "gene space". The differences in genome size are due mainly to the amount of repetitive DNA. We have shown that, because of being heavily methylated, repeats can be largely excluded from maize genomic shotgun libraries by using a McrBC+ E. coli host strains. In contrast, unmethylated genic regions are preserved in these genetically filtered libraries. Furthermore, we have evidence supporting the idea that these filtered libraries will contain most of the genes in the genome. This technique can be applied to other crops. In particular, we are aiming to shotgun sequence most of the low copy fractions of the maize and sorghum genomes using this approach. Selected clones will be then used to develop microarrays which will be useful to anchor genes to the physical map, obtain expression profiles and, in the case of maize, to perform a high throughput screening of transposon-tagged mutants in a large population of maize plants containing new insertions of transposon Mu (Maize Targeted Mutagenesis MTM; <http://mtm.cshl.org>). In addition, we are currently testing this technology in other plants in order to explore how broad the application of this approach may be.

89:40

Characterization of the phytochrome gene family in *Zea mays* cv B73

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Plants use the light environment to initiate and sustain developmental patterns. Phytochromes are the principle red and far-red photoreceptors mediating seed germination¹, hypocotyl elongation, and contribute to flowering time regulation and the shade avoidance response.² The phytochrome gene family has been examined in detail in the model eudicot, *Arabidopsis thaliana*, and consists of five genes PHYA-E. The gene family structure in monocots consists of only three members PHYA-C. Previous work in *Sorghum bicolor* showed that the *ma3R* allele conferring photoperiod-insensitive early flowering is due to a PHYB loss-of-function mutant³, suggesting that PHYB may play a similar role in flowering time regulation in maize. However, in maize an allotetraploid event⁴ resulted in the duplication of the PHYB locus. How the duplication at the PHYB locus will effect maize is not known. If the function of PHYB1 and PHYB2 are redundant then the loss of one should not affect normal plant development. Conversely, if PHYB1 and PHYB2 have diverged in function, it is probable that disruption of one copy would lead to a visible mutant phenotype. Thus our initial characterization of the PHYB signaling pathway in maize requires a detailed analysis of the PHYB gene family structure and organization. Using a B73 BAC library from CUGI, we have isolated both copies of PHYB and performed a preliminary examination on both the nucleotide and protein sequences for a phylogenetic study. References: 1Borthwick HA, Hendricks SB, Parker MW, Toole EH and VK Toole (1952) A reversible photoreaction controlling seed germination. Proc. Natl. Acad. Sci. USA 38:662-666. 2Smith H (1995) Physiological and ecological function within the phytochrome family. Ann Rev Plant Physiol Plant Mol Biol 46:289-315. 3Childs KL, Miller FR, Cordonnier-Pratt MM, Pratt LH, Morgan PW and JE Mullet (1997) The sorghum photoperiod sensitivity gene, *Ma3*, encodes a phytochrome B. Plant Physiol 113:611-619. 4Gaut, B and JF Doebley (1997) DNA sequence evidence for the segmental allotetraploid origin of maize. Proc Natl Acad Sci USA 94:6809-6814.

90:34

Sequence analysis of the chromosomal regions containing all members of the maize alpha zein multigene family

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Zeins represent the major fraction of storage proteins in maize seeds and alpha zeins are the most abundant proteins within this fraction. The alpha zeins are encoded by a multigene family that is organized as gene clusters of four subfamilies, *z1a*, *z1b*, *z1c*, and *z1d*. The *z1c* zein gene subfamily maps to two locations on maize chromosome 4S, at 27.3 and 51.8 map units. Previously, we have sequenced the two regions comprising this gene family from maize inbred line BSSS53. The centromeric, proximal region contains only one copy, but the distal region 22 copies of this gene family. When the restriction patterns of this gene family are analyzed in various inbred lines and teosintes, it appears that they fall into a few haplotypes. For instance, inbred B73 exemplifies a different haplotype than BSSS53. Southern blot analysis also indicates that haplotypes may differ in gene copy number. Hence, comparison between different haplotypes should provide insights into the amplification of this gene family. Therefore, the orthologous region of map position 27.3 from the B73 genome was sequenced. Indeed, significant sequence divergence could be found between these two inbreds. The divergence was caused by three rounds of gene amplification and different transposition events, which led to nearly a 50% chromosomal expansion in BSSS53. Chromosomal regions from B73 comprising the other three subfamilies, *z1a*, *z1b*, and *z1d* have also been cloned. Three probes were derived for each of these subfamilies by EST data analysis, and used to screen an MboI BAC library of inbred line B73. Positive clones were further sorted by DNA fingerprinting and Southern blot analysis into 5 groups, indicating 5 possible genomic locations for these subfamilies. Five BAC clones, comprising the regions of all five chromosomal locations and all members of these three subfamilies based on genomic Southern blot analysis, were selected for DNA sequencing. A total of 22 gene copies were identified with each BAC clone containing from 1 to 9 copies. This indicates that different degrees of gene amplification have occurred during the evolution of all the alpha zein genes. Distance analysis of zein genes from all four subfamilies provides a clear view of how the entire maize alpha zein multigene family has evolved.

91:226

A comparison of maize gene recovery from genomic sequence obtained from the undermethylated fraction of the maize genome vs. that obtained from whole BAC sequencing.

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Analysis of the maize genome will greatly influence our understanding of the molecular basis of agronomically important traits, gene regulation, transposable element function, genome evolution, plant development and biology. Recently, the maize community has voiced its support for the initiation of a maize genome sequencing project. However, the large size of the maize genome (~2.5Gb) and the expectation that upwards of 80 % of the genome is represented by repetitive elements has prompted the community to examine sequencing technologies expected to target gene rich regions as an alternative to whole genome sequencing. The maize research program at Monsanto has generated a collection of maize genome resources including physical mapping data, BAC genomic DNA sequence, EST sequence and sequences generated from reduced representation libraries that exploit differential methylation rates between repetitive and non repetitive regions of the maize genome. Initial comparisons of gene recovery from reduced representation sequence vs. maize genomic BAC sequence suggests that as few as 50% of the genes detected by BAC based sequencing may be present in the Monsanto reduced representation datasets. This suggests that a reduced representation approach to maize genome sequencing may limit the comprehensiveness of the resource produced. Details of this analysis will be presented.

92:220

Single nucleotide polymorphism discovery and mapping in maize.

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There is increased interest in using single nucleotide polymorphisms (SNPs) for detecting genes linked to economically important traits in plants. Single nucleotide polymorphisms are biallelic markers that have the potential for inexpensive genotyping using high throughput methods. The present study is aimed at developing SNP markers to integrate the genetic and the physical maps of maize. Two hundred unigenes from the "CornSensus" set served as templates for this preliminary study. Primers were designed to amplify ~300 bp from each unigene. The amplification products were sequenced with forward and reverse primers in 12 lines including B73 and MO17 (the parents of the IBM mapping population). Sequence quality was assessed using modified Phred and Phrap software. Primer sequences were trimmed with PERL scripts before aligning with the Clustal software. Only one locus was monomorphic. Sequence comparisons of 178 loci, consisting of 62,300 bp, contained a total of 1288 variants; 58% SNPs and 42% indels. This is an average of 7.2 variants per locus. The frequency of SNPs and indels is 1/84 bp and 1/115 bp, respectively. Seventy-five loci contained SNPs between B73 and MO17. The SNPs between B73 and MO17 were validated and used in multiplexed primer extension reactions on the IBM population. The scoring of the SNP mapping data was automated and integrated to a Laboratory Information System Management (LIMS) for mapping and for submission to MaizeDB. The pipeline of SNP production and mapping will be presented. Acknowledgments This work was funded by NSF Plant Genome Grant DBI 9872655.

93:147 Characterization and physical mapping of maize BAC libraries using high density BAC filter hybridization

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High-density filter sets from two maize B73 libraries containing 6X(HindIII) and 7X (EcoRI) haploid genome equivalents, respectively, were evaluated with a set of complex probes. The complex probes will provide information on chromosome architecture and organellar DNA content. A second set of probes containing ninety maize RFLP core markers were hybridized to the HindIII BAC filters. These markers will be used to facilitate the anchoring process of the genetic to physical map. The core markers have been extensively used to generate genetic maps in maize, provide a framework to anchor BAC contigs with the IBM map, and function as bin delimiters. After accounting for the clones with no inserts and organellar contaminants 98.6% of the HindIII BACs remain that can be used for physical mapping. The four centromeric repeat probes had similar hybridization trends in both libraries. The number of positive telomeric repeat sequences and rDNA were increase in the EcoRI library by four- and seven-fold compared to the HindIII library. Twenty-three of the single copy core markers identified an average of 7.2 positive clones with a standard deviation of 3.10 and a range of 3 to 15 positives clones. Forty-two markers with two to three copies based on RFLP mapping data had 3 to 23 positive BAC clones following hybridization experiments. Seven core markers are suspected to contain repetitive elements based on the high number of positive clones obtained from hybridizations. The wide range of positive signals identified by the maize core markers may be indicative of the effects of preferential cloning caused by use of the HindIII restriction enzyme.

94:172 A BAC pooling strategy: powerful tool for the maize integrated genetic and physical map construction.

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The construction of an integrated genetic and physical map of the maize genome (2500 Mbp) is the primary goal of our ongoing maize genome project. To accomplish this goal, we have used a BAC pooling strategy combined with a high-throughput PCR-based screening method to facilitate anchoring of the maize physical map to the genetic map. The BAC pooling strategy has been successfully demonstrated in sorghum, which has genome size one third of maize genome. This is the first report demonstrating the usefulness and efficiency of this BAC pooling approach in maize despite its large genome size and high amount of repetitive DNA. About 6X haploid genome equivalents (110,592 maize BAC clones) were pooled in six different dimensions (plate, face, side, row, column, and diagonal) to create 288 pools of BAC DNA. The quality of the BAC DNA pools, and their utility for identifying BACs containing PCR-based markers, was tested using primers that amplify maize SSR markers. These markers have been mapped on the IBM Genetic Map and are dispersed across the maize genome. Amplified PCR bands from the pools were deconvoluted into individual BAC address using Resolve script. Results showed 1 to 13 positive BAC clones per SSR primer pair. On average 5.45 BAC clones were identified with each SSR marker analyzed. Cross checking between our data and FPC fingerprinting data revealed that about 70% of the SSR markers identified BACs, that were located in single contig. Among the remaining SSR markers, some identified BACs that were located in two different contigs created by fingerprinting, or BACs located in a contig and a pool of singleton BACs. These make our data extremely valuable in merging contigs or singletons and contigs. This information will be integrated with fingerprinting data generated by CUGI to assemble the BAC contigs using FPC and contribute to the process of physical map construction.

95:139

New and Old Approaches to Selective Sequence Analysis of Gene-Containing Regions of the Maize Genome

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Maize has a moderately large genome of 2.3~2.7 X 10⁹ bp, and most of it is composed of retrotransposons. In order to understand and characterize maize genome structure and organization without sequencing the entire genome, we have utilized two technologies to selectively sequence the genic regions of the maize genome. One is methylation-spanning linker library (MSLL) technology which employs BAC end sequencing of clones that were derived from a series of libraries constructed with two restriction enzymes, Sall and HpaII, that are sensitive to 5 methyl cytosine in the sequences CG or CNG. Our data indicate that the end sequences of maize MSLL clones are greatly depleted in repetitive DNAs and enriched in genes relative to total genomic DNA. Another technology employs high Cot sequence analysis based on the DNA reassociation technique. Maize genomic DNA was renatured to a Cot value at which most repetitive DNA should reassociate, but not the unique genes or other low-copy-number regions. These unrenatured sequences were cloned and sequenced. As expected, the abundance of retrotransposons decreased dramatically from 57% to 16%, compared with an unnormalized shotgun library. We believe that these two technologies will allow recovery of most or all maize genes and also identify the junctions between genic islands and repetitive DNA blocks. These two technologies will permit low-cost sequencing of complex genomes that are otherwise resistant to shotgun sequencing.

Molecular Genetics

96:168

Analysis of Maize Inflorescent Genes Using Association Studies

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Association mapping methods were applied to four maize inflorescence development genes to determine possible associations between segregating polymorphisms and ear traits. Association studies focus on polymorphisms found in natural populations and establish how well they correlate with a phenotype of interest. The phenotypes in this study include the number of kernel rows per ear, ear length, and ear diameter. The inflorescence genes, leafy A (lfyA) and B (lfyB), ramosa 1 (ra1) and fasciated 2 (fae2), were PCR amplified in their entirety from a diverse group of 32 inbred maize lines. Regions with potential association were sequenced from an additional 70 inbred lines. The sequence for ra1 and fae2 showed relatively low diversity, while those for leafy A and B were highly diverse and had numerous insertions and deletions. One indel, a four basepair deletion, found in leafy A eliminates exon 3 and may be associated with flowering time and low row number. Association tests were performed between the polymorphisms and phenotypic data obtained over 7 field seasons. The results of these associations will be discussed.

97:105

Molecular analysis of maize lines involved in in vitro or in vivo double haploids production

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In vitro androgenesis and in vivo gynogenesis are very powerful methods to generate double haploid lines for breeding and for molecular studies. These two technologies can also be used to study gametophyte development and double fertilization mechanisms. Lines that carry good aptitude to in vitro androgenesis can be considered as tools to study the genes involved in the switch between normal pollen development and embryo development. Inducing lines for in vivo gynogenesis can be considered as mutants that allow the development of an haploid embryo with single fertilization of the endosperm. Here, we present molecular characterization of two isogenic lines with a good androgenesis aptitude. CDNA-AFLP technology was used for developmental and genetical differential analysis of genes expressed during the microspore culture process. Microsatellites were used for molecular mapping of loci involved in androgenesis. Results obtained on androgenesis are discussed and molecular mapping projects of genes involved in in vivo gynogenesis induction are presented.

98:167

Tubulin gene knockouts in maize

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Microtubules are cytoskeletal elements that are involved in essentially every aspect of plant growth. In maize, the alpha and beta subunits that comprise microtubules are encoded by large families of expressed genes. Our goal is to determine if disruption of any of the maize tubulin genes has an effect on the plants ability to grow and reproduce normally or on the plant's ability to withstand chilling. We have used a reverse genetic approach to identify plants containing a beta tubulin gene that is disrupted by the presence of a Mutator (Mu) transposable element. The gene targets in which we are most interested are those encoding tubulins implicated in chilling sensitivity, tub1, tub6, tub7, and two genes for tubulins that are prominent during reproduction, tub2 and tub4. Plants are screened for germinal insertions into exons, followed by outcrossing to dilute out copies of Mu in other genes. PCR screening for tub::Mu by PCR uses part of the Mu terminal inverted repeat and part of the gene specific 3' or 5'UTR as primers. To date we have five independent lines of tub1::Mu, three of tub2::Mu, two of tub4::Mu, and one each of tub6::Mu and tub7::Mu. We have produced homozygous mutants of tub1, tub2, tub4, and tub6 and heterozygous double mutants of tub1 tub6 and tub2 tub4. We are currently producing homozygous double mutants of tub1 tub6.

99:119

Evaluation of BMS cells as a model system to study organellar gene regulation

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Our laboratory studies regulation of plastid and mitochondrial gene expression by nucleus encoded factors. To this end, we wished to develop an in vivo maize system that would allow more rapid analysis than stable transformation of plants. Our first choice of a model was the Black Mexican Sweet (BMS) cell line due to its availability, prolific growth and the ability to easily produce transient and stable transformants. We assumed that BMS cells in culture would have plastids and mitochondria that are biochemically similar to "normal" in planta maize cells. While testing the fecundity of this assumption we found that the plastid gene expression profile of BMS varied dramatically from whole plants. Further tests revealed that the BMS plastid genome contains very large deletions. By our estimates, as much as 70% of the wild-type maize plastid genome is missing in BMS. In spite of the lack of plastid gene expression we have found that BMS cells can be used to test suspected organellar proteins for compartmentalization. Signal peptide sequences were placed upstream of the Green Fluorescent Protein (GFP) and BMS cells were transformed with these constructs using biolistics. 24 hours after transformation, cells expressing the transgene were easily identifiable via standard and confocal fluorescence microscopy. Presented will be evidence of distinct import of GFP into plastids and mitochondria of BMS cells as directed by the signal peptides of known and suspected organelle proteins. This assay offers an alternative to the current protoplast transformation approach to studying in vivo import in maize cells.

100:42

MARK, A MAIZE ATYPICAL RECEPTOR-LIKE KINASE EXPRESSED DURING EMBRYOGENESIS AND IN THE MERISTEMS OF THE ADULT PLANT, INTERACTS WITH MIK, A NEW GCK/SPS1 KINASE

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In the last few years an increasing number of receptor-like kinases (RLK) have been described in plants. Although RLKs display structural similarities with animal receptor protein kinases (RPK) and are supposed to function in a similar way, very few is known about their respective ligands, and in most cases almost nothing is known on the downstream signaling cascades they activate. In this communication we will present the characterization of an atypical receptor-like kinase from maize that we have named MARK (from Maize Atypical Receptor-like Kinase). MARK is an atypical RLK because it presents a putative intracellular domain containing the 11 conserved subdomains of protein kinases but presenting substitutions of some of the invariant and highly conserved aminoacids, suggesting that it contains an impaired kinase domain. Yeast two-hybrid screenings performed with the putative intracellular domain of MARK have allowed us to clone a new member of the GCK family of kinases that interacts with MARK, and that we have named as MIK (from MARK Interacting Kinase). The GCK proteins are Ser/Thr kinases related to the human Germinal Center Kinase (GCK) that, in some cases, are supposed to connect cell-surface receptors to intracellular signaling MAPK cascades. The interaction of MARK with the regulator domain of MIK could be a mechanism by which this atypical RLK could participate in signal transduction. Both MARK and MIK are expressed during embryogenesis and in the meristems of the adult plant, which suggests that they could participate in the control of the differentiation or proliferation processes in maize.

