45th ANNUAL MAIZE GENETICS CONFERENCE

PROGRAM and ABSTRACTS

13-16 MARCH 2003

GRAND GENEVA RESORT LAKE GENEVA, WISCONSIN

This conference received financial support from:

The National Science Foundation

Affymetrix, Inc.

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WE THANK THESE CONTRIBUTORS FOR THEIR GENEROSITY

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GENERAL INFORMATION

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

Talks and Posters.
All Talks and Workshops will be presented in Forum A.
Posters will be presented in Forum BC, adjacent to the talks.
Posters should be hung up on Thursday evening and should stay up until the end of the Sunday morning session if possible, but need to be removed by noon Sunday.

Informal Meeting Places.
On Thursday and Friday after the evening sessions we will have informal socializing in Forum A,B,C and the nearby lobby. On Saturday we will have informal socializing in the Evergreen Ballroom. Refreshments will be provided each night until 1am.
After 1am each night, rooms 6102/ 6104 will be available for subsequent socializing. These are "private party rooms" and alcoholic beverages may be brought in. However, you will have to stay in these rooms if you are carrying drinks and dispose of your trash and bottles in the party room.

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

David Jackson, Chair
Gunter Feix
Daniel Grimanelli
Sarah Hake
Martha James
Robert Meeley
Mike Scanlon
Patrick Schnable
Lynn Senior
Dave Weber
Marty Sachs, Local Organizer, ex officio
Karen Cone, Treasurer, ex officio
Mary Polacco, Abstract Coordinator, ex officio

Acknowledgements.
Many thanks to Mary Polacco, Denis Hancock and Steve Schroeder for handling the major effort of assembly and printing of the abstracts book. Thanks to the Missouri Maize Group for their help, and to Mike McMullen for preparing the poster. The meeting registration was outsourced to the MU Conference Center, and professionally handled by Lucy St. John and Jill Nunn. Thanks to Marty Sachs for catching all the little details that matter not only for local arrangements but the overall meeting as well. Thanks also to the maize community for being a great, cooperative group of scientists.

Next Maize Genetics Meeting: 11 – 14 March, 2004 Camino Real México
Mexico City, Mexico
http://www.caminoreal.com/mexico_i/
Local Organizer, Daniel Grimanelli <d.grimanelli@cgiar.org>

45th Annual Maize Genetics Conference.
March 13th-16th 2003.

Thursday, March 13 — 5:30-7:00 PM DINNER

7:00-7:15 PM **Announcements** — **Dave Jackson**

Session 1 **EVENING SESSION** **7:15-9:15 pm** Chair: **Sarah Hake**

7:15-8:00 PM **Neelima Sinha, University of California, Davis, CA**
The Development and Evolution of Leaves

8:15-9:00 PM **Vicki Vance, University of South Carolina, Columbia, SC**
Suppression of RNA Silencing in Plants: The Role of Small Regulatory RNAs

9:15 PM **Informal Poster Viewing** **(hang Posters Thursday Night)**

BEER & SNACKS AVAILABLE IN POSTER AREA UNTIL 1:00 AM

Friday, March 14 — 7:00-8:30 AM BREAKFAST

Session 2 **GENE REGULATION AND GENOMICS** **8:30-10:20 am** Chair: **Bob Meeley**

8:30-8:45 **Lyudmila Sidorenko, University of Arizona, Tuscon, AZ**
Identification of cis-acting regulatory sequences and trans-acting regulatory factors are the stepping stones to the elucidation of the mechanism of p1 paramutation

8:45-8:50 Discussion

8:50-9:05 **Julia Marcela Hernandez, Ohio State University, Columbus**
Transcriptional regulation of the flavonoid biosynthetic pathway: the dual role of the HLH coactivator R/B

9:05-9:10 Discussion

9:10-9:25 **David W. Galbraith, University of Arizona, Tucson, AZ**
The future of maize expression arrays: what can we learn from working with Zea and Arabidopis?

9:25-9:30 Discussion

9:30-9:45 **Trent Seigfreid, Iowa State University, Ames, IA**
Maize GDB: A Next Generation Maize Database

9:45-9:50 Discussion

9:50-10:00 **Brad Barbazuk, Donald Danforth Plant Science Center, St. Louis, MO**
The Maize genome Sequencing Project at the Donald Danforth Plant Science Center

10:00-10:10 **Cathy Whitelaw, The Institute for Genomic Research, MD**
Consortium for Maize Genomics - Approach Evaluation for Targeted Sequencing of Maize Genes

10:10-10:20 Discussion

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

Session 3 **DEVELOPMENTAL GENETICS** **10:50-12:30 pm** Chair: **Gunter Feix**

10:50-11:05 **George Chuck, Plant Gene Expression Center, Albany, CA**
The Control of Spikelet Meristem Development by the branched silkless1 Gene

11:05-11:10 Discussion

11:10-11:25 **John Fowler, Oregon State University, Corvallis, OR**
A Role for Maize ROP2 GTPase in the Male Gemetophyte

11:25-11:30 Discussion

11:30-11:45 **Peter Rogowsky, RDP, ENS-Lyon France**
ZmPRPL35-1 encodes a plastid ribosomal protein required for suspensor morphogenesis in maize embryos

11:45-11:50 Discussion

11:50-12:05 **Peter Bommert, University of Koln, Koln, Germany**
Thick tassel dwarf1 encodes a LRR-receptor kinase with high homology to CLAVATA1

12:05-12:10 Discussion

12:10-12:25 **Erik Vollbrecht, Cold Spring Harbor Laboratory, New York**
Molecular and evolutionary analysis of ramosa1 in inflorescence architecture

12:25-12:30 Discussion

12:30-1:30 PM - LUNCH

Friday, March 14 Cont'd

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

3:00-3:30 pm - BEVERAGES SERVED

Session 4 **MAIZE GENOMICS WORKSHOP** **3:30-5:30 pm** Chair: **Pat Schnable**

3:30-3:35 **Introductory Remarks**

3:35-3:50 **John Bon, Affymetrix**
Expression analysis in plants: a look at some approaches to array design using GeneChip technology

3:50-4:05 **Sean Coughlan, Agilent Technologies Inc.**
Global analysis of gene expression using high density in situ 60mer oligonucleotide arrays: Applications to Maize

4:15-5:30 **Genomic Posters**
Here many of the recipients of NSF funded genome grants for maize research will present the goals of their projects and highlight the deliverables to be provided to the maize community.

6:00-7:30 pm - DINNER

Session 5 **EVENING SESSION** **7:30-9:00 pm** Chair: **Mike Scanlon**

7:30-8:15 **Susan McCouch, Cornell University, Ithaca, New York**
Identifying genes and functional nucleotide polymorphisms underlying QTLs in rice

8:30-9:15 **Steven Henikoff, HHMI-FHCRC, University of Washington, Seattle, WA**
Traditional Genetics Meets Functional Genomics

9:30 **Informal Poster Viewing**

BEER & SNACKS AVAILABLE IN POSTER AREA UNTIL 1:00 am

Saturday, March 15 — 7:00-8:30 AM BREAKFAST

Session 6 — **GENOME ORGANIZATION AND EVOLUTION** — **8:30-10:10 am** — Chair: **Lynn Senior**

8:30-8:45 **Yves Vigouroux, University of Wisconsin, Madison, WI**
Population structure and gene diversity of american maize landraces

8:45-8:50 Discussion

8:50-9:05 **Volker Brendel, Iowa State University, Ames, IA**
The genomic origin of maize revisited

9:05-9:10 Discussion

9:10-9:25 **William Sheridan, University of North Dakota, Grand Forks, ND**
Global Analysis of the Maize Genome: Relating Genes and DNA Sequences to Chromosome Regions

9:25-:9:30 Discussion

9:30-9:45 **Shavannor Smith, Kansas State University, Manhattan, KS**
Identification and Characterization of Rp1 Genes with Novel Phenotypes in Maize

9:45-:9:50 Discussion

9:50-10:05 **Ning Jiang, University of Georgia, Athens, GA**
Identifying active DNA transposons in the genomic era

10:05-10:10 Discussion

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

Session 7 — **TRANSPOSONS AND CYTOGENETICS** — **10:40-12:20 pm** — Chair: **Dave Weber**

10:40-10:55 **Robert Meeley, Pioneer Hi-Bred Intl. Inc., Johnston, IA**
Diagnosis of hot spots for Mu integration in the maize genome and their association with binding sites for host-encoded nuclear protein(s)

10:55-11:00 Discussion

11:00-11:15 **Jianbo Zhang, University of Iowa, Ames, IA**
Transposition of Reversed Ac Element Ends Shuffles Exons and Rearranges Chromosomes in Maize

11:15-11:20 Discussion

11:20-11:35 **Rebecca Mroczek, University of Georgia, Athens, GA**
Distribution of retroelements in centromeres and neocentromeres of maize

11:35-11:40 Discussion

11:40-11:55 **Stephen Stack, Colorado State University, Fort Collins, CO**
Cytological crossover maps for all maize bivalents using recombination nodules

11:55-12:00 Discussion

12:00-12:15 **Zac Cande, University of California, Berkeley, CA**
The pathway of early meiotic prophase events in maize

12:15-12:20 Discussion

12:20-1:30 pm - LUNCH

Saturday, March 15 Cont'd

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

3:00-3:30 pm - BEVERAGES SERVED

Session 8 **QTL: Practical Aspects and New Approaches.** **3:30-5:30 pm** Chair: **Torbert Rocheford**

Oral workshop presentations of 10 min. approx. will be followed by an open discussion

Torbert Rocheford, University of Illinois, Champaign-Urbana, IL
Overview of Maize QTL Studies
Mike Lee, Iowa State Unviersity, Ames, IA
Map Construction and Use for Mutant Clone & QTL Mapping
Martin Bohn, University of Illinois, Champaign-Urbana, IL
Methodologies of QTL Analysis and Statistical Considerations
Mike McMullen, University of Missouri, Columbia, MO
QTL Approaches to study of a Pathway
Nick Lauter, University of Illinois, Champaign-Urbana, IL
High Resolution Mapping and functional Dissection of QTL Affecting Leaf Epidermal Traits
Ed Buckler, USDA-ARS, North Carolina State University, NC
Principles of Associative Genetic Analysis

6:00-7:30 pm - DINNER

Session 9 **EVENING SESSION** **7:30-8:30 pm** Chair: **Daniel Grimanelli**

7:30-8:15 **Hugo K. Dooner, Waksman Institute, Rutgers University, Piscataway, NJ**
Convergence of genetics and genomics at a bronze point in the map

BEER & SNACKS AVAILABLE IN EVERGREEN THEATER

Sunday, March 16

Session 10 **BIOCHEMICAL AND SEED GENETICS** **9:00-10:40 am** Chair: **Martha James**

9:00-9:15 **Tim Porch, University of Florida, Gainesville, FL**
Initial cloning and characterization of vp13 in maize

9:15-9:20 Discussion

9:20-9:35 **Christopher Goodman, Stanford University, Stanford, CA**
A multidrug-resistance associated protein involved in anthocyanin transport in Zea mays

9:35-9:40 Discussion

9:40-9:55 **Cynthia Gallagher, City University of New York, Bronx, NY**
Characterization of gene families that influence maize endosperm carotenoid content

9:55-10:00 Discussion

10:00-10:15 **Joao Leiva-Neto, University of Arizona, Tucson, AZ**
Expression of a dominant negative mutant of cyclin-dependent kinase A (ZmCDKA) reduces DNA endoreduplication during maize endosperm development

10:15-10:20 Discussion

10:20-10:35 **Bo Shen, Pioneer Hi-Bred International, Inc., Johnston, IA**
The supernumerary maize aleurone layer gene superal1 encodes an orthologue of the human CHMP family member of class E vacuolar sorting proteins

10:40 **FINAL ANNOUNCEMENTS**

10:45 **ADJOURN, Beverages availible.**

ABSTRACTS - TALKS AND POSTER PRESENTATIONS

TALKS:

Session 1. Thursday 7:15 pm March 13

Neelima Sinha	The Development and Evolution of Leaves
Vicki Vance	Suppression of RNA Silencing in Plants: The Role of Small Regulatory RNAs

Session 2. Gene Regulation and Genomics Friday 8:30 am March 14

1	Sidorenko, Lyudmila	Identification of cis-acting regulatory sequences and trans-acting regulatory factors are the stepping stones to the elucidation of the mechanism of p1 paramutation
2	Hernandez, Julia Marcela	Transcriptional regulation of the flavonoid biosynthetic pathway: the dual role of the HLH coactivator R/B.
3	David Galbraith	The future of maize expression arrays.
4	Seigfried, Trent	MaizeGDB: A Next Generation Maize Database
5	Barbazuk, Brad	The Maize Genome Sequencing Project at the Donald Danforth Plant Science Center
6	Whitelaw, Cathy	CONSORTIUM FOR MAIZE GENOMICS - APPROACH EVALUATION FOR TARGETED SEQUENCING OF MAIZE GENES

Session 3. Developmental Genetics Friday 10:50 am March 14

7	Chuck, George	The Control of Spikelet Meristem Development by the branched silkless1 Gene
8	Fowler, John	A Role for Maize ROP2 GTPase in the Male Gametophyte
9	Rogowsky, Peter	ZmPRPL35-1 encodes a plastid ribosomal protein required for suspensor morphogenesis in maize embryos
10	Bommert, Peter	thick tassel dwarf1 encodes a LRR-receptor kinase with high homology to CLAVATA1
11	Vollbrecht, Erik	Molecular and evolutionary analysis of *ramosa1* in inflorescence architecture

Session 4. Maize Genomics Workshop 3:30 pm Friday March 14
See posters #16-41; Genomics Resources

Session 5. Friday 7:30 pm

Steve Henikoff	Traditional Genetics Meets Functional Genomics
Susan McCouch	(Abstract not available).