101:32

Gene Expression Profiling after Decreased and Supplemental Ultraviolet Radiation in maize genotypes with varying amounts of flavonoids.

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Microarray hybridization was used to assess acclimation responses to four UV regimes by maize lines varying in flavonoid content. We found that 396 of the 2500 cDNAs tested were regulated by UV-B in at least one genotype. Among these, 209 transcripts are assigned putative functions, while 187 encode unknown proteins. Supplemental UV-B increased expression of stress response and ribosomal protein genes, while photosynthesis-associated genes were down regulated; lines lacking anthocyanins had more dramatic responses than did lines with anthocyanins, confirming the shielding role of these pigments. Sunlight filtered to remove either UV-B or UV-A+B resulted in significant expression changes in many genes not previously associated with UV responses. Some pathways regulated by UV-B and UV-A are shared with defense, salt and oxidative stresses, however, UV-B radiation can activate additional pathways not shared with other stresses.

102:41

Agrobacterium-mediated transformation of maize

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Maize cells have been shown to be receptive for integration of foreign DNA via direct DNA delivery and Agrobacterium-mediated transformation technologies. The former strategy generally results in high copy, complex insertion events, while the latter approach typically produces relatively simple integration events in the derived transformants. A complex integration event will have a higher probability of unstable expression of the desired phenotype over multiple generations due to an increased risk for gene silencing. Therefore, while direct DNA delivery transformation protocols for maize are generally robust, the lower quality of the derived transformants compared to Agrobacterium-mediated methods requires additional labor, greenhouse space and ultimately cost in the subsequent screening for desirable transformation events. The development of efficient Agrobacterium-mediated transformation protocols promises to significantly enhance the use of transgenic plants in maize functional genomics research. We have explored the efficiency of modifying published maize transformation protocols to integrate *A. tumefaciens* as a DNA delivery agent. Agrobacterium strain C58C1 carrying the Ti plasmid pMP90 and a pPZP212-derived binary vector was used to transform immature embryos from the genotype Hi II. The binary vector harbored the GUSPlus gene under the control of the maize Ubiquitin1 promoter and a CaMV 35S promoter::nptII selectable marker gene that conferred resistance to the antibiotic paromomycin. Following the inoculation step, the embryos were co-cultured overnight on the inoculation medium solidified with 1.2% agarose. The explants were subsequently placed on callus induction medium consisting of N6 components supplemented with 100 mg/L paromomycin and 50 mg/L carbenicillin. Following approximately two months of selection with biweekly transfers, regeneration of proliferating callus lines was initiated. Selection pressure was reduced during the regeneration steps to 50 mg/L paromomycin. Plantlets were rooted without further selection. A strong variation among ears was observed with transformation efficiencies across four independent experiments ranging from 0 to 4.7% on a transformant in soil per embryo basis. The combined efficiency across the four experiments was 1.6%.

103:72

Allele frequencies in fall armyworm populations

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Spodoptera frugiperda, commonly known as the fall armyworm, is a serious pest of over 60 documented host plants, particularly species of corn and other grasses. A larvae colony obtained from the Corn Host Plant Resistance Research Unit (CHPRRU) in Starkville, MS has been used to identify insect resistance genes in maize. To determine whether or not this experimental colony is representative of fall armyworms in the wild in identifying resistance genes that can be deployed for production, this project will compare the gene frequencies of three wild fall armyworm colonies to the experimental CHPRRU fall armyworm larvae. The wild colonies to be tested were collected from Tifton, Georgia; Holly Springs, Mississippi; and Columbia, Missouri. DNA extracted from each population was analyzed using Amplified Restriction Fragment Length Polymorphism (AFLP) with fluorescently labeled primers on the ABI 310 sequencer. Twenty-four *EcoRI/MseI* (+3/+3) primer combinations were tested on four DNA samples per location. The six consistently amplified primer pairs with the largest average number of fragments were used to perform the experiments. Primers were multiplexed for analysis. Data from the ABI 310 was analyzed using GeneScan. Fragments were tabulated by location and allele frequencies were calculated. Cluster and principle component analysis performed on the data were used to the degree of similarity among the wild populations and the lab colony.

104:179

Characterization of gene families encoding enzymes for carotenoid and isoprenoid biosynthesis in maize.

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Carotenoids, derived from plant food sources, are converted in humans to vitamin A and other vital compounds needed for growth and development. Endosperms of food crops, such as maize and wheat, are low in carotenoid content, and are especially low in provitamin A as compared with nonprovitamin A carotenoids. Metabolic engineering of the endosperm carotenoid biosynthetic pathway has potential for providing higher levels of the provitamin A carotenoid beta-carotene. To achieve this goal, it is necessary to characterize both genes encoding enzymes required for biosynthesis of beta-carotene and also those enzymes which convert betacarotene to nonprovitamin A carotenoids. We have screened a maize B73 BAC library with cDNAs encoding enzymes required for biosynthesis of carotenoids and for their isoprenoid precursors: 1-deoxy-D-xylulose 5-phosphate synthase (DXS), 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), isopentenyl diphosphate isomerase (IPP isomerase), phytoene synthase (PSY), zeta-carotene desaturase (ZDS), and beta-carotene hydroxylase (HYD). From DNA sequence and hybridization analyses, we are determining the size of the different gene families. We will then be looking at tissue- and temporal-specificity of expression to ascertain the role of each family member in contributing to carotenoid content in maize. These studies are contributing to the foundation required for metabolic engineering of carotenoid content and to manipulation of competing isoprenoid pathways that provide plant cells with compounds such as sterols, phytoalexins, hormones, etc.

105:166

Paramutagenicity of the p1 locus is correlated with enhancer element copy number

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Paramutation is a naturally occurring gene silencing phenomenon in maize. It is defined as an allelic interaction between an inducing or paramutagenic allele and a sensitive or paramutable allele that leads to a meiotically heritable reduction in gene expression of the sensitive allele. Several alleles of genes encoding transcriptional regulators of the anthocyanin pathway are known to be paramutagenic, including the p1 gene. We reported previously that two p1 alleles, P-pr and P-rr, participate in paramutation while the null allele P-ww is neutral. The paramutable P-rr allele confers dark red pigmentation in pericarp and other floral organs, whereas the paramutagenic P-pr allele, a spontaneous derivative of P-rr, causes reduced pigmentation in the same tissues. P-rr', the P-rr allele after exposure to P-pr, is phenotypically and molecularly indistinguishable from the P-pr allele. Although P-rr' acquires paramutagenicity and is therefore able to paramutate naive P-rr genes, P-rr' is less paramutagenic than P-pr. The P-pr allele is hypermethylated and reveals condensed chromatin in a distal enhancer element compared to P-rr, but is otherwise structurally identical to P-rr. The coding region of both alleles is flanked by two 6.2kb repeats each containing two 1.2kb repeats. Each 1.2kb repeat carries the distal enhancer for p gene expression. Several other p alleles that differ in copy number of the distal enhancer, ranging from 4 to 1 repeats, were tested for paramutagenicity. Since all alleles are null, only the paramutagenic strength for secondary paramutation can be studied. From genetic crosses and molecular analysis of these null alleles the following can be inferred: 1. The P protein is not involved in p1 paramutation. 2. The p transcript is not required for the induction of paramutation at the p1 locus. 3. The cis acting site necessary for p1 paramutation has to be outside the coding region. 4. Paramutagenic strength is correlated with the enhancer element copy number indicating a repeat induced silencing mechanism.

106:83

Identification of compartment specific genes in the maize kernel

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We are interested in the identification of genes whose transcription is restricted to a specific compartment, and/or developmental stage, in the maize kernel. To this aim, we have done differential screening experiments on 6000 clones from a cDNA library produced from 10 days after pollination (10DAP) maize kernels. Inserts from all the clones were PCR amplified and southern blotted. These filters were hybridised with the following cDNA probes: 8 DAP kernels subtracted with 21 DAP kernels 21 DAP kernels subtracted with 8 DAP kernels 10 DAP kernels basal-halves subtracted with 10 DAP kernels upper-halves 10 DAP kernels upper-halves subtracted with 10 DAP kernels basal-halves Roots plus leaves, un-subtracted We will present the results of this screening, as well as the preliminary results on the sequencing of those clones identified as putative compartment/developmental stage specific genes. This work was supported by the European Union grant QLK-2000-00302 and the Comunidad de Madrid grant 07G/0030/2000

107:86

Epigenetic Silencing and Heterosis in Maize

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For nearly a century, geneticists and breeders have known that the progeny of crosses between unrelated individuals often exhibit hybrid vigor or heterosis. In maize, the contribution of heterosis to commercial F1 hybrid yield is usually more than 50%, but the underlying genetic mechanisms responsible are still poorly understood. We have examined the relationship between yield heterosis in maize and gene expression using GeneCalling, a comprehensive platform for profiling expression of thousands of cDNAs. A large phenotypic variance in both yield and heterosis was established among hybrids by crossing a common female parent with different male inbreds that varied in genetic relatedness. The majority of cDNAs in the hybrids were expressed additively, i.e. within the range of the two parents, but, in each hybrid examined, more cDNAs were expressed at levels similar to the male parent than to the female parent. Heterosis and hybrid yield correlated negatively with the degree of paternally biased expression and positively with the proportion of cDNAs expressed at or near to mid-parent levels. Additional analysis indicated that epigenetic silencing of maternally inherited alleles that is associated with the genetic relatedness of inbred parents might negatively influence heterosis in a commercially important crop species.

108:87

Environmental Stress Influences Allele-Specific Gene Expression and Yield Heterosis in Maize

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Commercial maize breeding involves selection of hybrid genotypes that exhibit heterosis and high yield in both stressful and stress-free environments. Using GeneCalling technology to profile expression of thousands of cDNAs, we have discovered biases in allele-specific gene expression in hybrids that are influenced by both genotype and environment. Hybrids grown in 1997 had lower yield and exhibited less heterosis than the same hybrids grown in 1999. Consistent with the results reported in the companion poster (#86), heterosis and hybrid yield in each environment were negatively correlated with a paternal bias in gene expression and positively correlated with the proportion of genes expressed at or near to mid-parent levels. However, the extent of paternally biased gene expression was significantly greater in hybrids grown under the stressed conditions of 1997, whereas the extent of additive or near-additive gene expression was significantly greater under the less stressful conditions of 1999. These results were corroborated by comparison of gene expression biases in two hybrids grown in 1998 under different levels of stress imposed by increasing planting density. Finally, analysis of reciprocal hybrids suggested that allele-specific expression at individual loci might be more closely associated with genotype-of-origin than with parent-of-origin effects.

109:112

Functional Genomics of the *Zea mays*/Fusarium graminearum Interaction

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We are using a microarray approach to document how the maize plant responds to the fungal pathogen *F. graminearum*. This fungus is a broad host pathogen, causing ear and stalk rot in maize as well as head blight/scab in wheat and barley. Nine novel EST libraries have been produced from *Fusarium*-challenged maize silk and kernel tissue. Using contig analysis, EST sequences from these libraries were combined with ESTs from cold-stressed maize libraries to produce a unique set. Approximately 5000 unique cDNA clones were chosen to represent this unique set. These were spotted in duplicate on glass slides to produce a 10,000 - element microarray. Initial hybridization experiments using this array were performed to establish a baseline response for the susceptible inbred B73 to *Fusarium*, prior to examining the gene expression of more *Fusarium* tolerant inbreds. Control (sterile fungal media-inoculated) vs *Fusarium*-inoculated samples were compared in each hybridization. Multiple samples of total RNA and polyA RNA were tested from both silk and kernel tissue. Data were normalized using logarithmic transformation and density-dependent centralization. Averaged across experiments, the number of clones showing greater than 3-fold up/down-regulation was 173 for kernel tissue and 106 for silk tissue. These clones represent some duplication due to multiple gene families and/or incomplete contigs. Randomly chosen genes (with gene expression levels ranging from low to high) observed to be up-regulated from array data have had their expression patterns confirmed by Northern analysis. These include defence genes anticipated to be induced (e.g. PR-protein genes).

110:39

Coactivator dependent and independent transcription of flavonoid genes.

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The Myb-domain proteins C1 and P control the transcription of the genes involved in the flavonoid biosynthetic pathway in maize (Mol et al. 1998. Trends Plant Sci 3:212). In spite of the fact that these two proteins have Myb domains over 70% identical, they control distinct branches of the pathway and have different mechanisms of activation. C1 activates the branch of the pathway that leads to the accumulation of anthocyanins, but only if the helix-loop-helix coactivator protein R is present. P does not require R and only activates the genes involved in the production of phlobaphenes. We have identified the amino acid residues in C1 responsible for the specificity of the interaction with R. When those residues are mutated in P to match the C1 sequence, we obtained a protein that interacts with R, and through this interaction it is able to turn on the anthocyanin branch of the pathway. This novel P protein, P*, has retained the ability to activate a1 in the absence of R. Thus we have shown that the specificity of these proteins is defined by their ability to interact with the cofactor R (Grotewold et al. 2000. PNAS 97:13579). We are now taking advantage of the dual role of P* to understand how C1 and P activate a1 in co-activator dependent and independent fashions. We are using mutants in each of the three cis-regulatory elements identified in the promoter of the a1 gene to understand how R contributes to the regulatory specificity of C1.

111:214

Emerging Spectrum of Mu-Tagged Seed Mutants Derived from UniformMu Maize

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We have screened 10,000 UniformMu families for visible seed mutants. The UniformMu population was derived by introgressing Robertsons Mutator lines carrying bz1-mum9 into a pigmented W22 inbred to facilitate mutant identification in a uniform background. The Mu-active pollen parents of each backcross generation were purged of pre-existing mutants by examining selfed ears of each male parent to evaluate both the level of somatic activity and detect segregation of seed mutants. An overall mutation frequency of 12% novel seed phenotypes was observed from backcross 3 through 6. Beginning with backcross 4, visible seed mutants were identified, tested for heritability, and Mu-off kernels selected for propagation. Similar mutant phenotypes frequently were found within the same M1 family. To assure independence for each mutant isolate, we selected unique mutants from each family based on phenotype. This yielded approximately 600 independent isolates. Digital imaging (accessible through www.endosperm.org), phenotypic analysis, and heritability testing has thus far been completed for 310 mutants. We have broadly categorized these mutants into 29% empty pericarp, 18% small kernel, 17% embryo defective (germlless), 16% defective kernel, 8% rough endosperm, and 12% other classes. This spectrum represents a similar diversity of phenotypes observed in other studies of maize kernel mutants (Neuffer and Sheridan, 1980, *Genetics* 95:929-944; Martienssen et al. mtm.cshl.org; Walbot et al. *ZmDB*), thus the mutagenic UniformMu population used here is functioning effectively as an unbiased source of new Mu-tagged mutants.

112:37

Precise and Efficient Genetic Improvement of Cereal Seeds (SeedDesign)

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Cereal seeds are agriculture's most important renewable resource for food and industrial raw material. Conventional methods for the improvement of quality and yield have produced spectacular advances in the past. Future developments will involve tapping the vast potential of the modern technology of plant modification. The chances of successful modification increase if the activity of the transgene can be precisely directed to the targeted cells. This requires the isolation and characterisation of many more cell type-specific promoters than are currently available. SeedDesign aims to isolate at least two functional promoter sequences for each of the cereal seed compartments, thus allowing to efficiently modify seed composition. SeedDesign is a project funded by the European Union within FPV (QLK3-CT-2000-00302).

113:82

Searching for the ZmMRP-1 target sequence in the promoter of BETL-1

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The transfer cell layer is a specialized tissue that in maize kernels occupies the base of the endosperm, where facilitates the uptake of nutrients by the growing seed. BETL-1 was the first transfer cell specific gene identified. We have recently identified a transfer cell specific transcriptional activator (ZmMRP-1), which might be a key regulator of the transfer cell differentiation process. ZmMRP-1 is a DNA-binding protein that can trans-activate the promoters of several transfer cell-specific genes, including BETL-1, in transient expression assays. We summarize here our current knowledge on the function of ZmMRP-1 and present our preliminary results in the characterisation of the ZmMRP-1 target sequence. This work was supported by the Spanish MCYT grant BIO2000-0848

114:201

PCR Based Molecular Markers Distinguish Chloroplast and Mitochondrial Genomes between maize and Tripsacum

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Progeny from interspecific and intergeneric cross hybridizations result in novel nuclear-cytoplasmic genetic interactions. In contrast to the usually biparentally inherited nuclear genome, inheritance of mitochondrial (mtDNA) and chloroplast (cpDNA) genomes will vary depending on the mechanisms regulating transmission to, and subsequent maintenance by, an organism. Only rudimentary knowledge of these phenomena is currently available. Obtaining better information about mtDNA and cpDNA transmission is necessitated by the substantial impact that nuclear-organelle interactions have on genetic fitness and population dynamics. Moreover, this basic information will have immediate applications in both bioengineering based and traditional agriculture. Organelle transgenics is a relatively new avenue that is being taken to develop bioengineered improved cultivars and novel value-added crops. The attractiveness of this approach for creating these genetically modified organisms (GMOs) over nuclear transgenics is partly derived from high-level foreign protein expression capabilities and the expectation for transgene containment through strict uniparental maternal organelle inheritance. Paternal and bi-parental organelle transmission does occur however, and is possibly augmented by wide hybridizations. A rapid molecular assay for discerning organelle genomes would be highly useful for deciphering the genetic regulation of organellar inheritance. The assay and resulting information on transmission genetics would provide the means to address organelle transgene containment as well as other issues involving cytoplasmic contributions to germplasm enhancement. This research reports on the identification of cleaved amplified polymorphisms (CAPs) between maize and *Tripsacum* ssp. organelles. CAPs were detected for four of the five tested cpDNA loci. Mitochondrial DNA testing has thus far yielded at least two good candidates for maize-*Tripsacum* CAPs identifiable loci. Both chloroplast and mitochondrial markers were used to investigate the organellar origin(s) of the purported natural interspecific *Tripsacum*-maize hybrid, *T. andersonii*.

115:97

Zein protein interactions rather than asymmetric distribution of zein mRNAs on ER membranes influence protein body formation in maize endosperm

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Prolamin-containing protein bodies in maize endosperm are composed of four different polypeptides, the alpha-, beta-, gamma- and delta-zeins. The spatial organization of zeins within the protein body, as well as interactions between them, suggest localized synthesis of gamma-zeins could initiate and target protein body formation at specific regions of the rough endoplasmic reticulum. To investigate this, we analyzed the distribution of mRNAs encoding the 22-kD alpha- and 27-kD gamma-zein proteins on cisternal and protein body rough endoplasmic reticulum membranes. In situ hybridization revealed similar frequencies of the mRNAs in both regions of the endoplasmic reticulum, indicating the transcripts are more or less randomly distributed. This implies that zein protein interactions determine protein body assembly. To address this question, we expressed cDNAs encoding alpha-, beta-, gamma- and delta-zeins in the yeast two-hybrid system. We found strong interactions among the 50-, 27- and 16-kD gamma-zeins and the 15-kD beta-zein, consistent with their co-localization in developing protein bodies. Interactions between the 19- and 22-kD alpha-zeins were relatively weak, although each of them interacted strongly with the 10-kD delta-zein. Strong interactions were detected between the alpha- and delta-zeins and the 16-kD gamma- and 15-kD beta-zeins; however, the 50- and 27-kD gamma-zeins did not interact with the alpha- and delta-zein proteins. We identified domains within the 22-kD alpha-zein that preferentially bind the alpha- and delta-zeins and the beta- and gamma-zeins. Affinities between zeins are generally consistent with results from immunolocalization experiments, and they imply an important role for the 16-kD gamma- and 15-kD beta-zein in the binding and assembly of alpha-zeins within the protein body.

116:95

Mutants of maize with reduced seed expression have multiple phenotypes

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To better understand the processes involved in the tissue-specific regulation of gene expression, a mutant screen was done in our lab with the aim of identifying new factors affecting the maize anthocyanin pathway. Mutagenesis was carried out using a genetic background with B-Peru, an allele that confers anthocyanin pigmentation in the aleurone. This allowed us to easily identify mutants that showed reduced seed color relative to wild-type. 54 pale aleurone color (pac) mutants were identified, many of which show profound developmental defects. We are using RNA blots and RNase protections to identify those mutants that have reduced expression of either the regulators, b1, c1, and pac1, or the biosynthetic genes of the anthocyanin biosynthesis pathway. One of these mutants is of particular interest based on these experiments. pac5 shows reduced expression of B-Peru, as well as a1, c2, and bronze1. The pac5 mutant is pleiotropic; plants homozygous for pac5 show numerous defects in both vegetative and reproductive tissues. Recent work with pac5 and the remaining mutants will be presented.

117:84

Functional characterization of plant I-type kinesins

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We are using a combination of computational and molecular techniques to functionally characterize plant MCAKs or I-type kinesins. Animal I-type and fungal Kar3 kinesins are closely related and have been shown to bind and depolymerize microtubules at their plus-ends. In addition, animal I-type kinesins localize to kinetochores during cell division. To test whether plant I-type kinesins function similarly, we generated antibodies to two non-overlapping epitopes identical among all sequenced plant I-type kinesins. These antibodies are being used for in situ localization studies. We also are working to express a maize I-type kinesin in insect cell culture. This will enable us to assay its potential microtubule depolymerization activity. In addition we are characterizing four T-DNA insertions into the two I-type kinesins encoded by the Arabidopsis genome.

118:80

RAD51 is required for chromosome segregation but not for chromosome pairing or cell viability in maize

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RAD51, a RECA-homolog of yeast, plays a central role in homologous recombination and DNA repair. To define the functions of RAD51 in plants, a reverse genetic method was used to isolate Mu insertion alleles of the two maize rad51 homologs. A PCR-based screen was used to isolate deletions of exonic DNA adjacent to Mu insertions. Flanking deletion derivatives were obtained at a rate close to 1% (4/500). Deletions ranged from 69 to 177 bp. rad51 double mutants are male sterile and have seed sets of only 5-10% of wild type. Cytogenetic analysis of these double mutants revealed that pachytene cells were normal in that homologous chromosomes were completely synapsed. In contrast, bivalents prematurely dissociated during diakinesis and more than 25% of quartets carried cells that were either missing an organized nucleolus or had two nucleoli. These observations indicate that nondisjunction occurs at both meiosis I and II in cells that lack RAD51. Hence, although RAD51 not required for synapsis, it is required for proper interhomolog and sister chromosome segregation. This study demonstrates a significant difference between the roles of RAD51 in plants and vertebrates. Vertebrate cells that lack RAD51 can not propagate due to the accumulation of chromosome breaks. In contrast, somatic cells of maize do not require RAD51 for normal division.

119:10

Mutations Preventing Paramutation Activate a Transcriptionally Silent Transgene

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Paramutation is a natural gene silencing phenomenon involving an interaction between alleles that leads to a heritable decrease in the expression of one of the alleles. Previous studies isolated several trans-acting mutants affecting paramutation in maize. To address whether the paramutation mutants would affect transgene silencing, the mutants were crossed with a silent transgenic line to obtain transgenic stocks segregating each paramutation mutant. Our initial results demonstrate that mutations in 5 genes that prevent paramutation can activate a previously silent transgene. The silent transgene contains a 35S promoter driving the transcribed region of B-1, which when active results in dark purple plants. The b1 locus encodes a transcription factor that activates the anthocyanin pathway. Sibling plants with the transgene, and heterozygous or homozygous for each of the recessive mutations, were compared. The B-1 genomic transgene was activated in homozygous paramutation mutant plants (darkly purple), while it remains silent in heterozygous plants (green). Northern blots demonstrated a dramatic increase in transgene RNA in the homozygous mutants, relative to heterozygotes. Nuclear run-on assays on plants segregating each of three paramutation mutants (mop1-1, rmr1-1, rmr2-1), demonstrated that individuals homozygous for each of the three mutations had dramatically higher transgene transcription levels relative to their heterozygous siblings. These results indicate that the transgene is transcriptionally silenced and that the "absence" of the wild type gene products in each of the mutants relieves this silencing. In the paramutation mutants, another transcriptionally silenced transgene (35S Bar) within the same transgene array, is also activated. Experiments are in progress to address whether the B-1 genomic transgene can cause trans-silencing and to investigate of the transgene structure, methylation and chromatin structure.

120:114

Identification of a telomere DNA binding protein in maize

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Telomeres are specialized nucleoprotein complexes at the ends of linear chromosomes, characterized by the presence of highly conserved DNA repeats (TTTAGGG in maize). Telomeres have multiple functions in chromosome maintenance, including an important role in meiotic chromosome segregation. During meiotic prophase, telomeres cluster on the nuclear envelope in an arrangement called the bouquet, facilitating homologous chromosome synapsis and ultimately recombination and disjunction. In order to better understand the mechanisms responsible for meiotic telomere dynamics, we set out to identify genes encoding plant telomere-binding proteins. Here we describe the analysis of a maize cDNA (P5) that encodes a protein with homology to other known telomere-binding proteins. The entire open reading frame of P5 was expressed in *E. coli*, and the recombinant protein was purified in order to assess its telomere-binding activity. Electrophoretic mobility band shift assays (EMSA) showed that the recombinant P5 protein is capable of binding maize telomeric repeat DNA. Further studies are under way to define the sequence requirements for this *in vitro* binding activity. This work represents an important step in defining the protein composition of maize telomeres, and should provide molecular tools for the future analysis of telomere functions in meiosis.

121:5

Transcriptional regulation of DIMBOA biosynthesis in maize

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Benzoxazinones are secondary metabolites that play an important role in the defense mechanism of maize against insects and microbial pathogens. In maize the predominant benzoxazinones are DIMBOA-and DIBOA glucoside. Their biosynthesis is carried out by different enzymes (BX1-BX9) that are coexpressed with respect to time and tissue and therefore are supposed to be coregulated by the same transcription factors. A comparative promoter analysis helped to identify a conserved element that is located in the proximal promoters of all known Bx genes. The specific binding of nuclear proteins to this box could be shown by competitive gel shift experiments using nuclear protein extracts from maize seedlings. A yeast one-hybrid screen is now being performed to get more information about the putative transcription factor.

122:108

Gene Discovery in Maize using Serial Analysis of Gene Expression.

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{1} University of Delaware {2} Mass General Hospital {3} John Hopkins University {4} University of Illinois

Serial Analysis of Gene Expression (SAGE) assay is being used to identify and annotate the transcripts expressed in maize embryos and to study the expression profiles of the Illinois long-term selection oil strains looking for quantitative transcript differences between the high and low oil strains which have been divergently selected for 100 years. SAGE analysis of the various maize strains have identified over 6703 different tags across all the SAGE libraries. The gene and/or transcript corresponding to each tag that exists in the databases has been identified informatically either through Genbank blast analysis or through alignment to the ESTs in the ZmDB database. Several genes that did not have a hit were identified through 3-RACE (Rapid amplification of cDNA ends)cloning and sequencing. Of the 6307 SAGE tags that were identified, 3964 tags corresponded with a maize EST. About 62% of all the tags were identified. Approximately 2343 tags or 38% of the total tags were not correlated with any EST in maize. SAGE tags with lower frequencies were mostly unrepresented in the maize database suggesting that more genes can be discovered though SAGE sequencing efforts and by using RACE technique these genes could be potentially identified. The database presented here will assume further significance once the genes for all the tags are identified and annotated, and the genes can then be classified based on their position in a physiological pathway and by their differential expression.

123:48

Maize FIE (Fertilization Independent Endosperm) Homologues: Two Related Genes with Distinct Expression Patterns.

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The maize genome contains two homologues of the arabidopsis gene FIE (fertilization independent endosperm), ZmFie1 and ZmFie2. The temporal and spatial patterns of their expression suggest non-redundant functions of these genes. ZmFie1 is expressed only in developing kernels after pollination, reaching its maximum at 9DAP (days after pollination). Its mRNA accumulation is more abundant in the endosperm than in the embryo. Conversely ZmFie2 is expressed before pollination in the embryo sac. After pollination, its expression persists in the embryo, not in the endosperm. ZmFie2 is likely to be a functional homologue of the arabidopsis FIE gene, which prevents formation of an endosperm before fertilization and is important for seed development after fertilization. ZmFie1 may have a distinct role in maize promoting endosperm development after fertilization but not its repression before fertilization. Both ZmFie genes are regulated by imprinting but to different extents. ZmFie1 demonstrates strong monoallelic expression: only the maternal, not the paternal, ZmFie1 allele is transcribed during a kernel development. The ZmFie2 paternal allele is silenced during an early kernel development resuming activity after 10DAP. This type of a temporal imprinting is similar to the arabidopsis FIE.

124:177

Maize PCNA Gene Expression is Regulated by both PCF and Tb1 Transcription Factors

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PCNA (Proliferating Cell Nuclear Antigen) plays an important role in the cell cycle, as well as in DNA replication and repair. Pcn gene expression has been shown to be activated by a group of plant specific bHLH transcription factors called PCFs. Tb1 (Teosinte branched1) has a similar structure to the PCFs and is also a member of the TCP family (teosinte, cycloidea, PCF). Tb1 mutant plants display a dramatic phenotype similar to the maize progenitor teosinte, with extensively branched tillers as well as flowering effects. Here we demonstrate, by binding site selection and DNA binding studies, that Tb1 can also bind to the rice pcna promoter at the same sites as for PCFs. Consistent with the notion that Tb1 functions as a repressor to inhibit maize lateral branch growth, tb1 mutant plants have an elevated level of pcna gene expression. Since Tb1 does not heterodimerize with PCFs, we propose that Tb1 and PCFs compete for the same sites in the pcna promoter to regulate pcna gene expression.

125:213

TLKs: nuclear kinases with possible roles in chromatin remodeling.

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Tousled-like kinases (TLKs) are a family of ser-thr kinases that are evolutionarily conserved in animals and higher plants. Mutation of the single TOSLED gene of *Arabidopsis thaliana* produces a pleiotropic phenotype with severe developmental defects in the floral meristem and floral organs. Recent evidence from animal systems indicates that TLKs are activated during S phase and interact with Asf1, a protein found in a complex that is involved in assembling nascent nucleosomes [Sillje HHW and Nigg EA, *Current Biology* 11:1068-1073, 2001]. Both *Arabidopsis* and one of the human TLKs will phosphorylate histone H3 [Yaum, L et al, *Oncogene* 20:726-738, 2001]. One hypothesis for the *tsl* phenotype in *Arabidopsis* is that the enzyme is uniquely required for chromatin remodeling in the floral meristem, but not in the vegetative meristem. Maize has three TLK genes whose sequences, map positions, and expression patterns we are characterizing in an effort to determine the lineage of this gene family and the individual roles of each gene.

126:19

mis1 A RUST INDUCIBLE GENE IN MAIZE

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The maize gene *mis1* which maps to 6L near *umc237* at 129.90 was cloned by homology to *fis1* a rust induced gene from flax. The *mis1* gene is induced in leaves infected with either of the rusts *Puccinia sorghi* (common rust) and *P. polysora* (tropical rust). Homologous genes isolated from barley (*bis1*) and wheat (*wis1*) encode highly similar proteins (92-97% identity) and are also induced by species specific rust infection. The *Arabidopsis* homolog encodes an enzyme with pyrroline-5-carboxylate dehydrogenase activity. P5CDH is involved in the metabolism of proline to glutamate. (proline-->P5C-->glutamate) Transgenic flax plants containing a GUS reporter gene under the control of the *fis1* promoter show that induction is highly localised to mesophyll cells within and immediately surrounding the rust pustule. The level of GUS induction reflects the level of fungal growth. The promoters of rust inducible genes are being used to engineer synthetic resistance by targeting transgene expression to the sites of rust infection.

127:221

Studies on the molecular basis of heterosis

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Heterosis is the phenomenon in which hybrid offspring exceed the vigor of the better inbred parent. We are interested in developing a molecular model of heterosis. The immediate goal is to determine the types of changes in gene expression that occur in hybrids relative to the inbreds in order to formulate a molecular explanation. To this end we are examining triploid versions of two inbreds and their hybrids to test whether allelic dosage modulates target gene expression. Heterosis differs on the morphological level in the two types of triploid hybrids, indicating that allelic dosage has an impact on the phenomenon. Thus heterosis appears to be affected by dosage dependent regulation. Previous work has indicated that the predominant type of dosage dependent regulation is negative. Whether target gene expression increases or decreases depends on whether regulatory factors act positively or negatively. We are testing the levels of various RNAs in leaf tissues of Mo17 and B73 inbreds and their hybrids at both the 2x and 3x ploidies. Many nuclear genes show elevated expression in hybrids suggesting a less efficient negatively acting dosage regulation in hybrids.

128:176

SNP Marker Development using SAGE Unigene Tag Libraries (SUTL) in Maize

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We have generated 14 maize SAGE libraries that contain over 98,000 unique tags. Some of these tags are used in a novel approach called SUTL (SAGE Unigene Tag Library) to generate the 3 ends of genes. A cDNA library for the inbred B73 was constructed with the 3 ends of genes cloned into a plasmid. The 5 end of the cDNA inserts contains 14 base pairs (bp) of SAGE tag sequence. Oligonucleotides are designed against these 14bp plus the appropriate number of adjoining vector bases to produce gene specific primers of optimal annealing temperatures. Each gene specific primer is used in conjunction with a vector primer, 3' of the insert, to amplify the remaining region of cDNA insert out of the library. The cDNA sequences were determined using fluorescent dye terminator reactions and automated sequencers (ABI). Based on sequence size >150 bp, 384 of 864 5 oligonucleotides were selected for the amplification of inbred MO17 cDNA libraries using the SUTL method. Chromatograms for each genotypes SAGE-specific RACE-PCR product were assembled into contigs and analyzed for single nucleotide polymorphisms (SNPs), indels, and ambiguities. Thirty percent (117/384) of the projects formed contigs. The average contig overlap was 132 base pairs with 2 SNPs, 3 indels, and 5 ambiguities per 132 bp. The consensus sequences were exported for assay development of Taqman MGB probes using Taqpipe, a Taqman assay design systems (ABI). Taqman MGB assays were developed for 18% (21/117) of the projects or 5% in regard to the original 384. BLAST analysis of all 864 amplified B73 unigene sequences provided annotation for ~20% of the comparisons to EST and nucleotide databases. Allele validation will be conducted to confirm the accuracy and reproducibility of the automated assay designs.