Session 6. Genome Organization and Evolution 8:30 am Saturday March 15

12	Vigouroux, Yves	Population structure and gene diversity of American maize landraces
13	Brendel, Volker	The genomic origin of maize revisited
14	Sheridan, William	GLOBAL ANALYSIS OF THE MAIZE GENOME: RELATING GENES AND DNA SEQUENCES TO CHROMOSOME REGIONS
15	Smith, Shavannor	Identification and Characterization of Rp1 Genes with Novel Phenotypes in Maize
16	Jiang, Ning	Identifying active DNA transposons in the genomic era

Session 7. Transposons and Cytogenetics 10:40 am Saturday March 15

17	Meeley, Robert	Diagnosis of hot spots for Mu integration in the maize genome and their association with binding sites for host-encoded nuclear protein(s).
18	Zhang, Jianbo	Transposition of Reversed Ac Element Ends Shuffles Exons and Rearranges Chromosomes in Maize
19	Mroczek, Rebecca	Distribution of retroelements in centromeres and neocentromeres of maize
20	Stack, Stephen	Cytological crossover maps for all maize bivalents using recombination nodules
21	Cande, Zac	The pathway of early meiotic prophase events in maize

Session 8. QTL Workshop 3:30 pm Saturday March 15

W1	Torbert Rocheford	Overview
W2	Mike Lee	Map construction and use for mutant clone and QTL mapping
W3	Martin Bohn	Methodologies of QTL analysis and statistical considerations

W4	Mike McMullen	QTL Approaches to study of a pathway
W5	Nick Lauter	High Resolution Mapping and functional dissection of QTL affecting leaf epidermal traits
W6	Ed Buckler	Principles of associative genetic analysis

Session 9. 7:30 pm Saturday March 15

	Dooner, Hugo	**Convergence of Genetics and Genomics at a bronze Point in the Map**

Session 10. Biochemical and Seed Genetics. 9:00 am Sunday March 16

22	Porch, Tim	Initial cloning and characterization of vp13 in maize
23	Goodman, Christopher	A multidrug-resistance associated protein involved in anthocyanin transport in Zea mays.
24	Gallagher, Cynthia	Characterization of gene families that influence maize endosperm carotenoid content.
25	Leiva-Neto, Joao	Expression of a dominant negative mutant of cyclin-dependent kinase A (ZmCDKA) reduces DNA endoreduplication during maize endosperm development
26	Shen, Bo	The supernumerary maize aleurone layer gene superal1 encodes an orthologue of the human CHMP family member of class E vacuolar sorting proteins.
27	Lauter, Nick	High resolution mapping and functional dissection of QTL affecting macrohair density on leaf blades during shoot development

Posters

Bioinformatics

1	Buckler, Edward	THE GENOMIC DIVERSITY AND PHENOTYPE CONNECTION (GDPC): MIDDLEWARE FOR GENOMIC DIVERSITY AND PHENOTYPIC DATA
2	Costich, Denise E.	The Emerson Summer Genetics Program: Carrying on the legacy of maize cooperation through high school and undergraduate outreach
3	Garcia, Arturo	MaizeMeister: Phenotypic Data Collection and Seed Management System
4	George, Robert	Discovery and Expression Analysis of Alternatively Spliced Genes in Arabidopsis thaliana
5	Guo, Hena	Conserved Noncoding Sequence Comparisons as a Strategy to Identify Candidate Regulatory Elements in Cereal Gene Promoters
6	Joets, Johann	PROTIC : A database and web-based application to manage, analyse and web-publish plant proteome expression data.
7	Joets, Johann	ActionMap : A bioinformatic package for genetic mapping automation

8	Polacco, Mary	Maize Genome Database - Maize[G]DB -- Curation Issues – inputs and syntheses
9	Smith-White, Brian	Plant Genomic Resources at NCBI
10	Stapleton, Ann	ElucidateIt: A Bioinformatics Workflow and Analysis System
11	Tracy, Bill	Stalking an A-Maize-ing Plant. Corn In Culture And Science; an Integrated Curriculum; Science Enhancement for K-5 Teachers
12	Vincent, Leszek	Plant Ontologies and the Plant Ontology™ Consortium (POC)
13	Wang, Bingbing	Comparative analysis of splicing related proteins in plants
14	Wang, Cunxi	The maize DEK1 calpain sub-domain functions as a cystein proteinase.
15	Ware, Doreen	Comparative Physical Maps of Maize and Rice in Gramene.

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16	Barbazuk, Brad	The Maize Genome Sequencing Project at the Donald Danforth Plant Science Center
17	Bharti, Arvind K.	HIGH RESOLUTION PHYSICAL MAPPING OF THE MAIZE GENOME
18	Brutnell, Thomas	*Activator* (*Ac*) Mutagenesis in Maize
19	Butler, Ed	Finishing of the publicly-funded physical map of 'B73' at the Arizona Genomics Institute and Arizona Genomics Computational Laboratory
20	Carpita, Nicholas	FTIR and NIR spectroscopy to identify mutants in cell wall biogenesis
21	Chandler, Vicki	The Zea mays microarray resource
22	Dawe, Kelly	Functional Genomics of Maize Centromeres
23	Doebley, John	Evolutionary genomics of maize
24	Gardiner, Jack	AN INTEGRATED GENETIC AND PHYSICAL MAP FOR MAIZE
25	Lai, Jinsheng	Full-length cDNA sequencing for the functional genomics of endosperm development
26	Lai, Jinsheng	Sequence and phylogenetic analysis of the fie-orp intervals in the two subgenomes of maize with sorghum and rice as a reference
27	Latshaw, Susan	Molecular analysis of Mu-insertions derived from smk mutants.
28	Mcginnis, Karen	Functional Genomics of Chromatin Genes in Maize
29	Minx, Patrick J.	SEQUENCE COMPARISONS OF MAIZE AND RICE MITOCHONDRIAL GENOMES AND THE REARRANGEMENTS WITHIN THREE MAIZE MITOCHONDRIAL STRAINS
30	Okagaki, Ron	A RADIATION HYBRID SYSTEM FOR THE GENETIC AND

		PHYSICAL MAPPING OF THE MAIZE GENOME
31	Rabinowicz, Pablo	Methylation-Filtration results in gene enrichment in plants but not in animals because plant genes are hypomethylated and animal genes are often methylated
32	Rocheford, Torbert	Regulation of Inflorescence Architecture in Maize
33	Seigfried, Trent	MaizeGDB: A Next Generation Maize Database
34	Springer, Nathan	Applications of oligonucleotide microarrays for maize functional genomics
35	Walbot, Virginia	Maize Gene Discovery Project -- Update 2003
36	Walker, Nigel	Functional Genomics of Chloroplast Biogenesis: The Photosythetic Mutant Library.
37	Wang, Chung-Ju, Rachel	Physical localization of single copy sequences on 2-D maize pachytene chromosomes by fluorescence in situ hybridization
38	Wang, Kan	Establishment of Robust Maize Transformation Systems for the Public Sector
39	Wen, Tsui-Jung	Gene Discovery and Mapping in Maize
40	Whitelaw, Cathy	CONSORTIUM FOR MAIZE GENOMICS - APPROACH EVALUATION FOR TARGETED SEQUENCING OF MAIZE GENES
41	Yuan, Yinan	Gene Enrichment Technologies for Selective Sequence Analysis of the Maize Genome

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44	Dierking, Ryan	Comparison of cell wall proteins in drought tolerant and susceptible maize lines
45	Dinges, Jason	A Comprehensive Real-time PCR Expression Analysis of the Maize Starch Debranching Enzyme Gene Family
46	Heine, George F	Functional Significance of the Evolutionary Steps that Shaped the *R2R3 Myb* Gene Family
47	Herschberger, Nicholas	Photosynthetic Mutant Library (PML): A Reverse Genetics Resource for Chloroplast Biogenesis Genes
48	Houston, Norma	The Protein Disulfide Isomerase Multigene Family in Maize
49	James, Martha	Evidence for physical interactions among specific starch metabolizing enzymes

50	Kea, Molly	Utility of 2 Dimensional Isoelectric Focusing in Identifying Biochemical Networks.
51	Kirst, Mariana	Characterization of a putative maize ERAD protein
52	Kriechbaumer, Verena	Function of the nitrilases ZmNIT1 and ZmNIT2 of Zea mays in auxin biosynthesis
53	Licciardello, Nicholas	Color complementation in *E. coli* for the functional testing of a cDNA required for maize carotenoid biosynthesis
54	Manjunath, Siva	Enhancing Essential Amino Acids in Crop Plants
55	Rao, John	A maize ribosome-associated membrane protein is overexpressed in the floury-2 mutant
56	Sawers, Ruairidh	From Proplastid to Chloroplast: Understanding Plastid Differentiation in Maize through Microarray and Proteome Analysis (NSF Award - #0211935).
57	Schneerman, Martha	A Functional Genomics Program for the Illinois Long Term Protein Selection Strains
58	Vermerris, Wilfred	A genetic approach to dissecting the maize cell wall
59	Woodruff, Dana	Inhibition of *Aspergillus flavus* growth by genistin and diosmin
60	Wrobel, Russell	Center for Eukaryotic Stuctural Genomics: Facility for Structure Determination of Proteins from Arabidopsis thaliana and Other Model Eukaryotes.
61	Zhang, Xiaoli	The maize gene *sugary2* codes for starch synthase IIa

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63	Golubovskaya, Inna	absence of the first division1 (afd1) is a maize rec8-cohesin
64	Hiatt, Evelyn	Genetic and Cytogenetic Analysis of Abnormal Chromosome 10 Indicates that at Least Four Loci are Required for Meiotic Drive
65	Kato, Akio	Chromosome doubling in maize
66	Lamb, Jonathan	The maize B chromosome contains multiple centromeric elements located away from the functional centromere
67	Lee, Michael	Increased Meiotic Recombination in Maize Genotypes After Chronic Water-Deficit Stress
68	Pawlowski, Wojtek	'Master switches' of meiosis

69	Stack, Stephen	Cytological crossover maps for all maize bivalents using recombination nodules

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71	Barret, Pierre	Differential expression of a new HSP70-like gene and of the transposable elements Pong and PREM during in vitro androgenesis in maize NILs underlines new aspects of induction mechanisms
72	Bomblies, Kirsten	Quantitative analyses and transgenic overexpression support the maize FLORICAULA/LEAFY homologs as QTL candidates.
73	Braun, David	tie-dyed1 promotes the sink/source transition in developing leaves
74	Brooks, Lee	An analysis of CNS-lg3-i2 in non-ligule forming plants and plants with altered ligules
75	Buryak, Alla	Maize Sprouts Polarity as Factor Defining Exogenetic Phytohormones Influence
76	Cartwright, Heather	Pangloss1 and Pangloss2 are Required for Polarization of Subsidiary Mother Cells in the Formation of Maize Stomata.
77	Christophe, Reuzeau	TRAITMILL TM :AN APPLIED GENOMICS PLATFORM FOR THE IMPROVEMENT OF CEREALS.
78	Consonni, Gabriella	Maize mutants arrested in early embryogenesis disclose an irregular pattern of cell divisions and altered programmed cell death
79	Danilevskaya, Olga	Functional analysis of the maize FT/TFL homologs reveals potential players in the floral transition.
80	Deleu, Wim	Biochemical characterization of the FEA2 protein
81	Fajardo, Diego	Molecular Genetic Analysis of rgh Endosperm Mutants
82	Frank, Mary	Three Brick Genes Are Required for Polarized Growth and Division in Maize Leaf Epidermal Cells
83	Fu, Suneng	Functional analyses of EMPTY PERICARP2, an essential regulator of the heat shock response that is required for maize embyogenesis.
84	Gibbon, Bryan	The Protein Secretory Pathway is Upregulated in Several *opaque* Mutants
85	Grimanelli, Daniel	GENOMIC CLONING THROUGH APOMIXIS RESULTS IN EXTENSIVE EPIGENETIC VARIATION
86	Hake, Sarah	Clonal analysis of Wavy auricle in blade (Wab)
87	Henderson, David	ragged seedling2 fails to maintain the dorsoventral axes of leaf tissues in maize
88	Hollick, Jay	PARAMUTATION AND PLANT DEVELOPMENT REQUIRE rmr12 FUNCTIONS
89	Inada, Noriko	The characterization of pleiotropic shoot phenotype in *leafy coleoptile(lco)1-R*