129:64

A tissue culture-induced c2 white cob maize mutant is caused by a palindromic insertion sequence with an 8 base pair direct duplication

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Plant tissue culture is a mutagenic environment with a wide range of phenotypic and molecular variation observed among regenerated plants. Few studies have directly associated a molecular change with a mutant phenotype in regenerant-derived plants. The objective of this research was to characterize the molecular basis of a tissue culture-induced c2 mutation in maize identified based on a white cob phenotype segregating in regenerant-derived progeny. The c2 gene of mutant (white cob) and control plants (red cob) was sequenced using a PCR-based strategy. An insertion of 51 bps was found in exon 2 of mutant plants, but was not present in the control. The insertion is composed of a 21 bp palindromic (inverted repeat) sequence with a 1bp loop and has an 8bp direct duplication of the insertion site. The sequence is not homologous to any previously characterized transposon in maize.

130:160

A wheat DNA fragment exhibits reduced pollen transmission in transgenic maize

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An 8.2 kb fragment of wheat genomic DNA containing the Glu1-Dx5 gene has been transferred to maize using biolistic transformation. The Glu1-Dx5 gene encodes the 1Dx5 high molecular weight glutenin subunit, a seed storage protein associated with good bread making properties. The transgenic maize plants accumulate a novel protein that is immunologically related to 1Dx5 and is similar in size to this protein. This protein is found in maize endosperm, and not in other tissues examined. Surprisingly, in all three events studied, this transgene is transmitted very poorly if at all through the pollen of transgenic maize. In order to understand the mechanism of this poor pollen transmission, we have designed a series of chimeric constructs containing portions of the wheat DNA fragment in combination with the maize 27 kDa gamma zein gene. A construct in which the promoter of the 27 kDa gamma zein gene is fused to the 1Dx5 coding sequence and 3 non-coding sequences exhibits normal pollen transmission. This suggests that sequences responsible for the unusual pollen transmission of the wheat DNA fragment lie in the 1Dx5 promoter or upstream of this sequence.

131:126

Expression of a-zein promoters in transgenic maize

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Zeins, the largest fraction of seed storage proteins in maize, provide opportunities for understanding endosperm gene regulation and improving the nutritional quality of cereals. a-zeins, the major component in terms of abundance and complexity, are encoded by a multigene family consisting of more than 35 members depending on the maize haplotype. Based on sequence homology, a-zeins can be subdivided into four subfamilies, z1a, z1b, z1c, and z1d. Recently, our lab has undertaken the systematic genomic characterization of this gene family. The largest subfamily, z1c, has been completely sequenced for two haplotypes. A preliminary expression analysis of its members revealed that only a subset is expressed at significant levels. Out of those, despite an extreme conservation of coding and regulatory sequences, individual members respond differently to developmental and environmental cues. Hence, as a first step towards the identification of factors underlying zein transcriptional regulation, we have stably introduced chimeric genes consisting of a-zein gene regulatory sequences fused to the coding sequence of the green fluorescent protein into maize. Preliminary results indicate that these promoters have significant activity in embryogenic callus. In addition, several transgenic plant lines have been obtained with intense expression of the visible marker in maize endosperm. It is apparent that, irrespective of the transgenic event considered, each promoter drives a differential accumulation of transcript and gene product.

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Functional Genomics of Endosperm Development in Maize

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The abundance of seed mutants in maize provides a potent resource for functional genomics of seed development. Molecular genetic analysis of these mutants encounters two main challenges: 1) the large number of loci complicates genetic analysis and definition of complementation groups, and 2) the often-lethal phenotypes prohibit directed screens for new alleles of a locus. We are connecting cloned endosperm genes to biological functions through visible mutant phenotypes. First, we are conducting non-targeted transposon mutagenesis to identify 2,000-2,500 endosperm mutants in the UniformMu mutagenic background (5-6 alleles/locus). UniformMu is a Robertsons Mutator population that has been extensively backcrossed into color-converted W22. Each mutant is derived from a pedigreed, non-mutant progenitor, and thus, each isolate is assured to be independent. Second, we will isolate a large set of endosperm expressed genes through EST sequencing. These cDNAs will be used to construct a glass microarray representing endosperm-expressed genes. Third, we extract a representation of the Mu transposon insertions from each mutant using MuTAIL-PCR to amplify sequences flanking the transposon insertions within the mutant and its progenitor. The MuTAIL products are probes for the microarray and will genotype each mutant for the disrupted endosperm expressed genes that are specific to a mutant. Independent mutants with shared gene disruptions define hybridization groups that are likely to be allelic. Complementation tests will then be used to confirm that these mutants define an allelic group with transposon disruptions in the same gene. The hybridization group approach both identifies cloned cDNAs associated with endosperm mutant phenotypes and simplifies genetic analysis of these mutants by molecularly identifying complementation groups.

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Maintenance of p1 paramutation is not affected by the mop1 mutation

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We previously observed that the maize p1 gene can be heritably suppressed by exposure of the paramutable P1-rr allele (red pericarp and red cob) to a transgene carrying a 1.2 kb enhancer fragment of the p1 gene (Sidorenko and Peterson, 2001, Plant Cell:13, 319-335). Here, we tested whether the mop1 (mediator of paramutation 1) mutant affects the maintenance of paramutation of the p1 gene. Our results indicated that the P1-rr' phenotype was unchanged in homozygous mop1 background. In addition, mop1 did not increase GUS expression from the silenced paramutagenic transgene containing the 1.2 kb enhancer. This result is similar to the behavior of r1 paramutation: maintenance of the r1 paramutation state was not affected by mop1 (Chandler and Kermicle, unpublished data). In contrast, paramutation of b1 and p11 is relieved by mop1 (Dorweiler et al., 2000, Plant Cell:11, 2101-18). The differential effect of the mop1 mutant on paramutation of different maize genes may reflect the involvement of different transacting factors in the maintenance of paramutation at different maize loci. Interestingly, even though the mop1 mutation does not affect maintenance of r1 paramutation, it completely prevents the establishment of r1 paramutation. We are currently testing the effect of mop1 on the establishment of p1 paramutation, and the transmission of paramutation from paramutagenic to paramutable p1 alleles.

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Searching for the source of paramutagenicity in the p1 gene enhancer: enhancer subfragments induce two novel silenced phenotypes

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The maize p1 gene encodes a myb-like transcriptional activator that regulates the expression of structural genes for flavonoid pigment biosynthesis. One allele of the p1 gene, P1-rr (red pericarp and red cob), induces the accumulation of brick-red phlobaphene pigment in husks, silks, kernel pericarp, cob and tassel glumes. The regulatory sequences of the P1-rr gene extend about 6 kbp upstream of the coding sequence, and include a basal promoter region and two upstream enhancer fragments. Previous studies showed that transgenes containing a 1.2 kb fragment that includes the far-upstream enhancer can convert an endogenous P1-rr allele to a heritable suppressed state, P1-rr', which can in turn paramutate a nave P1-rr allele to P1-rr' (Sidorenko and Peterson, Plant Cell:13, 319-335, 2001). To delimit the minimum sequences necessary for paramutagenic activity, we tested four constructs containing deletions of the 1.2 kb fragment. The results indicate that three of the four deletion constructs retained the ability to silence the endogenous P1-rr allele. The smallest effective construct contained only 405 bp of the enhancer fragment. Interestingly, the three deletion constructs produced two novel suppressed phenotypes--P1-rr'(ww); white pericarp and cob; and P1-rr'(rw); red pericarp and white cob--in addition to the previously observed P1-rr' (patterned pericarp and red cob). The P1-rr'(ww) and P1-rr'(rw) phenotypes showed significantly less heritability than that of the P1-rr' phenotype. The paramutagenicity of the novel suppressed states is being tested.

Determination of the physiologically significant substrate of RF2A in fertility restoration**Skibbe, David {1} Liu, Feng {1} Yandeu, Marna {1} Schnable, Patrick {1}**

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In Texas (T)-cytoplasm maize, fertility restoration is governed by functional alleles of two nuclear encoded genes, *rf1* and *rf2*. The *rf2* gene (now termed *rf2a*) encodes a mitochondrial aldehyde dehydrogenase (ALDH) localized to the matrix (Cui *et al.*, 1996, Science; Liu *et al.*, 2001, Plant Cell). The physiologically significant substrate of this enzyme is not known because the RF2A protein can oxidize many aldehydes. Three additional maize ALDH genes (*rf2b*, *rf2c*, and *rf2d*) have been identified via a combination of computational and wet-lab approaches (Skibbe *et al.*, 2002, Plant Mol. Biol.). These ALDH genes are being used to identify the physiologically significant substrate of RF2A. This is being accomplished via a combination of *in vitro* enzyme and *in planta* complementation assays. Kinetic analyses have been performed on each ALDH to identify its preferred substrates. The *ptrf2a* complementation construct was produced by combining 2.3 kb of the 5 non-coding sequence of *rf2a*, the *rf2a* coding region, and 1.8 kb of the 3 non-coding sequence. Five of ten independent events containing this transgene complement *rf2a*-mediated sterility in T-cytoplasm. Complementation constructs containing the *rf2a* regulatory sequences and the *rf2b*, *rf2c*, or *rf2d* coding regions have been transformed into T-cytoplasm maize. ALDH genes that can complement *rf2a*-mediated sterility must be able to oxidize the physiologically significant substrate of *rf2a*. Identification of the physiologically significant substrate will provide insight into the molecular mechanism of fertility restoration.

136:101 A transcriptional enhancer and sequences required for b1 paramutation co-localize in a region ~100 kb upstream of the b1 transcription start site**Stam, Maik(1); Bebele, Christiane (2); Chandler, Vicki L.(2); Ramakrishna, Wusirika (3); Bennetzen, Jeff (3).** (1) Free University, Department of Developmental Genetics, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands; (2) University of Arizona, Tucson, Arizona 85721, USA; (3) Purdue University, West Lafayette, Indiana, USA

Paramutation is a mitotically and meiotically heritable change in the expression of one allele caused by interaction with another allele. At b1, a regulatory gene of the anthocyanin pigmentation pathway in maize, the high expression of an allele conferring dark purple pigment, B-I, is downregulated to the lower expression level of an allele conferring light purple pigment, B, in B'/B-I heterozygous plants. New B' alleles are like existing B' alleles, they paramutate naive B-I alleles with a 100% efficiency. B' is transcribed at a 10-20 fold lower rate than B-I (Patterson *et al.*, 1993, Genetics 135:881). Both paramutation and the high expression of B-I require sequences upstream of the coding region (Patterson *et al.*, 1995, Genetics 140:1389). Fine-structure recombination mapping localized the sequences required for B paramutation and B-I expression to a 6 kb region ~100 kbp upstream of the maize b1 transcribed sequences, suggesting the involvement of long range communication between sequences far upstream and the promoter proximal region. Within the 6 kb region B and B-I have 7 tandemly repeated copies of a 853 bp sequence, whereas B-P and b-K55 (both alleles that do not participate in paramutation) have only a single copy. A recombinant allele between B-P and B-I with three repeats showed a diminished ability to participate in paramutation, providing strong evidence that the repeats are involved in paramutation. There are no sequence differences between B and B-I in this region, establishing that they are epialleles. In the B-I allele the repeats have a more open chromatin structure and are more methylated than in the B allele. Analysis of new B alleles (spontaneously derived from B-I parents) indicates that the B methylation pattern follows the establishment of the B state. Investigations of chromatin structure revealed that new B alleles had an intermediate chromatin state relative to B-I (more open) and B (more closed). These findings provide support for our model that paramutation is mediated via heritable changes in chromatin structure. In the course of this study a BAC clone containing 100 kb of upstream B sequences was isolated, sequenced and characterized. Sequence features of this genomic region will be described.

137:96

Diversity and Recombination along chromosome 1 of maize

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We investigate the interplay between genetic diversity and recombination in maize (*Zea mays* ssp. *mays*). Genetic diversity was examined in previously published DNA sequences including a common sample of 25 individuals in 21 loci located on maize chromosome 1. Three types of markers were used: single nucleotide polymorphisms (SNP), indels and microsatellites. Small indels (1 to 5 bp) were numerous and far more common than large indels. Furthermore, several results suggest that large indels (>100 bp) are slightly deleterious. We examined 47 microsatellites, of which 33 were polymorphic. Diversity in SNPs, indels and microsatellites was compared to two measures of recombination: 4Nc, estimated from DNA sequence data, and R based on a quantitative Recombination Nodule (RN) map of maize synaptonemal complex 1. RNs mark sites of crossover events and therefore provide an independent measure of recombination rate relative to the number of recombination events per physical unit. SNP diversity was correlated with 4Nc ($r=0.65$; $p=0.007$) but not correlated with R ($r=-0.10$; $p=0.69$). In contrast, microsatellite diversity was correlated with R ($r=0.43$; $p=0.005$) but not 4Nc ($r=0.12$; $p=0.27$). We interpret these results in a temporal framework. Owing to the relatively slow mutation rate of SNPs, both 4Nc and q contain historical information, and we argue that the correlation between 4Nc and q likely reflects demographic processes such as the genetic bottleneck during domestication. In contrast, because both R and microsatellite diversity are relatively short-term measures, we propose that their correlation might reflect background selection acting on chromosome 1.

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Toward a function for rough sheath2 at the cellular level

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The rough sheath2(rs2) gene is essential for wild type leaf development and has been proposed to be a negative regulator of the rs1 knox gene and perhaps other genes in the leaf. rs2 belongs to a distinct subfamily of myb related genes which include the Antirrhinum gene phantastica and the Arabidopsis gene asymmetric leaf1, both of which act negatively in leaf development. Members of this family contain a highly diverged myb domain in the N-terminal portion of the protein and a large conserved C-terminal domain of unknown function. The conserved RS2 C-terminal domain was found to mediate homodimerization of RS2 protein. Deletion analysis was used to define a minimal dimerization region. A transgenic system was used to further elucidate the function of the conserved C-terminal domain and to study the subcellular localization of RS2.

139:18

Herbicide Target Discovery: A Model for Functional Genomics.

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Abstract: The goal of this project is to examine the cellular basis of plant growth and development by saturating for insertional mutants defective in essential genes. Over 123,000 T-DNA lines generated at Syngenta and 10,000 Ds transposon lines from Cold Spring Harbor Laboratory have been screened for embryonic and seedling lethal phenotypes. We have used TAIL PCR to recover flanking sequence from a large collection of lethal mutants. Approximately one-third of the recovered sequences have homology to genes with known functions, another third have homology to genes encoding hypothetical proteins, and the remainder have no significant homology to sequences in GenBank. Essential genes identified to date fall into all but one of the 15 major functional categories outlined by Bevan et al. [Nature (1998) 391:485]. As expected many of the identified genes have basic cellular functions. Based on sequence similarities, several identified genes fall into the disease/defense category, consistent with an important role for such genes in growth and development

140:216

A Diversity Map of the Maize Genome using Microsatellites

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Little is known about the genetic modifications that occurred during the domestication of maize, although one well-known but incompletely understood effect is the loss of genetic variation in maize as compared to its progenitor, teosinte. To better understand the forces that have shaped genetic diversity in maize, we analyzed diversity for 196 microsatellites spread throughout the maize genome and compared their diversity in maize to that in teosinte. Our analyses revealed a genome wide loss of genetic diversity that is indicative of a population bottleneck effect. Estimates of the bottleneck effect varied among different classes of microsatellites, probably as a result of differences in mutation rate among classes and the corresponding differences in the rate of recovery from the bottleneck. Coalescence simulations were performed to detect evidence of selection taking into account the demographic history of maize. Using these simulations, two different tests of selection were performed: one examines the degree of differentiation between maize and teosinte and the other examines the level of genetic diversity expected in maize knowing the level in teosinte. We found that the mutation model is a critical parameters for interpreting departures from neutrality at microsatellite loci and that overall maize diversity is mainly congruent with a bottleneck effect alone. However, we also identified some evidence of selection, even if the diversity loss due to selection has been moderate.