90	Irish, Erin	Hypomethylation at Pl-blotched is reset by shoot meristem culture-induced rejuvenation
91	Juarez, Michelle	Inbred modifier effects on adaxial patterning of the Maize leaf
92	Kessler, Sharon	Interactions between XCL1 and KNOX genes
93	Kladnik, Ales	Spatial and temporal control of programmed cell death (PCD) in developing caryopses of maize.
94	Kladnik, Ales	Sucrose synthase isozyme SUS1 in the maize root cap is preferentially localized in the endopolyploid outer cells
95	Koch, Karen	Mu-Tagged Empty Pericarp Mutants in the UniformMu Maize Population
96	Krolikowski, Katie	The indeterminate floral apex1 (ifa1) mutant phenotype is associated with a mutation in the Zea mays MADS box 14 (ZMM14) gene.
97	Lid, Stein Erik	The maize *Disorgal 1* and *Disorgal 2* genes restrict mitotic division plane in the aleurone layer and is necessary for normal epidermal cell development and plant stature.
98	M, Kirstin	Genetic Analysis of the Maize Rop2 Gene
99	Mao, Long	Comparative Analysis of MADS-Box Sequences in Arabidopsis and Rice
100	Moose, Stephen	Developmental Analysis of Transgenic Maize Lines that Overexpress Glossy15
101	Perez, Pascual	Functional genomics of rice grain development : Establishement of a database of mutant phenotypes and enhancer trap gus expression in mature seeds.
102	Sadeghian, Nasim	extended auricle (eta), an Essential Component in the Developmental Network Controlling Maize Leaf Development
103	Sandahl, Jeanne	A transient expression system for maize silks
104	Satoh, Namiko	Developmental analysis of the ramosa3-fasciated ear 1 mutant
105	Scanlon, Mike	Inhibition of polar auxin transport disrupts KNOX protein regulation, founder cell recruitment, and elaboration of leaf margins in maize shoots.
106	Sylvester, Anne	RAB2 contributes to orderly cell division and expansion during leaf development
107	Timmermans, Marja	Adaxial/Abaxial polarity specification in Maize leaf development
108	Tracy, Bill	Vegetative Phase Change and Response to Puccinia sorghi in Sweet Corn
109	Valdivia, Elene	Role of Pollen Allergens and Beta-Expansin Zea m 1 in Pollen Development and Fertilization
110	Whipple, Clinton	Transgenic analyses of a duplicate pair of maize MADS-box genes, Zag1 and Zmm2, suggest protein subfunctionalization of the C class in the traditional ABC model of flower development.
111	Woll, Katrin	Functional analysis of the maize root specific gene ZmGrp3

112	Xu, Zhennan	The Ac-tagged aberrant pollen transmission 1 (apt1) is a homologue of the Arabidopsis gene SABRE required for root cell expansion
113	Zimmermann, Roman	NAC genes in maize: highly cell type specific expression patterns in embryogenesis

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115	Dias, Anusha	Duplication and Divergence of the *R2R3 Myb* Gene Family in the Grasses
116	Falque, Matthieu	Large-scale Maize cDNA mapping for candidate gene approach
117	Fauron, Christiane	MAIZE MITOCHONDRIAL GENOMICS
118	Freeling, Michael	Conserved Noncoding Sequences (CNSs) in Grasses
119	Hahn, Elizabeth	Sequence diversity at the y1 locus of maize.
120	January, Eboni	INTERGRATING A MAIZE PHYSICAL AND GENETIC MAP USING PCR BASED MARKERS CONVERTED FROM SINGLE COPY RFLP MARKERS ON BAC POOL DNAs
121	Kurylo, Vasyl	Hormonal, Light and Organospecific Regulation of Ribosomal Protein S14 Gene Expression
122	Li, Bailin	An integrated physical map in maize
123	Song, Rentao	Genomic regions comprising the entire alpha zein gene family of Zea mays in a single inbred line
124	Song, Rentao	Haplotypes of a genomic region containing z1C gene family in maize
125	Yim, Young-Sun	Validation of in silico connection between maize physical map and genetic map by PCR-based screening on BAC pools.

Molecular Genetics

126	Balandin, Teresa	Keeping the babies healthy. Defence mechanisms operate at the base of the endosperm in developing seeds.
127	Bicar, Earl	Expression and inheritance patterns of a modified porcine a-lactalbumin transgene in maize kernels
128	Cao, Jun	Class III Aldehyde Dehydrogenase Genes of Maize
129	Charcosset, Alain	Use of DNA pooling to assess diversity within and among maize populations. Application to the investigation of maize introduction into Europe.
130	Clay, Catherine	Certain Mutations Preventing Paramutation Heritably Reactivate a Transcriptionally Silent Transgene
131	Cordia, John	Comparative mapping of drought responsive genes in *Zea mays* L. and *Arabidopsis thaliana*

132	Costich, Denise	Screening phenotypic diversity in light-responsiveness to identify potential phytochrome mutants in maize
133	Della Vedova, Chris	Is the maize C2-Idf mutation controlled by an RNA-based silencing mechanism?
134	Donahue, Kerry	Characterization of a Peptide Transport Gene from Oryza sativa
135	Gallagher, Cynthia	One gene family that may influence maize endosperm betacarotene content.
136	Gray, John	THE LETHAL LEAF-SPOT 1 (LLS1) PROTEIN IS LOCALIZED TO CHLOROPLAST MEMBRANES AND IS HIGHLY CONSERVED IN OXYGENIC PHOTOSYNTHETIC ORGANISMS.
137	Guenther, Eric	Identification of proteins associated with aflatoxin accumulation levels by 2-dimensional gel electrophoresis
138	Guo, Mei	Allelic Variation of Gene Expression in Maize Hybrids
139	Hallar, Brittan	Cell Death in the lethal leafspot1 mutant of maize and its ortholog in sorghum, dropdead1
140	Hernandez, Julia Marcela	DISSECTION OF PLANT PROMOTER FUNCTION *IN VIVO*
141	Hueros, Gregorio	Molecular dissection of the interaction between ZmMRP-1 and the promoter of BETL-1.
142	Jia, Hongwu	Expression Analysis of Glu-1Dx5 Transgenic Corn
143	Jia, Hongwu	Microarray Analysis of Maize Opaque2 In Developing Endosperms of Eight Inbred Lines
144	Johnson, Jeremy	Evaluation of Near-Isogenic Lines for Kernel Oil Concentration QTL in Maize
145	Karen, Cone	A DNA methyltransferase mutation increases expression of the epigenetically-regulated maize gene, Pl-Blotched
146	Kolomiets, Mike	Genomics analysis of lipoxygenase gene family
147	Li, Faqiang	A maize *Y1* homolog encodes a functional phytoene synthase.
148	Li, Jin	RAD51 is required for chromosome segregation but not for chromosome pairing or cell viability in maize
149	Licciardello, Nicholas	Color complementation in *E. coli* for functional testing of a cDNA required for maize carotenoid biosynthesis
150	Marian, Calin	Isolation and characterization of a maize cDNA encoding a telomere repeat DNA oligonucleotide-binding protein
151	Moody, David	Characterization of an oligopeptide transporter
152	Murigneux, Alain	Isolation of Genes Controlling Agronomic Traits in Maize
153	Muszynski,	Ectopic knotted1 expression does not perturb gibberellin biosynthesis in

	Michael	maize.
154	Odland, Wade	Chromosomal Distribution of Maize Repetitive Sequences
155	Pan, Jean	The Effects of Host Genetic Background on Aboveground Microsymbionts: Endophytes on Smut-resistant and Smut-susceptible Corn
156	Parkinson, Susan	Rmr7 is necessary for silencing at paramutable maize loci.
157	Rafalski, Antoni	EFFECTS OF SELECTION ON SEQUENCE DIVERSITY AND LINKAGE DISEQUILIBRIUM AROUND THE MAIZE Y1 LOCUS
158	Rhee, Yong	Methylation analysis of tissue culture-induced P white cob maize mutants
159	Rich, Patrick	Transgenic Luciferase Constructs That Respond to Abiotic Stress in Maize
160	Rupe, Mary	Gene Expression in the Maize Endosperm Revealed by Genome-wide mRNA Profiling
161	Saft, Elizabeth	Expression Profiling Across Diverse Maize Germplasm
162	Sangar, Vineet	Functional analysis of a sorghum myb orthogolous transcription factor promoter in transgenic maize
163	Settles, A. Mark	A novel screen for single-locus kernel composition mutants.
164	Shou, Huixia	Assessment of transgenic maize events produced by particle bombardment or Agrobacterium-mediated transformation
165	Skibbe, Dave	Determination of the physiologically significant substrate of RF2A in fertility restoration
166	Smith, Alan	Microarray Analysis of Trichostatin A and 5-aza-2-deoxycytidine Treatments in Maize Tissue Culture
167	Smith, Brady	Molecular and genetic characterization of a 'gain of function' Mu insertion P1-wr allele
168	Soto, Jennifer	Proteins associated with *Spodoptera frugiperda* resistance in Zea mays L.
169	Stam, Maike	The molecular characterization of the sequences required for b1 paramutation and high expression
170	Svabek, Catherine	Identification and Expression of Flavonoid 3'-Hydroxylase in Maize and Sorghum
171	Torney, Francois	MAR-induced variegation
172	Wang, Kan	LOCALIZATION OF A BACTERIAL PROTEIN IN STARCH GRANULES OF TRANSGENIC MAIZE KERNELS
173	Weil, Cliff	Targeting Induced Limited Lesions IN Genomes (TILLING) for Maize: Reverse Genetic Analysis of Point Mutations
174	Whitt, Sherry	The origins of sweet corn

175	Yandeau, Marna	Genotype-specific trans-acting Factors Influence Meiotic Recombination in the 140-kb a1-sh2 Interval and Elsewhere in the Genome
176	Zhang, Feng	Molecular analysis of the maize P1-rw allele
177	Zhao, Zuo-Yu	Nutritionally Improved Transgenic Sorghum

Quantitative Traits

178	Baumgarten, Andrew	Identification of QTLs controlling Ustilago maydis resistance in two populations of recombinant inbred lines
179	Buckler, Edward	Association mapping of starch candidate genes with kernel composition and starch viscosity traits
180	Clark, Richard	A large region upstream to the teosinte branched 1 (tb1) locus was selected during maize evolution
181	Coors, James	Genetic Control of the Number of Ears per Plant and Related Morphological Traits in the Golden Glow Maize Population
182	Darrigues, Audrey	Selection for Methionine and Tryptophan Content in Maize
183	Flint-Garcia, Sherry	Candidate Gene Association of Kernel Composition in Diverse Maize Inbreds
184	Fontaine, Anne-Sophie	The genetic and molecular basis of cell wall digestibility in silage maize
185	Fu, Yibing	Whole-Genome Mapping of Maize UV Responses
186	Gerau, Michael	Identification of QTL Responsible for Root Architecture
187	Hoekenga, Owen	Identification and characterization of Al tolerance genes in the Intermated B73 x Mo17 (IBM) population by quantitative trait locus mapping
188	Jones, Mark	Searching for Virus Resistance Genes-is mdm1 a Universal Virus Resistance Gene?
189	Manicacci, Domenica	Molecular evolution of genes encoding AGPase in endosperm of Zea mays
190	Paul, Chandra	Marker Assisted Selection for Resistance to Aspergillus flavus and Aflatoxin production in Maize.
191	Pletsch-Rivera, Laura	Xenia effect on phosphorus concentration in outcrossed maize seed
192	Pressoir, Gael	The impact of farmer management practices on maize landrace genetic diversity
193	Rafalski, Antoni	Allele Frequency Changes of Oil Candidate Genes in the High Oil Lines ASK Cycle0 – Cycle 28: Selection or Drift?
194	Ream, Thomas	A test for a heritable epigenetic component of heterosis
195	Reinders, Jon	Molecular Dissection of a Stalk Quality Quantitative Trait Locus on Maize Chromosome 3
196	Romero, Susan	Diverse Maize Germplasm Phenotypes: Population Structure and

		Heterosis
197	Scott, Trisha	Determination of QTL associated with root lodging in maize
198	Thornsberry, Jeffry	Association Mapping of Phenotypic Variation in Maize Flowering Time

Transposable Elements

199	Altun, Cagla	DNA Repair Genes and Ac/Ds Transposition
200	Brutnell, Thomas	*Ac* insertional mutagenesis of the *vp7/ps1* locus of maize
201	Dong, Qunfeng	Assembly and Analysis of *RescueMu* Transposon-tagged Maize Genome Survey Sequences
202	Feschotte, Cedric	Genome-wide analysis of mariner-like transposable elements in rice reveals complex relationships with Stowaway MITEs.
203	Gupta, Smriti	Discovery of Helitron Type Transposable Elements in Maize Genome
204	Lisch, Damon	Horizontal transfer of a Mu-like element (MULE).
205	Ma, Jianxin	STRUCTURES, AGES AND CHROMOSOMAL DISTRIBUTIONS OF LTR RETROELEMENTS IN THE RICE GENOME
206	Peterson, Thomas	Nested Deletions: A new tool for plant genomics research
207	Robin, Kevin	Mechanisms of Mu inactivation in the UniformMu population
208	Rudenko, George	Identification of the Transposase Controlling the Insertional Activity of *MuDR*/*Mu* Elements
209	Slotkin, Keith	*Mu killer (Muk)* epigenetically silences the *Mutator* family of transposons
210	Zhang, Xiaoyu	Distribution and Evolution of the PIF/IS5 Transposon Superfamily and Its Association with Tourist-like MITEs

Talk Abstracts

Session 1. Thursday evening.