141:46

Proteomic analysis of response to drought in growing maize leaves

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Plant response to drought involves many functions and genes (eg ABA synthesis, stomates closure, growth reduction, osmotic adjustment, leaf senescence, dehydrins, etc). First proteomic analyses allowed us to identify drought-regulated leaf proteins, and " candidate proteins " have been proposed according to correlations with the variation of phenotypic responses (Riccardi et al. 1998 Plant Physiol, 117 :1253-1263, de Vienne et al. 1999 J Exp Bot, 50 :303-309). All cells are not expected to respond in the same manner, since responses can differ according to various factors (eg sink/source tissues, exposure or not to light, differentiation stages). In maize leaves, cell division and elongation take place in the elongation zone at the base of the leaf. There is a gradient of cell age along the leaf. We took advantage of this to analyse the response of proteome to drought in tissues at different stages of differentiation. Profiles of protein expression were built by analyzing the proteome in 2 cm-long segments taken along the leaf. Multivariate analyses (principal component and hierarchical clustering analyses) were used to describe the main features of protein expression along the leaves of plants submitted to various treatments. The main variation is related to proteins whose expression is highly correlated (positively or negatively) to the position (distance to the base) along the leaf. Positively correlated proteins are probably related to the differentiation of the photosynthetic apparatus, since the bottom of the growing leaf is in darkness. Negatively correlated proteins might be involved in cell division and elongation. The second type of variation comprises proteins whose expression is maximal at various positions along the leaf. Caffeate-OMT is among them, and this might be related to a lignification zone. The main effect of drought is a shift of the expression profiles towards leaf base. This is consistent with the reduction of leaf growth caused by a decrease in cell division and elongation in the elongation zone (Ben Haj Salah and Tardieu 1997 Plant Physiol, 114 :893-900). Thus these changes can be considered as side effects. Our final objective is to identify and to study the expression of proteins whose modification of expression is a direct response to drought. Comparisons between droughted plants and control plants at different stages of leaf development will allow the detection of such proteins.

142:205 Maize EST Sequence Analysis

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ESTs are one route to gene discovery and are ideal for identifying genes that are expressed at moderate levels. There are currently 138,416 ESTs at Genbank. Of these, the Maize Gene Discovery Project has generated 97,002 from 20 cDNA libraries covering a broad range of developmental stages and tissue types. A major finding is the low overlap of gene expression between organs and between developmental stages during organ development. Different members of gene families appear to have specific and often distinct domains of expression. ZmDB is an ACEDB database created under the Maize Gene Discovery Project. ESTs are regularly assembled into tentative unique contigs (TUCs) at ZmDB using the ZmDBAssembler program (<http://www.zmdb.iastate.edu/zmdb/EST/assembly.html>). ESTs that do not assemble with any other ESTs are labeled tentative unique singlets (TUSs). The sum of the TUCs and TUSs equals the tentative unique genes (TUGs) represented by the whole EST population. The latest assembly produced 16,137 TUCs and 13,246 TUSs for a total of 39,383 TUGs. A unigene set of 13,006 clones from the first 13 cDNA libraries was created by selecting a representative EST for each TUG. These Unigene I clones can be ordered through ZmDB (<http://www.zmdb.iastate.edu/cgi-bin/order.cgi>) and are now distributed by Texas A&M. A second unigene set of 4,992 ESTs from the next two cDNA libraries is currently being prepared for distribution. Annotation of the ESTs and TUCs is done through homology searches against GenPept (<http://www.zmdb.iastate.edu/zmdb/EST/annotation.html>). Microarrays of ESTs from four cDNA libraries (endosperm, ear, 4-day root, and leaf primordia) have been created. The Unigene I set is currently being arrayed; Unigene1.1 slides are available now and 1.2 and 1.3 slides will be available later this year. Sequencing and microarray protocols can be found at <http://www.zmdb.iastate.edu/zmdb/protocols/>. This project was supported by the National Science Foundation as part of the Plant Genome Research Projects.

143:191

Effect of GFP expression in Transformation Efficiency of Maize

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Green fluorescent protein (GFP) from jellyfish *Aequorea Victoria* has been used as a convenient marker in various heterologous systems for non-lethal, non-invasive detection. In transgenic plant systems, GFP has been used for the early detection of transformation events, and efficient isolation of transgenic cells, assisting in the rapid optimization and improvement of a transformation system for a specific plant species. However, possible side effects from the GFP expression in plant cells have been speculated. In this preliminary study, maize cells were transformed via *Agrobacterium* harboring a T-DNA construct containing both CP4 (for glyphosate selection) and GFP genes, or with CP4 alone. Development of transgenic events from the cells transformed with both CP4 and GFP genes was monitored by the GFP expression. The transgenic nature of all regenerated plants were confirmed by the GFP expression, glyphosate resistance and/or Southern analysis. Comparing the transformation efficiencies between the treatments with and without the GFP gene, no significant negative effect associated with the GFP expression was observed. It suggests that GFP can be used as an effective reporter gene for research in transgenic maize.

144:146

Expression and Immunogenicity of a Synthetic *E. coli* Heat Labile Enterotoxin B Sub-unit (LT-B) as a model vaccine in Maize

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We have produced in transgenic maize the B sub-unit of the enterotoxigenic *Escherichia coli* (ETEC) heat labile enterotoxin (LT-B). Using a synthetic LT-B gene with optimized codon use, we examined the role of promoters and the SEKDEL endoplasmic reticulum retention motif in LT-B accumulation in callus and in kernels. Two promoters, the constitutive CaMV 35S promoter and the maize 27 kD gamma zein promoter, which directs endosperm specific gene expression in maize kernels, regulated LT-B expression. Transgenic maize synthesized LT-B polypeptides, which assembled into oligomeric structures with affinity for GM1 gangliosides. Ganglioside-dependent ELISA analysis showed that constitutive LT-B expression in callus peaked at 0.04% LT-B in total aqueous extractable protein (TAEP) and 0.01% in R1 kernels of transgenic plants. Maximum seed specific expression observed using the gamma zein promoter was 0.07% in R1 kernels. The SEKDEL motif did not enhance constitutive expression of LT-B but resulted in increased LT-B expression in kernels when combined with the gamma zein promoter. We monitored LT-B levels under greenhouse and field conditions over three generations. Significant variability in gene expression was observed between transgenic events, and between plants within the same event. A maximum of 0.3% LT-B in TAEP was measured in R3 seed of transgenic line carrying CaMV 35S promoter/LT-B construct. In R3 seed of transgenic line carrying gamma zein promoter/LT-B construct, up to 3.7% LT-B in TAEP could be detected. We orally immunized BALB/c mice by feeding transgenic maize meal expressing LT-B or nontransgenic maize meal spiked with bacterial LT-B. Both treatments stimulated elevated IgA and IgG antibodies against LT-B and the closely related cholera toxin B subunit (CT-B) in serum, and elevated IgA in fecal pellets. The transgenic maize induced a higher anti-LT-B and anti-CT-B mucosal and serum IgA response compared to the equivalent amount of bacterial LT-B spiked into maize. Following challenge by oral administration of the diarrhea inducing toxins LT and CT, transgenic maize-fed mice displayed reduced fluid accumulation in the gut compared to non-immunized mice. Moreover, the gut to carcass ratio of immunized mice was not significantly different from the PBS (non-toxin) challenged control group. We demonstrate that maize synthesized LT-B was similar in biochemical and antigenic properties to the native LT-B, and that maize can be used as a production and delivery system for functional antigens.

145:107

Genetic diversity and selection in the maize starch pathway

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For more than 6000 years Native Americans and modern breeders have exploited the tremendous genetic diversity of maize (*Zea mays* ssp. *mays*) to create the highest yielding grain crop in the world. Undoubtedly, selection for increased yield and kernel quality has had major effects on starch production. To evaluate the impact of selection at the gene level, we surveyed nucleotide diversity in 6 major genes involved in starch metabolism in the maize kernel, Amylose extender1 (Ae1), Brittle2 (Bt2), Shrunken1 (Sh1), Shrunken2 (Sh2), Sugary1 (Su1), and Waxy1 (Wx1). We found unusually low genetic diversity and strong evidence of selection across the pathway. Average diversity (π) in the starch loci was 2.3-fold lower than 20 random maize loci at silent sites (T-test; $p < 0.05$), and 4.8-fold lower at nonsynonymous sites. We also sampled genetic diversity in *Z. mays* ssp. *parviglumis*, the wild progenitor of maize. *su1*, *ae1*, and *bt2* exhibited a 3 to 7-fold reduction in diversity compared to *parviglumis*, in addition, *su1* and *bt2* had highly significant HKA test results consistent with artificial selection during domestication. *ae1*, however, had non-significant HKA results for diverse germplasm, significant HKA results for US germplasm, and a significant Tajima's D ($D = -2.29$; $p < 0.01$), a test indicating that selection in this gene may be ongoing. Low diversity in agronomically important genes suggests that a paradigm shift may be required for future maize breeding. Rather than purely relying on the diversity within maize, future maize breeding might greatly benefit from the introgression of selected gene regions from maize's wild relatives.

146:25

NUCLEAR-DIRECTED MODIFICATION OF MITOCHONDRIAL TRANSCRIPTS IN *cms-T* MAIZE

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T-cytoplasm maize (*cms-T*) has been developed as a model to study the molecular mechanisms underlying mitochondrial transcript processing and restoration of fertility in cytoplasmic male-sterile systems. Male sterility in *cms-T* results from the function of T-*urf13*, a T-cytoplasm specific, mitochondrial gene. Nuclear-directed modification of T-*urf13* transcripts is mediated by *Rf1*, *Rf8*, or *Rf**, which in combination with *Rf2*, suppress the male-sterile phenotype. *Rf1* is positioned near the centromere on chromosome 3, whereas *Rf8* and *Rf** are clustered together on 2L. This position on 2L also harbours *Rf3*, another nuclear gene that modifies mitochondrial transcripts, but its action is specific to S-cytoplasm maize. Computational analysis of a candidate *Rf1* cDNA was used to provide clues as to its possible function. Twenty-six residues of the predicted amino acid sequence revealed 96% similarity with mammalian small nuclear ribonucleoprotein E (snRNP E). The snRNP E protein is an 11-kD basic protein integral to RNA processing reactions and is one of four "core" proteins associated with the snRNAs of the U (uridine rich) family (U1, U2, U4, U5, and U6). U1 snRNA is initially bound to the 5'-splice site and is released upon recruitment of U4/U5/U6, a process that is consistent with the molecular phenotype of *Rf1*. Research funded by USDA-NRI/CGP 99-35300-7752.

147:217

Phenotypic Analysis of Mu-Tagged Small Kernel and Defective Kernel Mutants Derived from UniformMu Maize

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We report here on Mu-tagged small kernel (smk) and defective kernel (dek) mutants isolated from the UniformMu population. The smk mutants identified thus far (ca 18% of total Mu-tagged seed mutants) are shorter and/or have a reduced crown size relative to wildtype neighbors on a segregating ear. A prominent contributor to the size reduction in many of these smk mutants is a marked decrease in their scutellar volume. The embryo axis appears normally developed, and the scutellum has reached full length, but the scutellar thickness is often dramatically reduced. This feature suggests that a number of smk mutants may be moderately defective kernels (deks). The defective kernel (dek) mutants identified thus far (ca 16% of total Mu-tagged seed mutants) encompass a diverse range of phenotypes that include reduced grain filling and defective embryos. In many cases we note a pronounced variation in phenotypic severity between kernels having a single dek gene as well. On a given ear, an individual dek mutant phenotype can range from empty pericarps to nearly-wildtype kernels. The uniform inbred background of UniformMu should greatly assist in the interpretation of these variable penetrance dek loci.

148:124

Genotype-specific *trans*-acting Factors Influence the Rates of Meiotic Recombination and Distribution of Recombination Breakpoints in the 140-kb *al-sh2* Interval

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Rates of meiotic recombination can be affected by both *cis* factors such as DNA sequence heterologies and *trans* factors (genotype-specific modifiers not residing in the interval of interest). To identify *trans*-acting factors that affect recombination rates across the 140-kb *al sh2* interval and to test the hypothesis that such factors can influence the distribution of recombination events in a defined interval, an identical *al::rdt sh2* interval was introgressed for several generations into three inbreds: W64A, A632 and Oh43. The resulting near-isogenic inbred lines were crossed to *Al Sh2* (Line C). Colored shrunken recombinants were recovered by crossing large populations of the resulting F₁s by *al::rdt sh2*. Recombination rates among the three populations differed two-fold. High-resolution recombination breakpoint mapping is being conducted on confirmed homozygous recombinants from each population using PCR-based InDel Polymorphism (IDP) markers to determine if the patterns of meiotic recombination breakpoint distribution differ among the genetic backgrounds.

149:98

Cis-effects on Meiotic Recombination across Distinct *a1-sh2* Intervals in a Common Genetic Background

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Cis-factors such as large InDel polymorphisms and small nucleotide heterology can influence meiotic recombination. To identify and characterize *cis*-acting genetic effects on meiotic recombination across the *a1-sh2* interval, recombination events were isolated from a set of crosses between an *a1::rdt sh2* stock and four near-isogenic lines that carry structurally distinct *a1-sh2* intervals from the maize inbred Line C and three maize relatives (*Z. mays* spp. *mexicana* Chalco, *Z. luxurians* and *Z. mays* spp. *parviglumis*). Recombination rates ranged from 0.06 to 0.2 cM. The breakpoints associated with approximately 500 recombinants are being mapped to characterize the influence of *cis*-acting factors on the distribution of recombination events.

150:125

Novel structure of the maize P1-rw (red pericarp, white cob) allele

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The maize *p1* gene encodes a Myb-homologous transcription factor that regulates the flavonoid biosynthetic pathway. Alleles of the *p1* gene confer a variety of pigmentation patterns in pericarp and cob glumes, including P1-rr (red pericarp/red cob), P1-wr (white pericarp/red cob), P1-rw (red pericarp/white cob), and P1-ww (white pericarp/white cob). We are interested in determining the mechanism(s) leading to these organ-specific pigmentation patterns. The P1-rr and P1-wr alleles have been cloned and characterized previously. We analyzed the genomic structure, sequence and expression patterns of the P1-rw allele. The results showed that, like P1-rr, P1-rw is a single copy gene which is linked with a second paralogous gene (*p2*). The P1-rw gene is highly similar to P1-rr and P1-wr in the 5' regulatory region, and in the Myb-domain coding sequence. However, preliminary results suggest that the 3' coding region of P1-rw is more similar to the maize *p2* gene. Thus, the P1-rw allele may represent a new gene formed by recombination of *p1* and *p2*. Further molecular and expression analysis will be presented.

151:169

Using Chromatin Immunoprecipitation (ChIP) to identify putative functional centromere sequences in maize

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Centromeres are mainly composed of repetitive DNA sequences, and not all of them are essential for centromeric activity. We have used an immunoprecipitation assay to determine which of the centromeric repeats interact directly with the kinetochore, the proteinaceous organelle that generates chromosome movement during cell division. The kinetochore protein most closely associated with DNA is CENH3, a conserved histone H3 homologue that replaces histone H3 in centromeric chromatin. Polyclonal antibodies against maize CENH3 were generated using a synthetic polypeptide against the predicted protein. The antisera recognizes a 17 kDa protein that co-localizes with the kinetochore protein CENPC. Chromatin Immunoprecipitation (ChIP), a technique where native chromatin is digested into small fragments and immunoprecipitated, was used to identify centromeric DNA sequences. The data demonstrate that the tandem repeat array CentC and a highly-conserved centromeric retrotransposon interact specifically with CENH3. Knob repeats as well as total genomic DNA do not interact with CENH3 by our assay. These data provide strong evidence that specific DNA sequences are involved in organizing the centromere/kinetochore complex in plants.

Quantitative Traits

152:186

Investigating the durability of *Ustilago maydis* resistance in *Zea mays*

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Durable pathogen resistance is required to limit crop loss to pathogen infection over extended periods of time. However, host resistance is often quickly overcome by the evolution of virulence in pathogen populations. To investigate the genetic basis of a durable resistance trait, we are investigating the host/pathogen system presented by the interaction between *Zea mays* and *Ustilago maydis* (corn smut). *Ustilago maydis* resistance has been developed and maintained in maize populations for nearly a century. To investigate the genetic architecture of *U. maydis* resistance and its extended durability in *Z. mays* populations, we have generated two sets of recombinant inbred lines segregating for *U. maydis* resistance. RI lines were generated through crossing the inbreds A188 and W23 to CMV3 and selfing for eight generations. RI lines were planted in four repetitions, spread across two locations, and inoculated with a solution containing eight genetically distinct strains of *U. maydis*. Analyses show that significant genetic, environment, and genetic by environment effects regulate *U. maydis* infection. Several lines show resistance and susceptibility to *U. maydis* only within the tassel and ear tissue of the host. A bulk segregant analysis across approximately 240 SSR loci indicate that several loci, common to both sets of RI lines, control *U. maydis* resistance. The loci are found in bins 1.05, 2.04, 2.08, 3.04, 4.05, 6.01, and 9.03. Furthermore, some loci regulating *U. maydis* resistance map to genomic locations near previously described resistance gene analogs and defense genes.

153:59

Functional and molecular characterization of selection at the maize domestication locus teosinte branched 1 (tb1)

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In spite of their recent divergence within the last 10,000 years, maize and its wild relative (teosinte) have striking differences in body and inflorescence morphology. Maize and teosinte are cross-compatible, and quantitative trait locus (QTL) mapping has identified a single locus that determines many of the morphological differences between maize and teosinte. The teosinte branched 1 (tb1) gene is responsible for this QTL, and in maize levels of tb1 RNA are higher than in teosinte. Furthermore, molecular evolution studies suggest that sequences immediately 5' to the tb1 coding sequence, but not the coding sequence or 3' sequences, were selected during domestication. These results strongly suggest that differences in tb1 regulation were the target of selection during maize domestication. To better understand the molecular nature of changes at tb1 that occurred during maize evolution, we have identified maize-teosinte intragenic recombinant chromosomes at the tb1 locus in order to localize regulatory sites that differ between maize and teosinte. Intragenic recombinants were identified using an allele specific PCR screen of grided F2 plants that allowed as many as 12,800 chromosomes to be examined for recombination at sites within and extending 8 kb 5' to the tb1 coding sequence. We identified 4 recombinants within the coding region itself (a recombination frequency of 4×10^{-4}), but identified no recombination events in 5' sequences. Recombinant chromosomes will be analyzed for phenotypes in summer 2002. In addition, we have extended molecular evolution studies to include sites 8 kb and 35 kb 5' to the tb1 coding sequence. Our results suggest that this 5' intergenic region is in complete or nearly complete linkage disequilibrium. Recombination thus appears to be nearly absent from this intergenic region, and is consistent with our failure to identify recombinants 5' to the tb1 coding sequence in our field experiment. Sequence diversity at the 5' sites is low, and we are performing additional studies to determine the extent of the selected region at the tb1 locus.