Vicki Vance

SUPPRESSION OF RNA SILENCING IN PLANTS: THE ROLE OF SMALL REGULATORY RNAs

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RNA silencing is a remarkable type of gene regulation based on sequence-specific targeting and degradation of RNA with homology to the dsRNA that triggers the process. The term refers to related pathways found in organisms as diverse as fungi (quelling), plants (post-transcriptional gene silencing, PTGS), protozoans, and a variety of animals including *C. elegans*, *Drosophila*, mice and humans (RNA interference, RNAi). In plants, RNA silencing may have evolved as a defense against viruses, many of which replicate via dsRNA intermediates. Consistent with this idea, a number of plant viruses encode suppressors of silencing. Here we report studies using one such suppressor of silencing, the helper component proteinase (HC-Pro) of potyviruses, as a tool to understand the mechanism of RNA silencing. We show that HC-Pro suppresses several classes of RNA silencing and establish the point in the pathway where HC-Pro functions. Our recent results indicate that HC-Pro also impacts the microRNA pathway, enhancing the accumulation of these endogenous small regulatory RNAs. We have identified several cellular proteins that interact with HC-Pro in the yeast two-hybrid system. Studies of the role of these proteins in RNA silencing are providing clues about the mechanism and regulation of the silencing pathway

Neelima Sinha

The Development and Evolution of Leaves

Neelima Sinha, Tom Goliber, Sharon Kessler, Minsung Kim, Connie Champagne, Geeta Bharathan* and Kook-Hyun Chung.

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The Class I Knotted-like homeobox (KNOX 1) genes are highly expressed in the shoot apical meristem but not expressed in the emerging leaf primordium in tobacco, maize, or Arabidopsis. In tomato, KNOX1 expression (LeT6, TKN1) is seen in the early leaf primordium (Chen et al. 1997; Hareven et al. 1996). It is worth noting that tomato has compound leaves while the other organisms thus far tested have simple leaves. We have shown that this early expression of KNOX 1 genes in the tomato leaf primordium causes it to take on a compound fate in tomato (Sinha 1997). In order to thoroughly test this hypothesis we have completed an analyses at several different phylogenetic levels.

We have mapped the trait of compound leaves on the green plant evolutionary tree to identify genera in which compound leaves arose independently. This tree includes cycads and multiple independent origins in the dicot families. We have analyzed compound leaf producing shoot apices in all these clades except the monocots and found that in all instances except one (a derived clade in the Fabaceae) compound leaves always show expression of KNOX genes (Bharathan et al., 2002). In the derived pea clade the LFY/FLO gene regulates this function of generating leaf complexity. While KNOX genes appear to be important for generating leaf complexity (except in a derived clade in the Fabaceae) we find that other genes like PHANTASTICA might play a role in determining the form of the compound leaf generated. Transgenic plants overexpressing antisense PHAN suggest that PHAN, by modulating dorsiventrality, has a role in regulating the number of leaflets and their placement in a compound leaf.

In Neobeckia aquatica, leaves with different morphologies are produced depending on environmental conditions. Simple leaves are produced under high-light terrestrial conditions while lobed and compound or highly dissected leaves are produced under low-light terrestrial and underwater conditions, respectively. Experiments in our lab show that GA can induce the production of simple leaves on plants exposed to conditions that normally induce compound leaves (and Unaconalzole can lead to an opposite effect). The expression differences between these two phenotypic states are being explored. With these experiments we hope to understand a basic problem in plant biology - why some derivatives from the shoot apical meristem are simple, while others can be compound, and how these alternate morphologies may have arisen in evolutionary time.

Friday Morning

1 Identification of cis-acting regulatory sequences and trans-acting regulatory factors are the stepping stones to the elucidation of the mechanism of p1 paramutation

Sidorenko, Lyudmila {1} Wang, Yibin {2} Peterson, Thomas {2} Chandler, Vicki {1} {1} University of Arizona {2} Iowa State University

Paramutation is an epigenetic phenomenon in which one allele heritably reduces expression of the other allele. We are using the well defined maize p1 gene as an experimental system to address questions crucial for elucidation of the mechanism of paramutation: 1) what regulatory sequences are required for paramutation and 2) what trans-acting factors are involved in maintenance and establishment of paramutation. The p1 gene is a myb-like transcriptional activator that regulates the expression of structural genes for flavonoid pigment biosynthesis and leads to accumulation of the red phobaphene pigment in floral organs of a mature plant. The active state of the paramutable P1-rr allele results in uniform red pigmentation of pericarp and cob, while paramutated state, named P1-rrí, has streaky pericarp and pink cob. Major progress has been made towards identification of the sequences involved in p1 paramutation when we showed that a 1.2 kb enhancer fragment from P1-rr induces P1-rrí paramutation when introduced as a transgene. In the absence of the inducing transgene, P1-rrí was heritable and paramutagenic to a naÔve P1-rr allele (Sidorenko and Peterson, 2001, Plant Cell:13, 319-335). Subsequent experiments established that the bulk of paramutagenic activity is localized within a 400 bp sub-fragment of the 1.2 kb enhancer. Here we will present results of ongoing transgenic experiments that aim to define paramutagenic sequences to an even smaller fragment of approximately 200 bp. As a step toward identification of trans-acting factors involved in p1 paramutation, we have begun to test whether transacting mutants that affect paramutations of other genes also affect maintenance of the P1-rrí state. Intriguingly, the results indicated that the maintenance of p1 paramutation was not disrupted in homozygous mop1 (mediator of paramutation 1) background; this is in contrast to the observation that mop1 disrupts b1 and pl1 paramutation (Dorweiler et al., 2000, Plant Cell:11, 2101-18) and similar to r1 paramutation which persisted in the presence of mop1 (Chandler and Kermicle, unpublished data). Importantly, mop1 is required to establish paramutation at b1, pl1 and r1. Experiments to test whether mop1 is required for establishment of p1 paramutation are in progress. The other two mutations tested, rmr1 and rmr2 (required for maintenance of repression 1 and 2), also had no significant affect on the maintenance of p1 paramutation and this result is different from that for b1 (Chandler, unpublished data) and pl1 (Hollick and Chandler, 2000, Genetics:157, 369-378). Based on obtained results we hypothesize that maintenance of paramutation at the b1 and pl1 loci and r1 and p1 loci differ in their requirements for transacting factors encoded by mop1, rmr1, and rmr2 genes.

2 Transcriptional regulation of the flavonoid biosynthetic pathway: the dual role of the HLH coactivator R/B.

Hernandez, J. Marcela {1} Kim, Min-Gab {2} Chandler, Vicki L. {3} Grotewold, Erich {1,2} {1} Ohio State Biochemistry Program, Ohio State University {2} Dept. of Plant Biology, Ohio State University {3} The University of Arizona Department of Plant Sciences

The control of maize flavonoid biosynthesis provides one of the best regulatory networks in plants. C1, an R2R3 Myb protein, regulates the production of anthocyanins together with the cofactor R, an HLH protein. P, another R2R3 Myb, controls the production of phlobaphenes. We have established that the regulatory specificity of C1 is provided by its interaction with R, as demonstrated by P*, a P mutant that is able to interact with R, thus enabling it to activate the anthocyanin branch of the pathway. The structural gene *a1*, which participates in the synthesis of both anthocyanins and phlobaphenes and encodes for dehydroflavonone reductase, is a target gene for C1 +R and P. The promoter region of *a1* has a modular structure with the ^{ha}PBS (-55 to -65) and the ^{la}PBS (-116 to -124) providing binding sites for P and C1, and the ARE element sitting between them (-83 to -101). Mutations in the ^{ha}PBS and ^{la}PBS cause a drastic reduction in the ability of P to activate *a1*, yet affect C1 and R activation only moderately. Mutations in the ARE drastically reduce the activation by C1+R, whereas the activation by P does not change significantly. Using P* as a tool in transient assays we have uncoupled two functions for R: 1) R directly affects the C1 protein allowing C1 to activate transcription from the ^{ha}PBS. This role of R does not require any other interactions with DNA. 2) R allows C1 and P* to activate the anthocyanin-specific genes (e.g. *bz1*). This function of R involves other cis-regulatory elements, likely to include the ARE. This dual role of R provides models to explain how R2R3 Myb proteins with very similar DNA-binding preferences control specific sets of target genes.

3

The future of maize expression arrays: what can we learn from working with *Zea* and *Arabidopsis*?

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As part of NSF Plant Genome grant DBI- 9872657 (*Maize Gene Discovery, Sequencing and Phenotypic Analysis*), we have been producing amplicon based maize microarrays for the general academic and not-for-profit community. Using local funds, we have also been producing whole genome microarrays for *Arabidopsis* based on the Operon-Qiagen 70-mer oligonucleotide set. This talk will provide an overview of the experience that we have gained in developing and using these platforms, will compare their performance to those of competing expression platforms, and will provide recommendations as to the future directions that should be taken in maize expression analysis.

4

MaizeGDB: A Next Generation Maize Database

Seigfried, Trent {1} Polacco, Mary {2} Brendel, Volker {1} Brekke, Michael {1} Campbell, Darwin {1} Dong, Qunfeng {1}

{1} Iowa State University {2} USDA-ARS and University of Missouri - Columbia

MaizeGDB (Maize Genetics/Genomics DataBase) is an on-going project initiated by the USDA-ARS. The goals of this project are (1) to build a next generation maize database providing curated and integrated data such as sequences, maps, genetic markers, phenotypes; and (2) to provide a comprehensive online workbench for maize biologists to analyze the data. Since our development work started on April 1, 2002, we have begun to integrate the two existing major maize databases, ZmDB (http://www.zmdb.iastate.edu) and MaizeDB (http://www.agron.missouri.edu). Data sets from ZmDB and MaizeDB have gone through a process of evaluation and excavation before being added into MaizeGDB's re-designed schema. We have ported MaizeDB and ZmDB data into the Oracle-based MaizeGDB. A new intuitive web interface has been developed that provides easy access to the data and related information. Analytical tools are embedded within data display to facilitate in-depth study. Other developments include protocols and standards for data sharing, i.e., XML specifications for various data types and text downloads. We are developing web-based curation tools for both designated experts and general researchers. We work closely with a nation-wide MaizeGDB Steering Committee on both the scientific and technical aspects of the database. In addition, the Steering Committee members have been serving as beta testers of MaizeGDB as well as guiding the site development to meet the needs of the maize research community. MaizeGDB is publicly available for testing at http://www.maizegdb.org.

5

The Maize Genome Sequencing Project at the Donald Danforth Plant Science Center

Barbazuk, Brad Barbazuk {1} Whitelaw, Catherine {2} Quackenbush, John {2} Bennetzen, Jeff {3} Schubert, Karel {1}

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A consortium consisting of the Donald Danforth Plant Science Center, TIGR, Purdue University and Orion Genomics has recently been awarded an NSF plant genome grant to develop and evaluate high-throughput and robust strategies to isolate and sequence maize genes. The goal of the project is to examine the maize gene space by analyzing sequence obtained from methyl-filtered libraries produced by Orion Genomics and high Cot libraries produced at Purdue University. The methyl-filtered libraries, which contain inserts composed of non-methylated maize genomic DNA, have been shown to be gene rich. High Cot selection exploits the relatively low abundance of the gene sequences, which are present in a small number of copies in the genome. Clone sequencing and sequence processing is being performed at TIGR, while the Danforth Center will provide an overall analysis of the gene hit rate/coverage of each method. A summary of the sequencing strategy, sequence processing, current progress and projected deliveries will be presented, as well as an overview of the analysis methods and current results.

6

CONSORTIUM FOR MAIZE GENOMICS - APPROACH EVALUATION FOR TARGETED SEQUENCING OF MAIZE GENES

Whitelaw, Catherine {1} Quackenbush, John {1} Schubert, Karel {2} Beachy, Roger {2} Lakey, Nathan {3} Bennetzen, Jeffrey {4}

{1} The Institute for Genomic Research {2} The Donald Danforth Plant Science Center {3} Orion Genomics {4} Purdue University

Maize is both a classical genetic model for plant research and an economically important crop; however, the size and complexity of the maize genome deem it recalcitrant to whole genome sequencing. Current estimates indicate that genes constitute a mere 15-20% of the maize genome with the remainder consisting of highly repetitive DNA. The initial objective of the Consortium for Maize Genomics is to evaluate two approaches to sequencing the maize ëgenespaceí (methylation filtration and high Cot selection) in order to provide the most rapid and cost-effective alternative to sequencing the whole genome. At TIGR, we are generating paired end sequence reads from 250,000 methylation filtered clones and 250,000 high Cot clones. The sequences are clustered and assembled, both independently and in combination, at quarterly intervals. The resulting maize genomic assemblies and singletons are annotated based on homology searches, with subsequent development of improved methods for annotation. The results of these analyses, a BLAST-searchable database and Maize Assembly Annotator are presented in the TIGR Maize Database (http://www.tigr.org/tdb/tgi/maize). The results of the latest assemblies will be presented and discussed.

Session 3. Friday 10:50 am. Developmental Genetics.

7 The Control of Spikelet Meristem Development by the branched silkless1 Gene

Chuck, George {1} Hake, Sarah {1} {1} Plant Gene Expression Center, U.S. Department of Agriculture

Most of the world's food supply is derived from cereal grains such as wheat, rice, maize, barley, and sorghum. The seeds that are eaten are born in a unique structure called the spikelet, the fundamental floral unit of all grasses. branched silkless1 (bd1) is a maize mutation that alters the identity of the spikelet, causing indeterminate branches to form instead. Double mutant combinations with Tunicate greatly enhance the bd1 mutant phenotype, suggesting a role for Tunicate in spikelet meristem development as well. We have cloned bd1 and show that it encodes an ERF transcription factor that is expressed in a distinct domain of the spikelet meristem. Its expression pattern suggests the existence of signaling pathways from lateral domains of the spikelet meristem. Putative orthologues of bd1 have been identified in several grass species with highly divergent spikelet structures such as rice and sorghum, and may provide an entry point into the study of spikelet evolution in the panicoid grasses. Interacting proteins isolated by two hybrid screens indicate that BD1 may be phosphorylated in order to function. Finally, binding site selection studies show that BD1 binds to sequences similar to the GCC box promoter element known to be the target of several ERF proteins in dicots. This information will be useful to help identify downstream genes from gene chip experiments currently in progress.

8 A Role for Maize ROP2 GTPase in the Male Gametophyte

Fowler, John {1} Arthur, Kirstin {1} Vejlupkova, Zuzana {1}
{1} Oregon State University

Rop family GTPases are signaling proteins that are implicated in the regulation of pollen and vegetative cell growth, stress responses, and pathogen resistance. We are using a genetic approach to explore the functions of the nine known *rop*s in maize. We subdivided these genes, based on phylogeny, into three groups, each originating prior to the monocot/dicot divergence and persisting in both lineages. Our survey of *rop* expression in the maize sporophyte showed significant spatial and temporal overlap of the nine *rop* transcripts. In contrast, only a subset of *rop*s (including *rop2*) was highly expressed in mature pollen. With assistance from Pioneer Hi-Bred International, Inc. and the NSF-sponsored MTM project, we isolated and characterized 18 *Mutator* insertions in five *rop*s (*rop::Mu* alleles), none of which had any obvious phenotypes in the sporophyte. However, three out of five *rop2::Mu* alleles were associated with a male-specific transmission defect, suggesting that ROP2 is important for proper function of the male gametophyte. These three alleles formed an allelic series based on each one's relative transmission rate when crossed as a trans-heterozygote. Characterization of a derivative of an original *rop2::Mu* allele, and of ROP2-mRNA levels in mutant pollen, confirmed that mutation of *rop2* caused the mutant phenotype. Interestingly, the *rop2::Mu* transmission defect was apparent only when the plant was crossed either as a heterozygote, or using a mixture of wild-type and homozygous mutant pollen. Thus, the defect resulted from a competitive advantage for wild-type pollen compared to mutant, possibly during the development of the pollen tube. Our current data suggest that the cellular basis for the transmission defect occurs very late in tube growth, or at fertilization. We conclude that the male gametophyte is very sensitive to changes in ROP2 activity *in vivo*, whereas ROP functional redundancy appears widespread in the sporophyte.

9

ZmPRPL35-1 encodes a plastid ribosomal protein required for suspensor morphogenesis in maize embryos

Magnard, Jean-Louis {1} Heckel, Thierry {1} Massonneau, AgnËs {1} Lassagne, HervÈ {2} Perez, Pascual {2} Dumas, Christian {1} Rogowsky, Peter {1}

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In embryo specific (emb) mutants of maize the two fertilisation products have opposite fates: while the endosperm develops normally, the embryo shows more or less severe aberrations in its development resulting in non-viable seed. We show here that in mutant emb*-8516 the development of mutant embryos deviates as soon as the transition stage from that of wildtype siblings. The basic events of pattern formation take place since mutant embryos display an apical-basal polarity and differentiate a protoderm. However, morphogenesis is strongly aberrant. Young mutant embryos are characterised by protuberances at their suspensor-like extremity leading eventually to structures of irregular shape and variable size. The lack of a scutellum or coleoptile attest the virtual absence of morphogenesis at the embryo proper-like extremity. Molecular cloning of the mutation was achieved based on co-segregation between the mutant phenotype and the insertion of a MuDR element. The Mu insertion is located in gene ZmPRPL35-1 likely coding for protein L35 of the large subunit of plastid ribosomes. The isolation of a second allele g2422 confirms that a lesion in ZmPRPL35-1 indeed causes an emb phenotype. ZmPRPL35-1 belongs to a gene family present at two loci on chromosome arms 6L and 9L. The gene is constitutively expressed in all major tissues of wildtype maize plants. Lack of expression in emb/emb endosperm shows that endosperm development does not require a functional copy of ZmPRPL35-1 and suggests a link between plastids and embryo specific signalling events.

10

thick tassel dwarf1 encodes a LRR-receptor kinase with high homology to CLAVATA1

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Development of the aerial parts of higher plants depends on the activity of meristems, the formative regions that continuously initiate new organs at their flanks. Morphologically and functionally, meristems can be subdivided into distinct domains, i.e. a central zone maintaining the stem cells and a peripheral zone where organs are formed. In Arabidopsis, analysis of fasciated mutants has identified some of the genes involved in keeping the balance between stem cells and cells destined for elaboration of organs. Mutations in the genes involved in the CLAVATA signalling pathway lead to increased cell numbers in meristems. Here, we report on the isolation and characterisation of a LRR-receptor kinase with high homology to the Arabidopsis CLAVATA1 protein, by using a homology-based PCR approach. Mapping of the isolated gene places it in close vicinity to the td1-locus. Td1 mutants affect male and female inflorescence meristem maintenance, displaying severely fasciated inflorescence meristems. Sequence analysis of a Mutator-tagged td1-population shows a cosegregating insertion within the td1 gene. Investigation of the td1-R and td1-nickerson alleles reveals that they are identical and that they display a deletion within the 5í-signal sequence probably resulting in false protein targeting. The hypothesis that td1 is the maize orthologue of the Arabidopsis CLAVATA1 gene gains further support by a phylogenetic analysis of related LRR receptor-like kinases, which suggests that td1 and CLV1 are the closest relatives of known proteins from maize and Arabidopsis. Analysis of the expression pattern reveals that td1 is expressed within female and male inflorescences. The recent identification of the fea2 gene product as CLAVATA2 related protein supports the notion that the CLAVATA signalling pathway is conserved in angiosperms. Additionally, double mutant analysis of fea2/td1 plants indicates that both genes act in a common pathway.

11

Molecular and evolutionary analysis of *ramosa1* in inflorescence architecture

Vollbrecht, Erik {1} Martienssen, Rob {1}

{1} Cold Spring Harbor Laboratory

Plant shoot architecture is produced by the arrangement and modulated activity of shoot apical meristems to generate characteristic branched forms. We are investigating developmental, molecular and evolutionary bases of inflorescence architecture diversity, by identifying inflorescence architecture genes in maize and extending our analysis in a broad, comparative framework to other grasses. In the domesticated cereals and other grasses, the presence or absence of long inflorescence branches defines the fundamental panicle and spike architectures, respectively. In maize this distinction is the essential architectural difference between the tassel, which bears both long and short (spikelet pair) branches, and the ear, which bears only spikelet pair branches. Mutations in the *ramosa1* (*ra1*) and *ramosa2* (*ra2*) genes transform most second-order inflorescence branches from short to long. *ra1*, which encodes a small zinc-finger protein, imposes short branch identity in the spike as branches are initiated due to its expression in a discrete patch between nascent second-order meristems and the primary inflorescence axis. The *ra1* expression pattern and mutant phenotype suggest the *ra1* gene product defines a boundary that excludes a default indeterminacy signal from second-order branches. *ra2* mutants are phenotypically similar to weak *ra1* mutants with respect to second order branching, although *ra2* also represses internode elongation. In the spike, *ra2* positively regulates *ra1* expression levels. These and genetic data place *ra2* upstream of *ra1* in a single pathway that assigns spikelet pair identity to second-order meristem meristems. To investigate the role of *ra1* in inflorescence diversification, we studied the orthologous gene from other grass species, starting with the teosintes and *Tripsacum*. Molecular population genetic tests of selection indicate that *ra1* was a target of selection during maize evolution or domestication. More broadly within panicoid grasses, we selected species in the sugar cane tribe, some 20 million years removed from maize, for comparative analysis. In *Miscanthus sinensis* inflorescence architecture develops similarly to that of normal maize and *ra1* showed similar expression dynamics. In *Sorghum bicolor* whose fully branched inflorescence resembles a *ramosa* mutant, the ortholog is rearranged and expression was barely detected by RT-PCR during long branch initiation. Moreover *Sorghum* BAC sequencing demonstrated that rice lacks a *ra1* ortholog, consistent with riceís multi-spikelet architecture. These findings suggest a general role for the *ramosa* pathway in regulating branch length across the cereals, and implicate the *ramosa* pathway in the evolution of grass inflorescence diversity.

Session 4. Friday afternoon Genomics Workshop. See Genomics resources posters, # -##.

Session 5. Friday 7:30 pm.
Susan McCouch No Abstract.

Steve Henikoff {1}
Traditional Genetics Meets Functional Genomics
Till, Bradley J. {1}Greene, Elizabeth A.{1} Ng, Pauline C. {1} Reynolds, Steven H. {1} Young, Kim {1} Codomo, Christine A. {1} Enns, Linda C. {2} Johnson, Jessica E. {2} Burtner, Chris {2} Odden, Anthony R. {1} Taylor, Nicholas E. {1} Henikoff, Jorja G. {1} Colbert, Trenton {1} McCallum, Claire M. {1} Comai, Luca {2} Henikoff, Steven {1}
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Single-nucleotide changes can be induced in practically any organism using traditional chemical mutagens that have been widely used by geneticists for several decades. We have introduced a reverse-genetic strategy we call TILLING (for Targeting Induced Local Lesions IN Genomes) based on deleterious point mutations. TILLING uses chemical mutagenesis of reference individuals and screening for point mutations in a region of interest. Because base substitutions in proteins provide allelic series, and not just knockouts, this strategy can yield refined insights into protein function.

We have developed a high-throughput TILLING method, and have demonstrated its efficacy by establishing the *Arabidopsis* TILLING Project (ATP, http://tilling.fhcrc.org:9366), which provides point mutations as a service for the general *Arabidopsis* community. In just over a year of operation, ATP delivered >1500 sequenced mutations in 160 genes. From this large dataset, we are able to draw strong inferences about the occurrence and randomness of chemically induced mutations and to confirm the robustness of high-throughput TILLING.

We have also developed software tools for identifying potentially damaging mutations that have been applied to TILLING projects, both to choose optimal sequence segments for targeting and to ascertain whether mutations that are found are likely to cause a phenotype. These same tools can be used to identify deleterious single-nucleotide polymorphisms (SNPs) in natural populations. Our high-throughput TILLING strategy has been modified for SNP discovery and screening, allowing us to screen for both induced mutations and SNPs cheaply and efficiently.

To facilitate dissemination of TILLING technology, frequent workshops are held at the ATP facility in Seattle. In addition, we have begun a maize TILLING project in collaboration with Cliff Weil and others in the maize community. We anticipate that traditional mutagenesis followed by high-throughput detection will become an important general strategy for agricultural research and crop improvement.

Session 6. Saturday 8:30 am. Genome Organization and Evolution.

12

Population structure and gene diversity of American maize landraces

Vigouroux, Yves {1} Matsuoka, Yoshihiro {2} Goodman, Major {3} Sanchez G., Jesus {4} Doebley , John {1}

{1} University of Wisconsin {2} Fukui Prefectural University {3} Universitad de Guadalajara

{4} North Carolina State University

We analyzed the population genetic structure of maize landraces by genotyping 965 individual plants with 96 microsatellites. The plant samples represent the entire set of ~300 maize landraces native to the Americas from Chile to Canada. We used phylogenetics and a model-based approach to clusters individuals. Without using prior information about the origins of the plants, we detected four main genetic clusters corresponding to landraces from North American, the highlands of Mexico, the lowlands of Mexico and South America, and the Andean mountains. The highest genetic diversity occurs in highland Mexican landraces, and there is evidence of significant reductions in diversity in the Andean and North American clusters. Diversity between groups (landraces or clusters) accounts for only 6 to 8% of the genetic variation, indicating that a large amount of diversity exists inside accessions and landraces (94 to 92%). Isolation by geographic distance appears to be the main factor underlying the historic diversification of maize. Several cases of recent movement of landraces were also detected. Using the data, we have defined core sets of accessions that capture much of the diversity in maize.

13

The genomic origin of maize revisited

Guo, Wei {1} Petrov, Dmitri {2} Brendel, Volker {1}

{1} Iowa State University {2} Stanford University

The maize genome is thought to have a tetraploid origin, however, its mode of evolution remains unclear. Gaut and Doebley have suggested a segmental allotetraploid model based on a study of synonymous substitution distances (dS) of 14 duplicated maize loci (Gaut and Doebley, 1997, PNAS 94:6809-14; GD97). Since their seminal work, more gene sequences have become available and distance estimation methods have been improved. We reexamined the GD97 dataset using modern methods of distance estimation and statistical analysis and found that the original conclusions are not supported by the results of such refined analyses. Five of the 14 duplicated loci have very high GC content at third codon positions, a bias not corrected for in the earlier used Nei-Gojobori distance estimation method (1986 Mol Biol Evol 3:418). After correction with the maximum likelihood method that takes into account transition/transversion rate bias and base/codon frequency bias (Yang & Nielsen 2000 Mol Biol Evol 17:32) the hypothesis of a bimodal dS distribution was rejected by statistical analysis. We also examined an enlarged set of 37 mRNA and 85 EST contig-derived putative maize gene pairs, and the dS distances were found to be normally distributed with a mean dS of 0.21, interpretable as a single duplication event about 16 million years ago.