154:30

Quantitative Trait Locus Analysis of C-glycosyl Flavone Type in Maize Silks

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Resistance to corn earworm (*Helicoverpa zea* Boddie) has been attributed to high concentrations of C-glycosyl flavones and chlorogenic acid in maize silks. The most common C-glycosyl flavones isolated from maize silks are maysin, apimaysin and methoxymaysin, which are distinguished by their B-ring substitutions. For a better understanding of the genetic mechanisms underlying the synthesis of these compounds we conducted a quantitative trait locus (QTL) study with two populations: NC7A x Tx501 and Tx501 x Mp708. For chlorogenic acid, maysin and methoxymaysin concentration, the major QTL was located on chromosome 4 near umc1963 for both populations. For apimaysin, in both populations, the major QTL was located at the position of the pr1 locus on chromosome 5. The QTL alleles on chromosome 4 that increased methoxymaysin significantly decreased the synthesis of maysin and chlorogenic acid. This decrease in maysin concentration was four-fold greater than the increase in methoxymaysin. In addition, smaller effects were detected on chromosome 4 for apimaysin as well on chromosome 5 for maysin and methoxymaysin. Our results indicate that the QTL on chromosome 4 responsible for the increase in methoxymaysin synthesis alters the dynamics of both the phenylpropanoid and flavonoid pathways.

155:199

Variation in seedling photomorphogenesis among maize inbred lines

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Maize is grown in more countries than any other crop plant, where it has undergone selection in a diverse range of light environments that vary in intensity, spectral quality and duration. Since critical developmental responses throughout the plant life cycle are light-dependent, the extreme adaptability of maize could be attributed to a high level of diversity in the genes that underly photoperception. Phytochromes, the Red-/Far Red- absorbing group of photoreceptor proteins and blue light photoreceptors are known to control many aspects of development, from seed germination to seedling growth and the initiation of flowering. In this study we grow a diverse set of maize inbred lines (kindly provided by Dr. Major Goodman), representing all three major heterotic groups, in four light environments [White (fluence=3.7 mol m⁻²s⁻¹), Red (1.8 mol m⁻²s⁻¹), Blue (1.3 mol m⁻²s⁻¹), Far Red (1.2 mol m⁻²s⁻¹)] and in the Dark , for ten days. The inhibition of mesocotyl elongation provides a quantitative measure of light response in seedling de-etiolation. We found significant variation among lines in each light environment, as well as a significant line x light interaction, indicating that the pattern of response varies among the lines. This data set provides a baseline survey of the diversity of response present in the maize germplasm. Lines displaying divergent response patterns are identified for further molecular-genetic characterizations of light signal transduction pathways in maize.

156:121

Identification of a major QTL affecting oil concentration in maize

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As part of an ongoing program to develop maize with enhanced nutritional traits for use as livestock feed, Dow AgroSciences conducted research to identify sources of increased oil concentration in maize. A mapping population was derived from crossing an elite Mycogen inbred averaging 4% oil concentration to the Mycogen proprietary line 6UQ025 averaging 13.7% oil (percent reported on a dry weight basis) followed by backcrossing to the elite parent. A linkage map was developed for the resulting population using a well-distributed set of simple sequence repeat markers and Join Map version 2.0. Oil concentration phenotype was measured via near infrared reflectance analysis. Association between markers and the oil concentration phenotype were calculated via interval mapping using Map QTL version 3.0. Results of the mapping study identified a major QTL linked to, but not affected by, the waxy locus on chromosome 9. The QTL in this region accounts for 53 % of the variation in oil concentration, with an estimated additive allele substitution effect of 1.5 %. A marker assisted selection strategy was successfully applied to introgress the QTL into multiple Mycogen inbred lines originally averaging 4% oil concentration. The resulting BC4S1 lines are 95-97 percent converted to the elite parent, carry the QTL segment from 6UQ025 on chromosome 9 and range from 6.0-8.5 % oil concentration as measured with nuclear magnetic resonance analysis (a more precise measuring technique). Initial yield trial analysis indicate no loss of yield (yield drag) associated with the increase in oil concentration. This research provides a successful example of precisely crafting the plant genome by combining molecular marker and breeding techniques, resulting in the introgression of an enhanced value-added trait with no yield loss.

157:31

Phenotypic and Marker-assisted Selection for Stalk Strength and Second-generation European Corn Borer Resistance

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Maize (*Zea mays* L.) stalk lodging is breakage of the stalk at or below the ear, which may result in loss of the ear at harvest. The second-generation of the European corn borer (2-ECB) (*Ostrinia nubilalis* Hbner) is an insect pest of maize that increases stalk lodging by stalk tunneling. Rind penetrometer resistance (RPR) has been used to measure stalk strength and improve stalk lodging resistance. Quantitative trait loci (QTL) have previously been identified for both RPR and 2-ECB damage. The objective of this study was to compare the efficiency of phenotypic selection (PS) vs. marker-assisted selection (MAS) for both RPR and 2-ECB. Marker-assisted selection for high and low RPR was effective in three populations. Phenotypic selection for both high and low RPR was more effective than MAS in two populations. However, in a third population, MAS for high RPR using QTL from the same population was more effective than PS, and using QTL from an independent population was just as effective as PS in selecting for high RPR. Marker-assisted selection for resistance and susceptibility to 2-ECB using QTL from the same population was effective in increasing susceptibility, but not in increasing resistance. Marker-assisted selection using QTL from an independent population was effective in both directions of selection. Marker-assisted selection was as effective as PS in selecting for both resistance and susceptibility to 2-ECB. These results demonstrate that MAS can be an effective selection tool for both RPR and 2-ECB resistance. These results also validate the locations and effects of QTL identified for RPR and 2-ECB resistance identified in earlier studies.

158:91

Marker-assisted-selection for aflatoxin reduction in maize

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Many crops including fruits, peanuts, and corn can be contaminated by aflatoxin. This mycotoxin is a secondary metabolite of some strains of *Aspergillus flavus*. Since the toxin is a health hazard to humans and animals, the FDA has set 20 ppb as the maximum allowable level in a lot of corn. This often leads to economic loss for the producers. The correct genes in the plant and the fungus and the correct developmental stage of the plant are primary factors for aflatoxin production. Secondary factors that determine the level of toxin are heat and drought conditions. Elite germplasm can accumulate high levels of the toxin. Mp313E, a tropically derived inbred, has low toxin accumulation potential. Eight aflatoxin reduction quantitative trait loci (QTL) were identified in an F2:F3 population of Mp313E (resistant) x Va35 (susceptible) including a major resistance factor on chromosome 4. Our long term goal is to identify and characterize all the major QTL including *qaf1* on chromosome 4. Towards this goal, we have incorporated AFLP loci into the existing genetic map containing restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) markers. Twenty-four primer pairs were screened on the parental DNA and six pairs were then selected to used for the map. The average number of polymorphisms among these pairs was 49. Based on this information we have identified three EcoRI/MseI primer pairs that we are using for marker-assisted-introgression of *qaf1* into susceptible inbred backgrounds.

159:136

Mapping *Cercospora zeaе-maydis* resistance QTLs across multiple environments

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This confirmation study was undertaken to link molecular markers to *C. zeaе-maydis* resistance as a first step toward a marker assisted selection strategy. The resistant maize inbred, VO613Y, was crossed to the susceptible inbred, Pa405, to obtain a population of 144 F2:3 progeny lines. These lines were evaluated at Wooster, Ohio, USA, and Cedara, Republic of South Africa, for resistance to *C. zeaе-maydis* based on percent leaf area affected (PLAA). An F3:4 population, comprised of self-pollinated resistant and susceptible F2:3 lines, was evaluated at two Ohio locations over two seasons and at Cedara for one. Selective genotyping was initially performed on the resistant and susceptible tails of the population. Then 30 markers and 60 progeny lines were added to the population. We previously identified two quantitative trait loci (QTLs) for resistance, one on 1L and another on 2L. We confirmed a QTL located on 2L, found a novel QTL on 4L, and eliminated the locus on 1L. Two loci (2L and 4L) cumulatively explained 34% of the variance in the F2:3 generation and 51% in the F3:4 generation across all environments tested. These markers should be useful for introgression of *C. zeaе-maydis* resistance into elite maize germplasm via a marker assisted selection strategy.

160:51

GENETIC ANALYSIS OF MAIZE EAR LENGTH

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The length of the maize ear shoot (EL) is the product of the number of cupules per rank and the distance between cupules from the base to the tip of the rachis (cob). A bi-parental population was developed from two inbred parents that differ in EL by 14 cm. The parents are a result of 24 generations of divergent selection for ear length in sub-populations of the Iowa Long-Ear Synthetic (BSLE). The initial investigation focuses on detection of quantitative trait loci (QTL) for EL and other ear traits among 189 F2 plants using a genetic map composed of 165 SSR and RFLP loci. Seven EL QTL were identified on five chromosomes and cumulatively explained approximately 60% of the phenotypic variation. The QTL with the largest additive effect on EL variation is in the same bin (6.02) as *cdc48*, a gene involved in cell division. Two or more QTL were associated with each of the other traits. Analyses of data collected at four environments on the F3 progeny of the 189 F2 plants will be completed in order to confirm the presence and genetic effects of these QTL and to identify QTL that interact with environmental factors.

161:71

Associations between *Glossy15* polymorphisms and variation in juvenile leaf number

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Maize lines with fewer juvenile leaves are more resistant to fall armyworm and southwestern corn borer infestation during the whorl stage. This increased Lepidopteran resistance is likely due to differences in the chemical constituents of the wax layer of juvenile versus adult leaves. The goal of this project is to identify one or more single nucleotide polymorphisms (SNP) associated with reduced juvenile leaf number that can be used to screen for more insect resistant corn lines or in marker assisted selection. Since number of juvenile leaves varies between lines of maize, one hundred maize inbred lines (52 U.S. lines, 7 European and Canadian lines, and 41 Tropical/semiotropical lines) were used to identify the lines with the lowest and highest number of juvenile leaves. One hundred lines of maize were grown in two replicates and data was collected for the last juvenile, the first fully adult, and total leaf number of leaves. Number of transition and fully adult leaves were then calculated from collected data set. Juvenile leaf number varies between 3.00 to 7.36 leaves per plant with a mean of 4.90 units, while the adult leaf number ranges from 4.39 to 23.25 units with a mean of 11.54 units. There was no relationship between the origin of lines and juvenile leaf number. The maize *Glossy15* (*Gll5*) locus is a loss of function mutation that causes abbreviated expression of juvenile leaves. Fifteen primer pairs for PCR amplification were selected from the GenBank sequence of *Gll5*. Of the fifteen initial primer pairs, two amplified large robust single fragments. These were selected for use in sequencing of the various inbred alleles. Association analysis was first performed on a subset of 32 maize inbred lines to evaluate potential associations between *Gll5* sequence polymorphisms and variation in the number of juvenile or adult leaves.

162:158

Phytoliths as a Tool for Identifying Maize Lineages Archaeologically

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Maize is the most important domesticate to come out of the New World, yet its origins and diffusions are poorly understood. A new technique has been developed and used in archaeology using a morphometric classification scheme for rondel opal phytoliths, allowing the description of assemblages. These assemblages are produced in the cupule and glume of the maize inflorescence through the deposition of silica. This deposition is linked to a regulatory gene known as teosinte glume architecture (TGA) 1. A statistical analysis of these phytolith assemblages appears to reflect genetic differences between types of maize. Here, a number of assemblages have been examined to show how some high-altitude Bolivian maize types, that are defined based on morphology, are related.

163:28 Maize Resistance to *Aspergillus flavus* is Correlated to High Catalase Activit

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Maize resistance to *A. flavus* is a multigenic trait. Genetic mapping of the resistant maize inbred Mp313E against the susceptible inbred Va35 was performed at the USDA-ARS facility in Starkville, Mississippi, and indicated that QTLs for resistance were located on chromosomes 1 (bins 1.07 and 1.09) and 4 (bins 4.08 and 4.09). The *cat3* gene is mapped on chromosome 4 and encodes an isozyme of catalase. Catalase is the principal plant enzyme that is able to dismutate hydrogen peroxide, an important signaling molecule in plant-microbe interactions. Consequently, we hypothesized the involvement of catalase in the resistance mechanism. To visualize the path of fungal invasion, we used a GFP-tagged *A. flavus* strain to needle inoculate the developing ears of resistant and susceptible hybrids in the field. We collected the inoculated ears at 5 and 28 days after inoculation (DAI) and examined the cross-sections under a GFP-adapted microscope. Fungal invasion stopped in the rachis and did not enter the kernel in all resistant lines. In susceptible lines, the fungus appears to move through the rachis into the embryo. Interestingly, the endosperm was not invaded in either the susceptible or the resistant lines. These results demonstrated that invasion by *A. flavus* occurs early in ear development, at which time the resistance mechanism is triggered in resistant lines. Resistant and susceptible inbreds were grown in the field, self-pollinated, and a number of ears from each line were inoculated with *A. flavus* through the silk channels. Inoculated and noninoculated embryos from resistant and susceptible inbreds were collected at 18, 21 and 25 days after silking (DAS) and assayed for catalase activity. Embryos (inoculated and noninoculated) from the resistant lines exhibited significantly higher catalase activity levels than the embryos from the susceptible lines. Catalase levels also increased over the sampling period in the resistant lines. Inoculated embryos arising from the resistant lines exhibited higher catalase activities than their noninoculated counterpart. Activity gel assays also showed that the catalase from the resistant lines migrated slower than that from the susceptible lines. There was no correlation between resistance and catalase activity in mature embryos isolated from these lines. Taken together, these results suggest that catalase may play a role in the resistance response. To help elucidate its function in the resistance mechanism, we are currently isolating the *cat3* genes from the resistant and susceptible lines.

164:38

Genomics of Chilling Tolerance in Maize

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Chilling stress is a major problem affecting maize production in climatically marginal regions of Europe and is particularly deleterious in the initial growth stages of the maize plant during May and June. Chilling periods during grain-filling stage causes yield losses and decreases the energy content of silage maize. Maize varieties with high chilling tolerance would not only improve the productivity and quality of maize but also contribute in various ways to a more sustainable agriculture. The general goal of the project is to identify and characterize genomic regions and candidate genes for chilling tolerance and to apply the results in practical breeding. Therefore, a functional map for chilling tolerance will be constructed including candidate genes from databases and transcript expression analysis as well as QTL. The QTL will be identified and characterized in a mapping population of 700 doubled-haploid lines (DHL) derived from a cross between two contrasting elite parents. Candidate genes are identified by differential transcript expression using suppression subtractive hybridization and by retrieving sequences from databases. Both groups of candidate genes will be mapped in the above DHL population by means of STS, CAPS, SNP, or INDEL based markers. Co-localization of candidate genes with QTL will serve as a first validation step.

165:227

Using QTL analysis to study the effect of genetic background on a disease lesion mimic in maize

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Maize plants are subjected to many stresses in the field during growth. How they respond to such stresses can affect yield. If plants can be altered to have greater tolerance to stresses such as common field diseases, yields would be improved. The goal of my research is to identify quantitative trait loci (QTL) controlling the differential expression of disease lesion mimics seen in specific genetic backgrounds. Many disease lesion mimics show variation in severity in different backgrounds. Disease lesion mimics tend to be more severely expressed in early senescence backgrounds and less severely expressed in stay-green backgrounds. An *hsl*⁻¹* lesion mimic in a severe expressing line was crossed to MO20W, a lesion suppressing, stay-green line. Genotypes of 400 F₂ individuals were obtained with 102 simple sequence repeat (SSR) markers. A QTL analysis was performed to detect significant loci in the population for modification of lesion expression. The discovery of significant loci in our genetic model for plant disease should aid in improving plant response to lesion stress.

166:99

Population structure and strong divergent selection shape phenotypic evolution in maize landraces

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In order to conserve present and future evolutionary potential of species, it is necessary to investigate past and present evolutionary processes that shape quantitative trait variation. Understanding the dynamic of quantitative trait evolution as it occurred during and after domestication and still occurs is crucial to future crop breeding. We characterized population differentiation of maize landraces from Oaxaca in Mexico for quantitative traits and molecular markers. Q_{st} values (ratio of among farm variance on total genetic variance for quantitative traits) are much higher than F_{st} values (for neutral markers). In spite of continuous and recurrent high levels of migration among farms, strong divergent selection by the Mexican farmers occurs for most traits related to ear morphology. Comparing differentiation among populations for quantitative traits and neutral molecular markers provides an insight into phenotypic evolution. However, little is known about the evolutionary processes that shape genes (QTL) underlying quantitative trait variation undergoing divergent selection. Mexican landraces in some regions of Mexico offer a unique opportunity to study phenotypic association and phenotypic evolution. The characteristics of these populations include strong divergent selection at the farm level, very large effective population size of the whole population, and little population structure at unselected loci. Work is currently in progress to compare patterns of population differentiation between candidate genes for QTLs and that of neutral markers.

167:156

Searching for sugary enhancer1 (se1)a Near-Isogenic Approach

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The overall goal of this project is to provide a clearer understanding of the genetic and biosynthetic mechanisms involved in the accumulation of sugars in the recessive mutant phenotype sugary enhancer1 (se1) in maize. The molecular basis for this mutation is unknown. This endosperm carbohydrate mutation when homozygous in a sugary1 (su1) background has been observed to increase sugar content by 60-100%, improving consumer desirability while extending post-harvest maintenance of eating quality. One population being studied is based on F2:3 families generated from a cross of the near-isogenic inbreds IL451b (su1 su1 su1 Se1 Se1 Se1) X IL451b (su1 su1 su1 se1 se1 se1). The near-isogenic nature of the parents should allow for a more direct approach in identifying those loci associated with the se1 trait since in theory this trait is the only difference between the parents. 308 F2:3 families were grown in replication during Summer 2000 and fresh harvested at 21 days after pollination (DAP) to quantitatively evaluate kernel sugar content. Prior to planting, families had been visually evaluated for lighter kernel color and slower dry down previously associated with the se1 trait. Kernel samples for families harvested at 21 DAP were based on equal numbers of kernels taken from a bulked set of ears. Samples were freeze dried, ground to a fine powder, and kernel sugars were extracted with 80% ethanol. Sucrose, glucose and fructose were quantified in each sample using HPLC. Preliminary data from Summer 2000 are presented, as well as discussion of additional research objectives involving data from Summer 2001, and incorporation of DNA marker data.

168:76

Protein Differences Between Lepidopteran Resistant and Susceptible Maize

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Protein patterns from juvenile and adult leaf tissue of corn (*Zea mays L.*) inbreds that are resistant or susceptible to fall armyworm (*Spodoptera frugiperda*) were analyzed through two-dimensional electrophoresis. The goal of this comparative protein research is to identify putative *S. frugiperda* resistance genes in maize. These resistant genes can then be integrated into elite maize lines in order to reduce: the pest population, cost of pesticide application, and hazards to the environment. Mp705 (a resistant line) and Oh28 (a susceptible line) were used for comparison. Three replications of 2-dimensional IEF protein gels were run for each. Spot position and intensity were analyzed. Intensities were averaged across replications. Putative resistance factors were identified by subtraction of susceptible from resistant data. Comparisons within lines were also made on juvenile versus adult leaf tissue. Presence of higher numbers of adult leaves has been associated with *S. frugiperda* resistance in this material. Putative resistance proteins from both sets of comparisons will be tested against 2-dimensional protein patterns from F2 derived progeny of Mp705 x Oh28 segregating for resistance to confirm their association with resistance.

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TGA1, Silica Deposition, and the Archaeology of Maize

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The effects of the *tga1* genetic locus on the development of cupule and glume form in maize and teosinte by Dorweiler et al. (1993) and Dorweiler and Doebley (1997). This analysis also demonstrated that silica deposition in the cupule and glume of maize is under direct genetic control. Blind tests conducted on phytolith assemblages recovered from W-22 maize cobs, each exhibiting one of three expressions of this genetic locus, showed that phytolith assemblages recovered from the chaff of a cob could be correctly identified as to which genetic expression of *tga1* was its source. This has important implications for tracing the initial domestication of maize archaeologically. Collection of phytolith assemblages from the cupule and glume of several races of maize shows that the effects of *tga1* interact with other aspects of maize genetic selection. Races, or sometimes racial complexes, consistently produce taxonomically specific assemblages of silica bodies. These assemblages are recoverable from food residues inside utilized pottery, tooth calculus, and coprolites. Using these assemblages the movement of maize lineages across South America can be traced.

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Analysis of putative maize flowering time genes using association tests.

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Flowering time is an agronomically important quantitative trait, which is potentially influenced by a large number of maize candidate genes. Association testing, using diverse inbred lines, provides a rapid means of dissecting the effects of these putative flowering time genes at very high resolution, provided that the effects of population structure are controlled in the statistical analysis. Three positional candidate genes, believed to be involved in flowering time will be discussed: *Dwarf8*, *Indeterminate1*, and *Teosinte Branched1*. Each candidate gene was PCR amplified and sequenced from at least 32 maize inbred lines. Association tests were used to assess the effect of sequence polymorphisms on phenotypic variation. Statistical methods of controlling population structure (Pritchard *et al.*, 2000) were adapted for use with quantitative traits, to eliminate spurious associations. The results of these association tests, population structure, linkage disequilibrium, and nucleotide diversity will be discussed for each of these genes. Polymorphisms in *Dwarf8* (Thornsberry *et al.*, 2001) and *Indeterminate1* were significantly associated with differences in flowering time. Understanding the effects of such nucleotide polymorphisms and their presence in the germplasm will improve selective breeding techniques for these quantitative traits.

171:116

Genomics of Nitrogen Use Efficiency in Maize

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In most industrialized countries the use of nitrogen fertilizers has been dramatically increased during the last decades. This resulted in negative effects on the environment. Therefore, the development of cultivars with improved nitrogen use efficiency is an important contribution to sustainable production systems with reduced input of fertilizers. Results from a long-term collaboration between KWS SAAT AG and the University of Hohenheim showed that sufficient genetic variability for adaptation to low soil N supply exists in current European maize breeding materials. Based on that information, inbred lines contrasting in N-use efficiency had been developed. In this project, two dent lines selected for extreme adaptation to low-resp. high-N conditions were crossed and a doubled-haploid (DH) line population (N = 720) was derived from the F1 by maternal haploid induction. This material is presently being used to generate a genetic map of the population. Aim of this study is to identify and characterize genomic regions and candidate genes that have an influence on nitrogen use efficiency in maize and to apply the results in practical breeding. Therefore, Quantitative Trait Loci (QTL) are determined and characterized in the foregoing DH-line population. Furthermore, candidate genes are identified by data base searches and and polymorphic markers based on PCR are developed and mapped in the population. In parallel experiments new candidate genes for nitrogen use efficiency are identified by differential gene expression in parent lines grown under low and high nitrogen supply, respectively. Co-localization of candidate genes with QTL data will serve as a first validation step.

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Associations for starch content and pasting properties in diverse maize inbreds

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Starch content in the maize kernel is a complex trait controlled by many genes. The market for cornstarch and its unique properties continues to grow with diverse applications, which range from livestock feed to adhesives and film. In this study, we focused on 6 genes: Amylose extender1 (Ae1), Brittle endosperm2 (Bt2), Shrunken1 (Sh1), Shrunken2 (Sh2), Sugary1 (Su1), and Waxy1 (Wx1), all of which are involved in the biosynthesis of starch. In order to dissect this pathway using an association approach, Ae1, Bt2, Sh1, Sh2, Su1, and Wx1 were sequenced in a diverse set of 32 inbred lines. Association tests for starch content and various pasting properties were performed and polymorphisms with putative associations were identified (P-values < 0.05, 100 permutations of the data). These polymorphisms were then scored in a larger set of 102 inbreds and associations were reexamined. Sites in Ae1, Bt2, Sh1, Sh2, and Wx1 show significant associations for kernel properties such as overall starch content (measured by near infrared light reflectance over 4 field environments). Significant associations for various starch pasting properties were also found in Bt2, Ae1, and Su1. Association approaches can quickly identify and evaluate useful polymorphisms in genes and begin to elucidate how allelic variation may affect phenotype in important biochemical pathways.

Transposable Elements

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Regional Mutagenesis Utilizing Ac in Maize

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The goal of this project is to develop a uniform maize population for both forward and reverse genetic screens. We will exploit the tendency of Ac to move to closely linked sites in the genome, to provide a tool for regional mutagenesis. The low forward mutation rate associated with Ac will enable near-isogenic comparisons between any mutants recovered in this program. By distributing Ac elements at 10 cM intervals throughout the maize genome, any mapped gene, EST or QTL will serve as a target for Ac mutagenesis. Each Ac has been maintained in an inbred W22 germplasm as a single active element at a well-defined genetic and physical map position. To date we have developed more than 150 Ac lines and have mapped 20 elements located on 9 of the 10 chromosomes.

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Mariner-like transposases are widespread in flowering plants and are responsible for the amplification of Stowaway-like MITEs

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Complete and partial sequences of mariner-like elements (MLEs) have been reported for hundreds species of animals, but only two have been identified in plants. Based on these two plant MLEs and several related sequences identified by database searches, plant-specific degenerate primers were derived and used to amplify a conserved region of MLE transposase genes from a variety of plant genomes. Positive products were obtained for 9 dicots and 31 monocots out of 64 plant species tested. Phylogenetic analysis of 68 distinct MLE transposase sequences from 25 grass species is consistent with vertical transmission and rapid diversification of multiple lineages of transposases during grass evolution. Surprisingly, the diversification of MLEs in grasses was accompanied by repeated and independent acquisition of introns in a localized region of the transposase gene. We also present evidence that plant MLEs are the likely source of transposase for the amplification of Stowaway-like MITEs.

TRANSPOSON, Ac/Ds, -MEDIATED GENE TRAP SYSTEMS FOR FUNCTIONAL GENOMICS IN RICE

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Maize Ac and gene trap Ds has been introduced into rice genome by Agrobacterium-mediated transformation. CaMV 35S promoter was used to express Ac cDNA as a transposase source. For gene trap Ds, a partial intron with three alternative splicing acceptors were fused to GUS. A genetic marker for Ds (BAR) was inserted into trap Ds. To develop a large scale of Ds transposant lines in rice, three strategies have been employed and evaluated. In the first of all, a large scale of genetic crosses have been made between homozygous Ds or Ac starter lines that carry either a single copy of gene trap Ds or a single copy of Ac. The data suggest that the mobility of Ds is highly variable among starter lines. A couple of Ds lines generated high frequency of germinal revertants up to 25%, in the F2 segregation generation. However, the same genetic crosses that were repeated later, the frequency of germinal reversion in the same crosses noticeably decreases down to 10%. This indicates that the activities of Ac or Ds were subjected to modification in rice genomes. In contrast, when transgenic plants were generated by introduction of Ac and Ds in a single T-DNA vector, nearly 70% of Ds elements were excised from original T-DNA insertion sites. The last strategy is to select rice lines carrying high copy of Ds. The possibility is being examined whether a multi-copy Ds can be maintained and normally transmitted into subsequent generations. To maximize the efficiency of characterizing Ds tagged genes, optimal condition of iPCR (inverse PCR) have been established to clone Ds flanking DNA in rice genomes. Using cloned Ds insertion sites, it was found that 45 Ds transposed from 4 independent loci were 9 chromosomes except chromosome 8. Our data demonstrated that a maize transposable element family, Ac/Ds, could be efficiently utilized for saturation mutagenesis in rice. A national consortium has been organized to develop internationally competitive scale of population mutagenized by Ds, and to construct databases of molecular information on Ds insertion sites. The project includes cloning of 6,000 Ds insertion sites per year and selection of 10,000 Ds lines per year.

176:143 Dasheng and RIRE2: a novel non-autonomous LTR element and a candidate autonomous partner in rice

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Nonautonomous elements are a common feature of class 2 (DNA) element families. Their relationship with autonomous partners has been well-established since McClintock's characterization of the Ac/Ds family in maize. In contrast, the origin of nonautonomous class 1 (retro) elements remains a mystery. Dasheng is one of the highest copy number LTR elements and one of the most recent elements to amplify in rice (*Oryza sativa*). However, the internal region of Dasheng does not encode any proteins that are normally associated with retrotransposons (e.g. gag, pol), indicating that Dasheng is a nonautonomous element. To search for the autonomous partner of Dasheng, a comprehensive survey of transposable elements in rice was performed with the genomic sequences of Nipponbare (*O. sativa* ssp. japonica) from the Rice Genome Research Program. Among all the recognizable transposable elements, RIRE2, a previously reported gypsy type LTR retrotransposon, is the best candidate. The two elements have similar LTRs and almost identical cis-sequences required for transposition (PBSs and PPTs). Furthermore, although amplification of Dasheng appears to be more recent than that of RIRE2, some of the RIRE2 elements transposed very recently, as evidenced by the sequence similarity of their LTRs. These elements appear capable of autonomous transposition. The distribution of RIRE2 and Dasheng in the rice genome is also very similar, as would be expected if RIRE2 transposition machinery amplified Dasheng elements. Both are clustered in pericentromeric regions. Finally, a chimeric element with both Dasheng and RIRE2 sequences was found among rice genome sequence, suggesting the two elements may share transposition machinery. Taken together, these data strongly suggest that RIRE2 is the autonomous element responsible for the recent amplification of Dasheng.

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Insensitivity of Maize Mutator Transposon Activities to Endogenous and Transgene-Encoded Antisense RNA

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ABSTRACT The maize MuDR/Mu transposable elements are highly aggressive, and their activities are held in check by host developmental and epigenetic mechanisms. The Mutator regulatory element, MuDR, produces antisense messages in addition to its sense transcripts, *mudrA* and *mudrB*. We report that internal deletions in MuDR arise frequently in somatic tissues; preferential loss of the intergenic region results in chimeric sense *mudrA*/antisense *mudrB* and sense *mudrB*/antisense *mudrA* transcripts. Heritable internal deletions are extremely frequent (>10⁻² per element) and also encode antisense transcripts. Surprisingly, expression of endogenous or additional transgene-encoded antisense transcripts neither lowers sense transcript levels nor inhibits Mutator excision activity over the three generations examined. Thus unlike many host genes, MuDR is immune to antisense regulation and such transcripts are not dominant negative regulators of Mutator activities. Our findings suggest that MuDR has evolved a defense mechanism against antisense-mediated gene suppression and host-imposed RNA surveillance.

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Analysis of RescueMu Germinal Insertions

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RescueMu is a transposable element genetically engineered to contain pBlueScript, enabling it and flanking maize DNA to be plasmid rescued in *E. coli* (Raizada et al. 2001). As part of the NSF-funded "Maize Gene Discovery" project (<http://zmdb.iastate.edu>), RescueMu has been utilized to obtain new genomic sequence information via gene tagging. Sequences flanking RescueMu insertions, obtained from sequencing plasmid libraries rescued from plant DNAs pooled in a 48 x 48 grid format, come from a collection of germinal and somatic events (<http://gremlin3.zool.iastate.edu/zmdb/library-plate/index.html>). Here we more closely examine a subset of 25 putative germinal insertions that are in or very close to coding regions based on BLAST searches to the EST databases at GenBank. We confirm that a subset of these are truly germinal by using PCR amplification and southern analysis to verify their heritability in the progeny of the candidate grid plants. A close analysis of the sequence from rescued plasmids has revealed a high frequency of small somatic insertions and deletions flanking the original site of the RescueMu germinal insertions. Analyses of the effect of the insertion on gene expression will be performed along with searches for mutant phenotypes segregating with the RescueMu insertions

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KARMA, a novel LINE-type retrotransposon activated by tissue culture in rice

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The activation of endogenous retrotransposon elements upon tissue culture and stress conditions is a common feature in all eukaryotes. In normal conditions, however, most retrotransposons are subjected to tight epigenetic regulation, so that transcription and transposition rarely occur. All active elements reported so far for plants belong to the LTR (long terminal repeat) class of retrotransposons. Here we report the identification of KARMA, a novel non-LTR retrotransposon of rice, which is activated upon DNA demethylation caused by tissue culture. Different from other known plant retrotransposons, KARMA is not silenced soon after activation and continuously transposes in subsequent generations of plants regenerated from tissue culture. Although KARMA is transcribed in cultured cells, transposition was not observed in the analyzed cell culture lines but only in regenerated plants, suggesting that transcription is not sufficient for transposition of KARMA and that some sort of post-transcriptional regulation may be required.

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Molecular mapping in a japonica/japonica recombination inbred population of rice

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Molecular maps of *Oryza sativa* (domesticated rice) have been developed using indica/japonica intersubspecific and interspecific crosses, but not with japonica/japonica intrasubspecific crosses. Molecular mapping based on the variation from crosses between two related varieties belonging to the same subspecies is important for a breeding program that exclusively utilized japonica germplasm. A molecular map consisting of 57 AFLP markers and 98 simple sequence length polymorphism (SSLP) markers has been constructed using a F8 recombinant inbred (RI) population of 231 lines derived from a cross between two temperate japonica parents, Suweon365 and Chucheongbyeon. A set of SSLP markers was used to construct the framework map. The AFLP markers were derived from eleven EcoRI(+2) and MseI(+3) primer combinations. The proportion of polymorphic bands between the parents averaged 21.3% and 12.9% for SSLP and AFLP, respectively. This level of polymorphism was lower than that observed between temperate and tropical japonica. The map contains 1,683cM of the 12 rice chromosomes, with an average interval size of 11cM but with relatively fewer markers for chromosomes 2, 3, 5 and 6. This map will be continuously refined through the addition of microsatellite and MITE anchored markers. This population is also being used for quantitative trait loci (QTLs) analysis. Together this information, when combined with the molecular markers, will be utilized for marker-assisted selection and for breeding of high-quality cultivars.