14 GLOBAL ANALYSIS OF THE MAIZE GENOME: RELATING GENES AND DNA SEQUENCES TO CHROMOSOME REGIONS

Sheridan, William {1} {1} University of North Dakota

We are constructing a cytological physical map of the maize genome by creating many new compound B-A-A translocations. The B chromosomes are supernumerary chromosomes found in some populations of maize. An exchange between a B chromosome and one of the essential A chromosomes results in a B-A translocation. B-A-As are compound translocations produced by recombination between B-A translocations and A-A translocations. Because of the peculiar behavior of the B centromere, B-A (and B-A-A) pollen parents regularly generate progeny that are deficient for the translocated A regions. As a result, B-As have long been used to cytologically define genetic loci, but usually only to chromosome arm. The new B-A-A translocations will enable us to subdivide each chromosome arm into 25 to 40 regions defined cytologically by the breakpoints of the compound translocations. The Maize Mapping Project (a consortium of the U. of Missouri, U. of Arizona and U. of Georgia) aims to create a DNA physical map of the maize genome. These workers have constructed DNA libraries comprised of bacterial artificial chromosomes (BACs) containing maize DNA inserts. The BACs are being anchored to the maize molecular genetic map using unique simple sequence repeats (SSRs). This integrated genetic and DNA physical map will identify the relative positions of genes, sequences, and BAC inserts along the length of the DNA molecules. Our goal is to relate these markers and molecular constructs to small-defined segments of the cytological map for each of the chromosome arms. To this end we will use the same SSR sequences that are being employed to anchor the BACs in the Missouri-led Maize Mapping Project to screen the progeny of B-A-A crosses for polymorphisms. This will enable us to locate the SSR sequences to each of the cytologically defined regions and thereby construct a unified genomic map, which will relate the genetic maps and DNA physical map to the cytological physical map of the maize genome. We will describe the project in more detail and report on our progress in creating new B-A-A translocations that will subdivide four maize chromosome arms (1S, 5S, 6L, and 10S) into several segments.

15 Identification and Characterization of Rp1 Genes with Novel Phenotypes in Maize

Smith, Shavannor {1} Sun, Qing {1} Hulbert, Scot {1} {1} Kansas State University

The rp1 rust resistance locus of maize consists of a cluster of NBS-LRR genes. Different rp1 haplotypes can be very different structurally due to mispairing and recombination in meiosis. Different maize haplotypes may carry as few as one to more than 40 rp1 genes, most or all of which have no detectable phenotypes. The phenotypically undetectable genes may be functional but simply do not recognize any of the currently prevalent rust biotypes. We are characterizing recombinant haplotypes with novel phenotypes to identify the genes controlling the phenotypes and to characterize the types of recombination events that give rise to them. We have generated and characterized novel haplotypes with nonparental race specificities (Hrp1-DI1, DI28 and D2I). Comparisons of these haplotypes to the parental haplotypes has indicated most of the novel race specificities are due to the reassortment of the Rp1 genes into novel combinations. We have also characterized haplotypes with more unusual phenotypes. Two of these confer defense reactions to any rust isolate and confer lesion mimic phenotypes under normal growing conditions, and a third induces defense responses spontaneously (Hrp1-Kr1N). These phenotypes are controlled by genes derived from recombination events in their LRR-coding regions. We have constructed genes similar to some of these in vitro and are now testing them in transgenic plants to examine the structural basis for their novel regulation of defense responses. We are also testing them in transient transformation assays in maize and other cereals to determine the extent to which Rp1 genes can function in different taxa. The identification and characterization of genes with novel phenotypes will shed light on how complex resistance genes function and evolve.

16 Identifying active DNA transposons in the genomic era

Jiang, Ning {1} Bao, Zhirong {2} Zhang, Xiaoyu {1} Eddy, Sean {2} McCouch, Susan {3} Wessler, Susan {1}

{1} University of Georgia {2} Washington University {3} Cornell University

Transposable elements were first discovered through genetic analysis of unstable mutant alleles of several maize genes. Yearís later, molecular characterization of these mutant alleles led to the isolation of members of several active DNA transposon families including Ac/Ds, Spm/dSpm, Mutator and Dotted. We now know that although members of these families are present in all maize strains, only some strains harbor active elements. In these cases, the autonomous, transposase encoding family members are either not in the genome or they have been silenced by epigenetic mechanisms. Unlike the situation in maize, active DNA transposons had not been identified by prior genetic analyses of rice. We reasoned that this might be because rice TEs have been more effectively silenced. The availability of two draft genome sequences allowed us to test this notion and to develop a methodology to isolate active but silenced TEs from organisms where there is significant genome sequence available. Using this methodology, we isolated the first active DNA transposon family from rice, called Ping/Pong and the first active MITE called mPing. In addition to Ping/Pong, the search has identified other promising candidates that are now being tested for activity. As more maize genomic sequence becomes available, this methodology should be of great use in identifying additional active DNA transposon families in maize.

Session 7. Saturday 10:40 am. Transposons and Cytogenetics.

17 Diagnosis of hot spots for Mu integration in the maize genome and their association with binding sites for host-encoded nuclear protein(s). Abbaraju, Hari Kishan Rao {1} Melo-Oliveira, Rosana {1} Kurth, Karla {1} Aalbers, Kimberley {1} Tymeson, Mary {1} Meeley, Robert {1}

{1} Pioneer Hi-Bred International, Inc. - A DuPont Company

Cumulative data from PCR-based reverse genetics against our TUSC population of Robertson's Mutator lines reveals non-random insertion behavior, both across the genome, and within specific loci. In a significant percentage of our gene targets, so-called Mu insertion 'hot spots' are detected, typically in 5' untranslated regions (UTR) or in the proximal portions of promoter. Results suggest a clear forward bias for transposition to these sites, particularly because both somatic and germinal transpositions produce equivalent clusters of insertions. Our diagnoses are based on detailed pedigree, genetic, and molecular data for several selected hot spots, including our best-characterized region in the 5'UTR of the empty pericarp-2 (emp2) locus. The Emp2 hot spot is a collection of highly clustered somatic and germinal transpositions that appear to arise with a distinct forward bias, based on unpublished TUSC results and published work on a number of mutant alleles from the cluster (Fu et al., 2002). Coincident with this Mu integration hot spot, a 148bp fragment of the Emp2 5'UTR shows specific nuclear protein binding as detected by electrophoretic mobility shift assays (EMSA). EMSA patterns between non-Mu and Mu-active nuclear extracts from young seedling tissue are indistinguishable at this time, but the Emp2 region of interest shows a strong DNase I footprint and hypersensitive site adjacent to the sites of Mu integration. Interestingly, competitor probes from three unrelated genes Sxd1, zmLD, and zmArgC compete specifically in EMSA for nuclear protein binding at Emp2. These three genes also have strong hot-spot insertion clusters in their 5' ends, yet their primary DNA sequences share limited homology. Control fragments from either pBluescript, Glossy-8, or flanking Emp2 regions do not compete in Emp2 EMSA. EMSA assays directed against zmLD 5í DNA show nearly identical results, and yeast-1-hybrid screens are now underway to help gain insight into the identities of those proteins binding at the Emp2 5'UTR. We seek to integrate our reverse-genetics experience with how host-encoded proteins might influence insertion site preference or contribute to the overall activity of the Mu system. We postulate that at least one DNA binding protein participates as a host factor with tangible influence in marking multiple preferred sites for Mu integration. This model is a modification of the transposon 'homing' phenomenon described for engineered P elements in Drosophila (see Kassis, 2002), which we extend by invoking a range of interactions between Mu transposase proteins and host factor(s) that effect certain points of local chromatin accessibility, insulation, or nucleosome remodeling. Study of these hot spots is complementary to other approaches addressing the role of host factors and Mu transposition.

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Transposition of Reversed Ac Element Ends Shuffles Exons and Rearranges Chromosomes in Maize

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Transposons are discrete elements delineated by terminal sequences that serve as sites for recognition and cutting by an element-encoded transposase. When transposase acts upon the two ends of a single transposon, the net effect is element excision. If transposase acted upon transposon termini arranged in a reversed orientation, various chromosomal rearrangements could be produced. However, transposition involving reversed transposon termini has not been reported previously in a eukaryote. Here we show that in maize, a pair of Ac termini in reversed orientation and separated by 13 kb can undergo transposition reactions resulting in inversion, deletion, and other local rearrangements. We also identified a case of exon shuffling, in which a flanking deletion created a functional chimeric gene by fusing the 5í and 3í portions of two linked paralagous genes. In each of these cases, the rearrangement breakpoints are bounded by the characteristic footprint or target site duplications typical of Ac transposition reactions. These results show how transposition reactions involving reversed transposon ends could contribute significantly to genome evolution by generating deletions, inversions, duplications, and other rearrangements, and by creating new genes through shuffling of coding and regulatory sequences.

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Distribution of retroelements in centromeres and neocentromeres of maize

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Fluorescent in-situ hybridization was used to examine the distribution of six abundant LTR retroelements on maize pachytene chromosomes. Specifically, the Opie, Huck, Cinful-1, Prem-2, Grande, and Tekay retroelement families were examined with respect to the satellite repeats within centromeres (Cent C), knobs (180bp and TR-1 repeats), and along a portion of the abnormal 10 (Ab10) chromosome. All families were significantly underrepresented in centromeric satellite arrays, showing an average 3-fold reduction in this domain relative to euchromatin. In contrast, the retroelements CRM and Cent-A, known to interact with kinetochore protein CENH3, were readily detected within the centromere. Knobs, which show neocentromere activity and meiotic drive when abnormal chromosome 10 (Ab10) is present, also tend to exclude retroelements. An exception is Cinful-1, which is abundant in TR1 arrays. In addition, there was no evidence that Prem-2, Cinful-1 or Huck elements accumulate the portion of Ab10 that controls neocentromere activity, further suggesting that the meiotic drive system is not an unusually favorable niche for retroelements. Our data support the view that satellite arrays within centromeres and neocentromeres are under selection for functions in chromosome movement. The CR and Cinful-1 elements may have evolved mutualistic relationships with the organism, or other mechanisms to evade host defenses in these niches.

20

Cytological crossover maps for all maize bivalents using recombination nodules

Anderson, Lorinda {1} Brigham, Brian {1} Carter, Jenna {1} Hooker, Kristina {1} Lai, Ann {1} Rice, Mindy {1} Stack, Stephen {1} {1} Colorado State University

Cytological markers such as chiasmata, MLH1 foci, and recombination nodules (RNs) are useful for defining the frequency and distribution of crossovers along the length of chromosomes. Of these, RNs provide the highest resolution currently available because they are observed by electron microscopy of synaptonemal complexes (SCs) in extended pachytene chromosomes. The most useful cytological crossover maps are those in which each bivalent can be unequivocally identified and related to a specific linkage group. To date, such maps have been generated only for tomato (using RNs) and mouse (using MLH1 foci). To achieve this goal for maize, we have prepared an SC karyotype for the maize inbred line KYS, in which each SC can be identified based on its relative length and arm ratio. Each SC was related to the proper chromosome and linkage group using inversion heterozygotes. Using this karyotype, we mapped RNs on more than 2000 SCs to produce high resolution maps of RN frequency and distribution on each bivalent. The average RN frequency per bivalent is closely correlated with SC length. The crossover frequency using RNs is about 10% higher than estimated using chiasmata. While the total length of the RN map is about two-fold shorter than current linkage maps, the correlations between the RN and linkage maps are good ($r2 > 0.63$) when the cM lengths of the bivalents are compared. Each bivalent has a unique distribution of crossing over, but all bivalents share certain general characteristics such as a high frequency of distal RNs and a severe reduction of RNs at and immediately adjacent to kinetochores. The frequency of RNs at knobs is either similar to or higher than the average frequency of RNs along the SCs. These RN maps represent an independent measure of crossing over along maize bivalents and provide a means to integrate genetic linkage maps with chromosome structure.

21

The pathway of early meiotic prophase events in maize

Cande, Zac {1}

{1} University of California, Berkeley

We have made great progress towards the cytological, genetic and recently, the molecular understanding of meiotic prophase in maize. Based on analysis of the phenotypes of over 30 mutants and knowledge from other organisms, we have developed a model of early meiotic prophase events in maize and have placed genes at the stage where we think they are likely to first function. After meiocyte cell fate determination, the cell cycle is switched from mitotic to meiotic. The am1 gene controls this switch, as meiocytes of most mutant alleles of am1 go through a mitotic division or arrest in mitotic interphase. am1 meiocytes do not install RAD51 foci so we know that the meiotic cytoskeleton, chromatin structure, and recombination are controlled by am1. We have initiated cloning am1 and found it is a novel protein (see Pawlowski poster). Afd1 controls meiotic chromosome reorganization and sister chromatid cohesion (SCC). The mutant bypasses the early stages of meiotic chromosome formation blocking the installation of RAD51 foci and it is epistatic to all other meiotic mutants tested except am1 . We are in the midst of cloning afd1 and it appears to be a homologue to yeast rec8 type cohesin (see Golubovskaya poster). We believe that these two genes function before meiotic prophase, possible in pre-meiotic S phase. During meiotic prophase several important events occur; the homology search, pairing, synapsis and recombination. While these events are interrelated, we assume the search for homology is one of the earliest events of prophase. We know that both the telomere bouquet and early recombination events are required for the homology search because mutants unable to create a bouquet (pam1) or that cannot localize RAD51 to their chromosomes (afd ,phs1, segII, etc) cannot complete the homology search, and subsequently cannot synapse. In an effort to define steps in the homology search and subsequent events, we have begun to order our meiotic mutants. Phenotypic criteria were used to place the phs1, dsyCS, and segII mutants upstream relative to other desynaptic mutants such as mtm99-14, mtm99-25, mtm99-30, dsy9901, dsy1, dsy2, and as1. pam1 is placed in a pathway separate from other desynaptic mutants. Research is in progress to develop new techniques for ordering mutants and analyzing their wild type function during meiotic prophase.

Session 8. Saturday afternoon QTL workshop Torbert Rocheford.

Torbert Rocheford
Overview of Maize QTL Studies

Department of Crop Sciences, University of Illinois

The primary purpose of this community workshop is to help enable maize researchers that have not performed QTL analysis previously to begin this type of study. Another purpose of the workshop will be to address community QTL resource needs. My overview is designed to give the big picture of QTL analysis, providing a flow chart of how the different approaches and talks in the workshop are interrelated. I will discuss how QTL analysis relates to traditional maize genetic resources such as mutants and also to more contemporary genomic resources and developments. The fundamental difference between segregation for a qualitative and a quantitative trait and how we need to approach analysis of variance will be presented, but methodological detail will not be provided. Examples of candidate genes for QTL will be discussed to show how different maize genetics resources are integrated in genetic approaches to study in this arena. After each speaker there will be plenty of time for questions, and a panel discussion will convene at the end of the session. Interaction and questions on concepts and future directions and needs such as database resources are encouraged. Computers may be available to demonstrate software and websites after the session.

Mike Lee
Map Construction and Use for Mutant Clone & QTL Mapping

Iowa State University

Genetic maps have been important resources for various types of investigations including those seeking to interconnect the abundance of genotypic and phenotypic information emerging from genomics and quantitative genetics (e.g. Takahashi et al. 2001. PNAS 98:7922-27). Maize and the maize research community provide options and some infrastructure for mapping and integrating information from different sources (Casa et al. 2000. PNAS 97:10083-89). This workshop session will briefly review introductory aspects of map construction, some resources for mapping (Sharopova et al. 2002. Plant Mol. Biol. 48:463-481; http://www.maizegdb.org/) and common issues and problems encountered when relating QTL to other loci on genetic maps.

Martin Bohn

Methodologies of QTL Analysis and Statistical Considerations

Department of Crop Sciences, University of Illinois

The objective of QTL mapping is the identification of associations between Mendelian genes or molecular markers and genes that are involved in the inheritance of quantitative traits. Genotypes from segregating populations derived from bi-parental crosses are fingerprinted with molecular markers and phenotyped for quantitative characteristics. For each molecular marker, genotypes are sorted into marker classes and class means for the quantitative character are determined. A molecular marker is linked with a putative QTL, if the marker classes are significantly different. Over the last 15 years, this basic single marker QTL mapping procedure was significantly refined to increase power of QTL detection, precision of QTL localization, and to reduce the bias of QTL effect estimates. Simple Interval Mapping (SIM) uses genetic linkage map information to determine the distance between a QTL and a molecular marker. Composite Interval Mapping (CIM) approaches increase the power of QTL detection, taking into account the genetic background. Resampling procedures were implemented in the QTL software programs to obtain less biased estimates of QTL effects and the amount of genotypic variance explained by detected QTL. Relevant factors that determine the power, precision, and accuracy of QTL studies are (i) the size and type of mapping population, (ii) the number and size distribution of QTL, (iii) the heritability of the characteristic under study, (iv) marker coverage of the genome, (v) and the method used to identify QTL. How these factors influence the outcome of a QTL study will be described.

Mike McMullen: QTL Approaches to Study of a Pathway

Plant Genetics Research Unit, USDA-ARS ; University of Missouri, Columbia, MO 65211

The development of molecular markers for crop plants has enabled research on the genetic basis of quantitative traits. However, despite more than a decade of these studies, called quantitative trait locus (QTL) analyses, the molecular basis for variation in most agronomic traits is still largely unknown. Our research group has addressed this deficiency by using QTL analysis to study the role of specific genes in the flavone pathway in controlling resistance to the corn earworm in maize silks. Our results indicate: 1) The importance of transcription factors as genes underlying QTL. 2) The importance of the interconnections of biochemical pathways and substrate flow in understanding QTL effects. 3) The importance and biological bases of epistasis for QTL. For details see Proc. Natl. Acad. Sci. 1998. 95:1966-2000; Genome 2001. 44:667-676; Crop Sci. 2002. 42:1669-1678 & 1679-1687.

Nick Lauter: High Resolution Mapping and Functional Dissection of QTL Affecting Leaf Epidermal Traits

We investigate the molecular mechanisms that regulate changes in the distributions and densities of specialized epidermal features and cell types during shoot development. To complement our traditional developmental genetic analyses of *glossy15*, *dwarf1*, *macrohairless1* and *macrohairless2*, we have undertaken multiple QTL investigations to identify, map and characterize the functions of other genetic factors affecting these distributions and densities. Here we report, as *users* of the community resource, several results that demonstrate the improved resolution of map positions for QTL detected using the IBMRI resource. For example, QTL analyses of the wax and macrohair markers for vegetative phase change each detected a QTL which corresponds to *glossy15* on chromosome 9L, while analysis of macrohair density on leaf 9 detected a QTL which likely corresponds to *macrohairless1*, which maps 5cM distal to *gl15* on a non-intermated map. The increased length of the IBM genetic map allows the clear resolution of these two separate effects on macrohairs, whereas a non-intermated map would not. This improved map resolution allows more robust inferences concerning function and pleiotropic action of individual QTL when related traits are investigated. Since such inferences require further investigations, we present two strategies that employ the IBMRI resource for further experiments. Both center on the identification of IBMRI lines carrying specific combinations of QTL alleles identified in silico, which are then treated as Nearly Isogenic Lines (NILs) for breeding or used directly for molecular genetic analyses. We believe our success with using this tool to analyze epidermal traits will be generalizable, since B73 and Mo17 are phenotypically quite similar for some of the traits for which we detected numerous robust QTL

Ed Buckler: Principles of Associative Genetic Analysis

USDA-ARS, North Carolina State Univ.

Association analysis uses the rich evolutionary history of recombination events to dissect QTL, and it can provide very high resolution and examine a wide range of alleles. The basic methods will be discussed for candidate gene association analysis including germplasm selection, characterization of linkage disequilibrium and population structure, phenotypic evaluation, candidate gene sampling, and statistical analysis. The maize resources available for association studies will be discussed including germplasm and software. A few examples of maize association analysis will be shown. Finally, the strengths and weaknesses of association analysis will be highlighted, as will the complementary strengths and weaknesses of linkage QTL analysis. The merger of association and linkage QTL analyses may provide the most powerful means to dissect complex traits in maize.

Session 9 Saturday evening

Hugo Dooner

Convergence of Genetics and Genomics at a bronze Point in the Map

Waksman Institute, Rutgers University

Our lab applies the tools of classical genetics to maize genome analysis. We have used mutations in the bz gene, a recombination hotspot in the genome, to try to identify general rules for intragenic recombination in maize. In conjunction with bz mutations, we have also used the transposon Ac as a marker to characterize recombination between genes in the bz genomic region. The bz gene is part of a gene-rich island in the maize genome. Intergenic recombination in this gene island is of the same order of magnitude as recombination within bz. The high gene density of this island, which is even higher than the Arabidopsis average, may help to explain its high recombination rate. Separating the bz gene island from other gene islands on the proximal and distal sides are large nests of retrotransposons similar to those first described at Adh1. Recombination across these retrotransposon clusters is greatly reduced relative to recombination in the bz gene island, suggesting that retrotransposon nests are recombinationally inert. Analysis of the bz region in different maize inbred lines has revealed a surprisingly plastic genomic organization in maize. Not only is the location and make-up of retrotransposon clusters polymorphic among lines, the gene content of a particular region can vary as well. The discovery of this +/- form of gene variability has intriguing theoretical and practical implications. We are also taking advantage of Ac's property of inserting preferentially into genes to develop a useful resource for maize functional genomics. We have constructed a bz mutable allele with a Ds element engineered to facilitate the selection and mapping of Ds transpositions and the isolation of the DNA adjacent to the transposed element. Based on this marked bz-m allele, a set of transgenic lines is being created that will enable localized saturation mutagenesis across the entire maize genome. Our research is supported by NSF grants MCB-0212785 and DBI-0211547.

Session 10. Sunday morning.

22 Initial cloning and characterization of vp13 in maize
Porch, Tim {1} McCarty, Don {1} Settles, A. Mark {1}
{1} University of Florida, Gainesville
ABA is necessary for seed maturation and dormancy and ABA deficiency is often characterized by the formation of viviparous seed in maize. Maize viviparous mutants have provided key insights into ABA biosynthesis and sensing. Three of the 15 or more viviparous loci have been cloned and two have been found to be ABA biosynthetic enzymes, while one is crucial in ABA signaling. The viviparous13 (vp13) mutant presents a novel phenotype characterized by normal accumulation of carotenoids and a distinctive lethal phenotype at the seedling stage. Five vp13 alleles have been isolated from a number of Mutator populations and introgressed into W22. Initial measurements of endogenous ABA levels suggest that vp13 mutant seedlings have reduced ABA accumulation. In addition, vp13 mutants are sensitive to exogenous ABA. These data suggest that vp13 is a biosynthetic mutant. We utilized MuTAIL PCR to amplify Mu-flanking sequences from both mutant and normal siblings. We isolated a partial clone of the Vp13 locus by subtracting these MuTAIL products. This clone shows homology to the cnx1 genes that are involved in Moco biosynthesis. Moco biosynthetic enzymes do not cause vivipary in Arabidopsis, illustrating the distinct differences in establishing seed dormancy in maize versus dicot species.

23
A multidrug-resistance associated protein involved in anthocyanin transport in Zea mays.
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{1} Stanford University
Anthocyanin biosynthesis is one of the most well-studied enzymatic pathways in biology but little remains known about the molecular mechanisms of its final stage; the transport of the anthocyanin pigment into the vacuole. We have identified a multidrug-resistance associated protein, *ZmMRP1*, involved in this transport process. *ZmMRP1* expression is controlled by the regulators of anthocyanin biosynthesis and mirrors the expression of other anthocyanin structural genes. Localization of the ZmMRP1 protein *in vivo* shows its presence in the tonoplast, the site at which anthocyanin transport occurs. Mutants generated using antisense constructs have a distinct pigmentation phenotype in the adult plant that results from a significant reduction in anthocyanin levels but no alteration in the ratio of anthocyanin species produced. Surprisingly, no aleurone phenotype was observed in mutant plants. This appears to be due to the presence of second, highly homologous gene - *ZmMRP2* - that is also co-regulated with the anthocyanin pathway but is expressed exclusively in the aleurone. This is the first description of a plant MRP with a known endogenous substrate and, as such, provides a new model system for examining the biological and biochemical mechanisms involved in the MRP-mediated transport of plant secondary metabolites.

24 Characterization of gene families that influence maize endosperm carotenoid content.

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Carotenoids, synthesized by plants and other organisms, include over 600 structures, many of which are vital to human health. Carotenoids are essential for plant growth and development; carotenoids function as accessory pigments in photosynthesis, as photoprotectors preventing photooxidative damage, and as precursors to abscisic acid (ABA). Endosperms of major food crops, including maize, are low in carotenoid content and therefore potential targets for improvement via marker-assisted selection or transgenic metabolic engineering approaches. The biosynthetic pathway takes place on plastid membranes by nuclear-encoded enzymes that require appropriate plastid-targeting domains. The marked difference in plastid membrane architecture between endosperm and photosynthetic tissue suggests possible differences for pathway assembly depending on tissue/plastid type. As part of an ongoing effort to investigate regulation of the pathway, we are characterizing gene families and enzymes for the entire pathway, including isoprenoid precursors. We wish to determine the contribution of each family member to tissue specificity of carotenoid accumulation and more specifically to assembly of pathway enzymes with regard to plastid membrane localization. Current efforts are focused on characterization of various gene families in combination with functional testing of protein products to confirm enzyme activities. We are also attempting to associate isolated genes with known genetic loci that affect carotenoid accumulation or condition blocks in the pathway. This research was funded in part by the National Institutes of Health.

25 Expression of a dominant negative mutant of cyclin-dependent kinase A (ZmCDKA) reduces DNA endoreduplication during maize endosperm development

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The study of maize endosperm is appealing because of its importance in agriculture, and the insight it provides regarding important biological processes. During endosperm development, cells transition from a mitotic to an endoreduplication cell cycle, and this coincides with an increase in cell size and accumulation of storage proteins and starch. In this study, we generated transgenic maize plants over-expressing HA epitope-tagged maize cyclin-dependent kinase A (HA-ZmCDKA) or a dominant negative form (HA-ZmCDKA D146N) of this enzyme. Both genes were ectopically expressed using a highly active endosperm-specific promoter (27-kD gamma-zein). Anti-HA and anti-CDKA immunoblots showed high levels of transgenic protein accumulation beginning around 12 days after pollination (DAP). Histone H1 kinase assays on HA immunoprecipitates revealed the point mutation (HA-ZmCDKA D146N) completely abolished kinase activity. Additionally, a considerable reduction of p13suc1 adsorbed kinase activity was observed in HA-ZmCDKA D146N endosperms. Flow-cytometric analysis performed on developing HA-ZmCDKA D146N endosperms showed a significant reduction in DNA endoreduplication, when compared to control endosperms segregating on the same ear. By 18 DAP, the mean ploidy of HA-ZmCDKA D146N endosperms (5.9 C) was 50% of the control endosperms (11.9 C), and the maximal ploidies observed were 24C and 96C, respectively. This dramatic reduction in endopolyploidy was reflected in nuclear size, as seen in DAPI-stained endosperm sections analyzed by fluorescence microscopy. A detailed characterization of the anatomical and physiological effects created by the reduced level of endoreduplication in HA-ZmCDKA D146N endosperms will be reported.

26

The supernumerary maize aleurone layer gene superal1 encodes an orthologue of the human CHMP family member of class E vacuolar sorting proteins.