181:171 Epigenetic Regulation of MuDR Activity

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Epigenetic regulation can be defined as heritable changes in gene activity in the absence of variation in chromosomal position or DNA sequence. Such changes are a characteristic feature of the *Mutator* system of transposons, which is regulated by the autonomous *MuDR* class of elements. Epigenetic changes of *MuDR* activity are easily scorable, non-essential to normal plant development, and subject to genetic regulation by specific maize loci. Further, a Minimal Line is available that carries a single *MuDR* element at a known chromosomal location (*p1*) and a single non-autonomous reporter element, allowing for comparisons without the effects of variations in copy number and position of *Mu* elements. Because of this, Mutator activity in the Minimal Line represents an ideal model system for the analysis of mutations that affect epigenetic regulation. We have identified two loci that can affect *MuDR* silencing. *MuK* is a single dominant locus that can reliably silence our single *MuDR* element. Silencing of *MuDR* is associated with methylation of the TIRs of both non-autonomous and autonomous elements. Steady state RNA levels of silenced *MuDR* elements are reduced, as is DNaseI hypersensitivity. Silencing is progressive during development; in F1 progeny of crosses between plants carrying active *MuDR(p1)* and those carrying *MuK*, *Mu* elements are less methylated in immature seedling than they are in adult plants. In subsequent generations of test crossing, *MuK* segregates independently of the silenced *MuDR* elements. Further, even in the absence of *MuK*, the *MuDR* elements remain silenced through multiple generations of outcrossing, demonstrating that the loss of activity is both stable and heritable. Reciprocal crosses reveal that, although *MuK* has an easily scorable effect of excision frequency when used as a female, its silencing effect is only manifest in during growth of progeny seedlings when *MuK* is used as a male. A second locus, *mop1*, was originally identified as a mutation that prevents paramutation, but has also been found to result in the loss of methylation at both *Mu1* and *MuDR* termini. Although methylation is lost immediately in a mutant background, reactivation of *MuDR* elements, as assayed by excisions of a reporter element, occurs only gradually, following several generations of exposure to the mutation.

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Maize Targeted Mutagenesis: progress in maize gene knockouts

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The Maize Targeted Mutagenesis project is a large Mutator (Mu) population and screening service created by a collaboration between Cold Spring Harbor Laboratory, Syngenta, and UC Berkeley. The screening service is open to all academic researchers and has been fully operational for 1.5 years. Insertions into genes of interest are detected by nested PCRs on 3-dimensionally pooled DNA samples. Transmission of an insertion from the F1 to the F2 is confirmed before the relevant maize line is sent to a researcher. To date approximately 40% of genes screened have a transmitted insertion present in the collection. Insertions predominate in the 5' portions of genes and no bias for intron or exon sequences is evident. A correlation between ear phenotypes and residual Mu activity exists and may reflect suppressibility of Mu-induced alleles, a property seen with insertions near the 5' ends of genes and in introns. The results of phenotypic screening of ears of almost all lines and seedlings of 8000 lines are also searchable in the database, located at <http://mtm.cshl.org>. Progress will be reported on establishment of an array-based screening system.

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Mapping of 225 MITE markers from 5 different families in rice

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MITEs are estimated to make up 4-6% of the *Oryza sativa* genome. Primers for transposon display (TD) analysis were designed to recognize conserved regions in the alignments of the MITE families Explorer, Castaway, Tourist, Olo, Wanderer and Gaijin. Comparison of TD profiles revealed that different MITEs showed characteristic levels of insertion site polymorphism (18-56%) between indica (IR64) and japonica (Azucena), thus providing evidence for temporally distinct rounds of amplification throughout the genome. The genomic positions of a total of 225 elements from the Castaway (57), Explorer (34), Tourist (44), Olo (36) and Wanderer (54) families were determined in an IR64 x Azucena doubled haploid mapping population and found to be distributed over all 12 chromosomes. No differences in distribution of elements from different MITE families were detected. One exception is Explorer for which elements are located preferentially in lower chromosome arms. A total of 13 (6 %) elements from 4 families are located in the centromeric regions of 6 chromosomes and more in the pericentromeric, suggesting that MITEs are present in euchromatic as well as heterochromatic regions. Computer searches of BAC/PAC clones which had been anchored in silico (TIGR) were performed. Analysis of the identified sequences shows that the distribution of elements not detectable by mapping agrees with the patterns seen on the genetic map. The fact that *Oryza* is comprised of wild and cultivated species, that the genome of *O. sativa* will be completely sequenced and that several MITE families are described makes the genus *Oryza* a suitable system to study the evolutionary dynamics of MITEs and to determine how MITEs contribute to genome evolution in *Oryza*.

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Unconventional transposition during DNA replication can generate complex chromosome rearrangements

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Maize plants carrying active *Ac/Ds* transposable elements spontaneously generate large, complex chromosome rearrangements. We have previously shown that large duplications and deletions can be formed by a non-standard transposition event involving an *Ac* 5' end in one sister chromatid and an *Ac* 3' end in the other sister chromatid. This type of transposition is termed non-linear transposition (NLT; Zhang and Peterson, 1999 Genetics). The *Drosophila* P element can undergo similar transposition events termed Hybrid-Element Insertion (HEI; Preston and Engels 1996 Genetics). Here, we present evidence that NLT can generate additional types of complex chromosome rearrangements that contain an inverted duplication plus an additional insertion at the duplication breakpoint. We propose that these rearrangements arise when NLT occurs during DNA replication. The NLT events insert replicated chromosomal regions into unreplicated target sites, where they can undergo an additional round of replication to generate an insertion. Termination or displacement of the "extra" replication forks will result in two free chromosome ends; fusion of these free ends would then form an inverted duplication separated by a sequence of variable length. This model is consistent with the known tendency of *Ac* to transpose during DNA replication, and it can explain the origin of several complex maize chromosome rearrangements isolated by McClintock and studied by others (sh-m5933; Burr and Burr, 1984 Cell; Doring et. al. 1990 MGG). These results further demonstrate the impact of transposable elements on genome structure.

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Initiation of silencing of the MuDR/Mu transposon family

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Multiple mechanisms contribute to genomic stability through suppression of transposable elements. Coordinate epigenetic silencing is the main regulatory mechanism controlling the dispersed, multi-copy MuDR/Mu elements responsible for Mutator activity in maize. Once silenced, MuDR/Mu elements are rarely reactivated spontaneously. Silencing includes (1) the transcriptional inactivation of both the *mudrA* (transposase) and *mudrB* (helper) genes controlled from nearly identical promoters located within the terminal inverted repeats (TIRs) of the MuDR element, (2) DNA methylation of Mu TIRs, and (3) cessation of transposition. Here we report that RNA interference may also regulate MuDR/Mu. We found transposon-specific RNAs of ~25 nucleotides in all maize lines tested, independent of Mutator activity. The properties of these small RNAs and preferential accumulation of the antisense strand are consistent with a role in translational regulation. Such translational regulation could explain the finding that despite wide variation in *mudrA* and *mudrB* transcript abundance, protein levels are similar among all lines. In plants with active elements, initiation of silencing coincides with nuclear retention of non-polyadenylated MuDR-encoded RNA. MuDR/Mu silencing was previously shown to be progressive during plant development and is often initiated in the flowers, which are produced last in the life cycle. During silencing, there is also a progressive decrease in transposon-encoded RNA but a relative increase in the fraction that is nuclear. Genetic analysis shows that high levels of transposon-encoded nuclear RNA in a parent plant are predictive of the loss of somatic excision activity of Mu elements in the progeny. We infer that nuclear RNA triggers host defenses leading to transcriptional silencing of MuDR elements and that this results in coordinate transposon inactivation and DNA methylation.

186:184 Towards identification of the transposase responsible for MuDR/Mu element insertion

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Mutator activity is controlled by the autonomous element MuDR. It contains two genes *mudrA* and *mudrB*, which are transcribed from promoters located in the ~215 bp terminal inverted repeats (TIR). *mudrA* encodes several MURA transposases resulting from alternative splicing. The *mudrA* gene has two transcription start sites at positions +169 and +252 and three introns. It was assumed that the first exon was a non-coding 5' untranslated region and that translation started with the ATG at position +450, within the second exon; two large proteins are predicted from initiation at +450, MURA823 from completely spliced transcripts and MURA736 when the third intron is retained. Transgenic maize containing 35S:MURA823 (Raizada and Walbot, 2000) or S-tagged MURA823 and MURA736 transcribed from the native TIRA promoter program Mu somatic excision from anthocyanin reporter alleles. No Mu insertion events have been observed, even when these MURAs are crossed into lines that express all forms of MURB (35S:*mudrB* or deletion derivative d112, Lisch et al., 1999). Additional analysis of *mudrA* RNA indicated that transcripts initiated at +169 have an alternatively spliced first intron that eliminates the ATG at +450. An ATG at position +224 opens a short reading frame of just 48 aa. Surprisingly, antibody against this short polypeptide detects a large ~120 kD polypeptide in maize tissues; this is the same size as the protein recognized by antibodies to the main ORF in MURA823. We hypothesized that a translational frameshift could incorporate the novel amino terminus into the main ORF, resulting in the predicted protein MURA854. To determine if translational frameshifting occurs in vivo, we constructed transgenic maize plants with either the native cDNA (frameshift required) or a frameshift corrected form. Both cDNAs incorporated an S-tag and were expressed using the native TIRA promoter. MURA854 protein is produced in vivo from both constructs. MURA854 programs somatic excision of Mu elements (from the F1 cross of the transgene X reporter gene stock). MURA854 lines have just been crossed with [1] *mudrB* lines to determine if MURB is essential for germinal insertion and [2] RescueMu lines to monitor for somatic insertion.

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Efficient Recovery and Sequencing of *RescueMu* Maize Genomic Clones

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Background: *RescueMu*, an engineered genetic transposon, is an integral component of the sequencing effort at the Maize Gene Discovery Project (MGDP). Because it is proposed to insert randomly throughout the genome but preferentially into genes, this component of the sequencing effort provides a parallel, complementary approach to the EST portion of the project by sequencing host DNA next to *RescueMu*. Up to this date, the MGDP has 58,743 sequences defining 22,569 insertion sites. **Protocol Overview:** Families with high mutator activity are elucidated using Southern Blot analysis. These mutator-enriched families are grown in 48-row X 48-column grids (2304 plants). DNA samples are prepared from leaves pooled from each grid row and column. DNA containing *RescueMu* is treated with BamHI and BglII and ligated to form individual plasmids. These plasmids are then transformed into *E. coli* host cells for sequencing beyond the Terminal Inverted Repeat of the *RescueMu* insert (See *Genomic DNA Extraction* protocol at http://www.zmdb.iastate.edu/zmdb/protocols/rmu/plasmid_rescue.html.) Rows of two grids (G and H) have been sequenced, with a third (I) in progress. In addition, 4 columns of the grid are sequenced to begin to identify likely germinal insertions (Raizada et al., 2001). In Grid G, we estimate there are ~2200 germinal insertions. Through the ZmDB website, you can order library plates containing grid row and column libraries. Along with plasmid sequencing, PCR can be used to screen library plates for an insertion in a gene of interest. Seed may be obtained from the Maize Genetics Cooperative Stock Center, conducted jointly with the MGDP. The Maize Gene Discovery Project is an NSF plant genome project. For additional information, see <http://www.zmdb.iastate.edu>.

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Generation of allelic diversity at the pink scutellum1 locus through Ac insertional mutagenesis

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The maize transposable element Ac will often transpose to genetically linked positions, making it an ideal tool for regional mutagenesis. As part of our ongoing development of an Ac-based regional mutagenesis program (see abstract for Brutnell et al.) we performed a targeted mutagenesis of the ps1 locus of maize. ps1 mutants of maize are a viviparous class of mutants that accumulate lycopene in the scutellum and embryos. With the aim to clone the ps1 gene and to generate multiple alleles at this locus, an Ac insertional mutagenesis was performed. Approximately 400 independent transposition events were selected from a donor Ac, resident approximately 1 cM from the ps1 locus on chromosome 5S. A screen of the F2 individuals identified seven independent alleles that segregated for the pink scutellum phenotype. Six of these alleles are viviparous and result in embryo lethality, presumably due to the failure to accumulate ABA. The scutellum and the embryos in these alleles are pink in color. However, one weak allele was identified that failed to condition the viviparous phenotype of the kernels. The scutellum and embryo of plants carrying this allele are orange in color suggesting a lower accumulation of lycopene compared to the severe alleles. Utilizing Ac as a molecular tag, the ps1 gene was cloned and characterized.

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RETROARRAYS - MICROARRAY ANALYSIS OF LTR RETROTRANSPOSONS FROM THE MAIZE GENOME

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Long terminal repeat (LTR) retrotransposons belong to the class I transposable elements and have features in common with retroviruses. They transpose via RNA intermediates, with their transcriptional activity conferred by promoters within the long terminal repeats. The majority of the maize genome is composed of diverse families of LTR retrotransposons, often arranged in nested patterns. We have constructed a DNA array (the "Retroarray") with diagnostic probes for numerous LTR retrotransposons. The Retroarray consists of 64 probes printed in quadruplicate, 44 of which represent 19 elements from 12 different families. A pilot study was performed to identify major sources of experimental variation in a typical microarray experiment. The layout of the Retroarray then followed standard concepts in experimental design, allowing estimation of experimental effects via linear model theory. This approach permits the identification of significantly expressed retrotransposons, as well as those undergoing significant differential expression, without the need to specify an a priori fold change cut-off. Using total RNA samples from different maize organs at various stages of development and under several treatments, few of the more abundant LTR retrotransposons from the Ji, Cinfu, Opie, Huck and Tekay families were found to be expressed. Some retrotransposons exhibited differential expression under different treatments. In addition, the Retroarray was used to determine differences in LTR retrotransposon copy number between different maize inbreds and teosinte. The data suggest that even in the genomes of closely related maize inbred lines, some LTR retrotransposons are present in differing copy numbers.

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RescueMu-tagging grids - what are they and how to make use of them for your own research?

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Our maize gene discovery project is advancing into its fourth year and has produced a wide range of genomics data (see our other posters) as well as utilities for the maize community. Utilizing engineered Mu elements, more than ten superior tagging grids (one full grid = 48 rows x 48 columns = 2304 plants) have been generated. These grids (F through Q) are composed of progenies from actively transposing founders (comparable to the Mu1 elements), each containing one or more transposed elements without the original transgene array(s) in their background. All plants were selfed and deposited at the Maize COOP. Corresponding plasmid libraries for grids "G", "H", and "I" have been completed and are available through our website (<http://www.zmdb.iastate.edu>). This poster is designed to familiarize users with the composition of individual grid, what to expect from the library plates, and the methodology employed in screening your mutant(s) of interest. Once a specific plant is identified, seed request can be directly forwarded to the Maize COOP.

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DNA repair of *Ac/Ds* excision sites in the yeast *Saccharomyces cerevisiae*

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Both autonomous *Ac* and non-autonomous *Ds* elements transpose in yeast cells, and we have utilized this system to examine three aspects of transposition. In striking contrast to the typical situation for repairing DNA breaks in yeast, approximately half of the transposon footprints from a total of 39 independent events occur through non-homologous end-joining repair even when a homologous repair template is provided (360 bp on one side and 600 bp on another side of excision site at the 5'UTR of *ADE2*). These results suggest that free DNA double-strand breaks may not be intermediates in host DNA during transposition, but may instead occur only as aberrant events. Alternatively, the transposition complex may recruit host factors that target the repair of an excision site into the end-joining pathway. We have also compared transposition of both an autonomous and a nonautonomous element from the yeast *ADE2* gene located on a single-copy plasmid and from the yeast chromosome. The repair of the excision sites is essentially the same in all four cases. However, the frequency of Ade⁺ reversion for both *Ac* and *Ds* alleles is 2-3-fold higher on plasmids, suggesting a higher transposition frequency from the plasmid. Finally, analysis of 96 independent non-homologous end joining (NHEJ) footprints indicates that, as in plants, a predominant *Ac/Ds* footprint is formed and is determined by flanking sequences.

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PIF-like elements are widespread and associated with Tourist-like MITEs.

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Miniature inverted-repeat transposable elements (MITEs) are non-autonomous elements prevalent in plants and animals. Despite their wide distribution, the origin and transposition mechanism of MITEs have yet to be clearly elucidated. The best clue so far comes from maize, where an active DNA element family, P Instability Factor (PIF), was found to be associated with a Tourist-like MITE family called miniature PIF (mPIF), and their shared features strongly suggest that PIF and mPIF were amplified by the same or a closely related transposase. To further investigate this possibility, we have conducted a survey for PIF-like elements in plants and animals, which combined both database mining and a PCR-based screening approach. The result of this survey revealed the presence of PIF-like elements in a wide variety of monocots, dicots, invertebrates and vertebrates. Significantly, like the association between the maize PIF and mPIF, several other plant and animal PIF-like elements were found to have clearly given rise to Tourist-like MITE families in their respective genomes. Taken together, these results suggest that PIF-like elements are members of a new eukaryotic DNA element superfamily widespread in plants and animals, and that the transposases encoded by these elements are responsible for the origin and spread of Tourist-like MITEs.

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