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Screening of the Pioneer TUSC Mu-collection identified the superal1-1 (sal1-1) mutant line carrying up to seven layers of aleurone cells in defective kernel endosperms, compared to one layer in wild type grains. Cloning of the superal1 gene was accomplished using Mu-tagging, and the identity of the cloned gene was confirmed by isolating an independent sal1-2 allele by reverse genetics. Homozygous sal1-2 endosperm have two to three layers of aleurone cells in normal, well filled grains. In situ hybridization experiments reveal that the superal1 gene is ubiquitously expressed in vegetative as well as in zygotic grain tissues, no difference being detected between aleurone cells and starchy endosperm cells. The Superal1 gene encodes a homologue of the human Chmp1 gene, a member of the conserved family of the class E vacuolar protein sorting genes implicated in membrane vesicle trafficking. The mammalian CHMP1 function in the pathway targeting plasma membrane receptors and ligands to lysosomes for proteolytic degradation. These data suggests a role for endosome trafficing in the developmental pathways affected by the sal1 gene, including aleurone layer formation, embryogenesis as well as vegetative leaf formation.

Poster Abstracts

Bioinformatics

1

THE GENOMIC DIVERSITY AND PHENOTYPE CONNECTION (GDPC): MIDDLEWARE FOR GENOMIC DIVERSITY AND PHENOTYPIC DATA

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Databases and software tools that integrate information on genomic diversity with phenotypic data provide an opportunity to help bridge genomics and plant breeding. GDPC provides a middleware interface that gives access to data on genomic diversity (SNPs, RFLP, AFLP, etc.) and phenotypic data that may be collected in field, genetic, or physiological experiments.
The goal of this project is to simplify access to our data (i.e. loci, taxi, experiments, phenotypes, etc.) by creating a middle layer between the software tools and the data itself. This middleware separates the tools from the data format, allowing developers to concentrate on the tools purpose. Software tools request data from the GDPC interface in a standardized format. Since the middleware manages the interface between the tools and the data format, the tools, once designed, are able to work with any number of databases. GDPC also provides a way for the database developers to write connections for their database, allowing any given database to work automatically with software tools supporting the GDPC interface. This would allow tools to take advantage of new databases (with supporting connections) without additional software development. Finally, interactions with the databases would be separated from the analysis and presentation, while allowing software tools to work with larger sets of genomic and phenotypic data.
The early release version of GDPC is available at http://www.maizegenetics.net.

2

The Emerson Summer Genetics Program: Carrying on the legacy of maize cooperation through high school and undergraduate outreach

Costich, Denise E. {1} Ahern, Kevin {1} Conrad, Liza {1} Markelz, Nicole H. {1} Sawers, Ruaridh {1} Sheehan, Moira J. {1} Singh, Manjit {1} Brutnell, Thomas P. {1}

{1} Boyce Thompson Institute for Plant Research

The Emerson Summer Genetics Program has been developed at BTI and Cornell in honor of Rollins A. Emerson, who in the early 1930's brought together a team of scientists at Cornell including Barbara McClintock, George Beadle, Charles Burnham and Marcus Rhoades. Through a constant sharing of ideas and materials, these scientists helped to lay the foundation of genetics over the course of the century. In the same spirit of community fostered by Emerson, the Emerson Summer Genetics Program aims to increase public understanding of science through the direct interaction of students, scientists and educators. An important goal of the program is to encourage scientific discourse among students, teachers, young scientists and senior researchers from the US and abroad. During the summers of 2001 and 2002, Brutnell lab members mentored a total of 18 undergraduate and high school students on the Ac Tagging and Phytochrome Projects. In addition to participating in lab and field experiments, the students also formed journal clubs and gave presentations at the Colonel's Cup Challenge, an undergraduate symposium named in honor of the founder of the Institute, Colonel William Boyce Thompson. Most of the students also gave presentations about their experiences at their home institutions. We are currently recruiting students for Summer '03.

3

MaizeMeister: Phenotypic Data Collection and Seed Management System

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Accurate and complete phenotypic data are essential to genetic analysis. Often analysis of quantitative traits becomes complicated due to large population sizes and the measurement of multiple traits. The vast amount of data required can be overwhelming. Technology can be exploited to improve the phenotypic data collection process, be it simply through better tools or information management systems.

MaizeMeister is a system with the ability to collect phenotypes quickly and accurately with little manual data entry using information-handling technologies such as bar codes, personal data assistants (PDAs), and computers.

Currently, MaizeMeister uses mostly bar codes with its data entry tools to minimize data entry errors. MaizeMeister is comprised of four components:

- The **desktop application** has an easy-to-use interface used to design field seasons, enter data via synchronization with PDAs or digital-output tools, register harvested ears, and keep a seed inventory record. All data are stored in an Access database.
- A **bar coded plant tag** with a unique bar code identification number (PlantID) is attached to each plant. The plant tag has removable sections that can be used for ear and sample collection. The bar code travels with tissue samples and harvested ears so you can track where the samples/ears came from.
- The **PDA data collection program**, PhenoData, is a PalmOS application for collecting phenotypic data in the field and requires a PDA with a built-in bar code reader. PhenoData works in conjunction with a set of data entry tools for data collection.
- Specifically designed **bar coded data entry tools** eliminate as much keyboard entry as possible.

All software and source code is freely available for download at www.maizegenetics.net

4

Discovery and Expression Analysis of Alternatively Spliced Genes in Arabidopsis thaliana

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Alternative splicing is a process by which some eukaryotic genes encode multiple transcripts via differential selection of splice sites during pre-mRNA processing. Gene regulation via alternative splicing is extensively documented in vertebrates and has been linked to important roles in cell growth and differentiation. Genome wide expression analysis of human ESTs indicates that 30-60% of human genes are alternatively spliced, suggesting this process plays an integral role in increasing the functional complexity of the human genome. The availability of the nearly complete Arabidopsis genome sequence along with a large collection of ESTs have provided a novel resource to study this process in plants from a genome wide perspective. A database of potentially alternatively spliced Arabidopsis genes discovered by computational analysis is available at the AtGDB (http://www.plantgdb.org/AtGDB/prj/ZSB03PP/alternativeSplicing/). To test the efficacy of our computer prediction, we selected 18 genes from AtGDB for experimental analysis. These genes were selected based on two criteria: (1) the encoded protein products of the genes shared significant sequence similarity to characterized genes in the database, (2) the EST predicted alternative splicing utilized an alternative donor or acceptor site in one intron. RT-PCR analysis of total RNA extracted from Arabidopsis root, shoot and flower tissues using primers flanking the alternatively spliced regions of these 18 genes provided experimental evidence of alternative splicing in 12 cases. The results of these studies and the impact of alternative splicing on plant gene expression are discussed.

5

Conserved Noncoding Sequence Comparisons as a Strategy to Identify Candidate Regulatory Elements in Cereal Gene Promoters

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The identification and analysis of conserved noncoding sequences among genes from related species is one strategy to identify candidate promoter elements that regulate gene expression. We surveyed for CNS among a large set of orthologous gene sequences from maize, rice, sorghum, wheat and barley to assess their general properties and utility in identifying promoter regulatory elements. A number of phylogenetic footprinting tools were evaluated for their ability to predict 30 known regulatory elements present in 10 maize-rice gene pairs, with the VISTA tool performing best. Comparisons of 81 maize-rice gene pairs found that maize-rice CNS are relatively short (mean of 11.9-bp) and represent a small proportion (mean 8.7%) of promoter sequence. Extension of these comparisons to 18 orthologous gene sets containing sequences from at least three cereal species showed that the frequency, length, and nucleotide substitution rates of cereal CNS are consistent known phylogenetic distances among the cereals. Our dataset was used to develop criteria to assess the statistical significance of CNS identified in cereal gene promoters, based on the frequency of conserved sequence blocks in comparisons of orthologous gene promoters relative to their occurrence in random promoter sequence comparisons. We have also initiated tests for CNS function in regulating promoter activity and will present our progress to date for CNS identified in the maize Shrunken2 gene and its rice ortholog.

6

PROTIC : A database and web-based application to manage, analyse and web-publish plant proteome expression data.

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Identification of gene involved in plant traits is a first step to understand biological processes underlying these traits. Transcriptome and proteome studies allow the analysis of gene expression in function of plant tissue, genotype, environmental condition, etc. Then correlation may be evidenced between traits and genes expression patterns. Other data about these genes such as functional annotation, co-localization with QTL, etc.. must then be taken into account to support or to invalidate these gene as candidate.
Large international effort have provided community with databases and softwares dedicated to transcriptome data mining. However, less effort has been done for proteomics. Although proteomics is far from being as automated and high-throughput as transcriptomics is, the advantages of proteomics are well recognized. Transcript and protein levels do not always correlate, and post-translational modifications can not be deduced from cDNA sequence or transcriptome analysis. To our knowledge, databases developed so far for 2-D PAGE and compliant with plant proteomics do not deal with quantitative data or relations between protein spots. Then new developments are needed to store and analyse proteome data and to compare them with transcriptome data.
In a first step, we developed the PROTeome bioinformaTICs Protics; a database and a web-based application to manage, track, query and web-publish proteomics data.
The scope of the database is to store complete sets of data generated by experiments, from experiment design description to spot identification and quantitative variations. Schema was designed mainly around 2D-gel electrophoresis, proteins spot quantification and MS identification of spot but the database may also be fed with results from other protein identification methods like Edman microsequencing.
In order to ease discovering of correlation of protein expression levels between experiments, we included in the database schema possibilities to aggregate different types of object like plant, sample, gel or spot. Then data from those groups can be easily extracted whenever needed, for instance to conduct statistical analysis. One of the important features of proteomics analysis is relations between spot, such as ìthe two spots are the same protein but in different post-translational modification stateî. These spot relations can be evidenced only by specific analysis. As this information may be very important when comparing experiments, we managed to store it in the database. This system can be extended to any type of relation that could exist between two proteins from the same or different samples. The Protic application can then automatically build relations network using transitivity. Thus if a non identified spot is related to an identified one by, for example an allelism relationship, then the first one will inherit of the identification result of the second one and so on.
Database feeding may be achieved by automated uploading of different formatted files or using web forms in case of low amount of data. Data files are either files from widely used proteomics softwares like Melanie or tabulated files from spreadsheets.
Protic interface provides users with a set of web forms to query the database and a graphical tool to query 2D-gel by clicking.
Protic is based on Oracle or Postgresql DBMS and will be freely available.

7

ActionMap : A bioinformatic package for genetic mapping automation

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Genetic linkage map computation may be achieved by using different softwares such as MapMaker (Lander, Green et al. 1987), JoinMap (Stam, 1993) or GMendel (Holloway and Knapp, 1994). We choose MapMaker because it is a free software adapted to our mapping populations and widely used by the maize genetics community. But for a high number of loci, genetic mapping remains time-consuming. Furthermore, manipulating numerous files to import data and export results may cause mistakes. Thus, automation of genetic linkage map computation is interesting to increase the throughput as well as the reliability of large mapping projects. However, building a map de novo requires too much human expertise to be easy to automate. But once a robust framework map is built, MapMaker command steps required to assign new markers to the framework are similar for each marker. It becomes then possible to automate these command steps. We thus developed ActionMap, which is made of a relational database to store raw data and mapping results, and a software that automates the assignation of markers to framework maps. The Actionmap package is used by the GÈnoplante maize high-throughput mapping project. Framework raw data of maize community mapping project will be added soon to the database. ActionMap is packaged as a web server, which may be used on line or locally. The users can then access the

database maize and mapping automation software via usual web browsers. The web interface is used to input data, to set up the automatic process, and to display and export the results.
ActionMap is a at http://moulon.inra.fr/~bioinfo. Package may be used on line or downloaded for free.

Holloway J. L. and S.J. Knapp, 1994, GMendel 3.0 Users Guide, Department of Crop and Soil Science,Oregon State University, Corvallis OR 97331, USA.
Lander E. S., Green P., et al . (1987). MAPMAKER : an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1(2) : 174-81
Stam P., 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. The Plant Journal 3: 739-744

8

Maize Genome Database - Maize[G]DB – Inputs and Synthesis.

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Curation of hypothesis-driven research and high-throughput sequencing or other projects share many aspects, but from a database perspective need to be handled differently. (Inputs 1) Reading the literature and extracting information requires expertise in genetics, biochemistry, physiology, plant development ; extensive familiarity with the organism being described; expertise in nomenclature and Ontologies with skill in handling conflicts/grey areas; skill in systematic integration. Typically databases fund such positions, rather than rely on well-intended, but time-constrained, researchers. (Inputs 2) In contrast high-throughput data is best handled by the funded project, performing necessary checks of semantic consistency, missing data and relevant validations in a collaboration with the integrating genome database, eg MaizeGDB. This takes advantage of considerable expertise on-hand for the research project while in full-tilt, without unduly burdening either the central genome database or the research project. Examples of each curation mode will be presented, both as practiced and as foreseen. (Syntheses)
Systematic integration of data permits ready generation of syntheses otherwise difficult to enable.. The IBM neighbors maps, a consensus genetic map product , grounded on the IBM high resolution genetic map will be presented.

9

Plant Genomic Resources at NCBI

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We wish to describe the plant genomic resources available from the NCBI Web site Plant Genomes Central at http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/PlantList.html. These resources are 1) classic genetic maps for corn, rice, wheat, oat, barley and soybean, 2) sequence maps for Arabidopsis thaliana and the International Rice Genome Sequencing Project, 3) text searching of any map data, 4) BLAST-mediated location of potential homologs. The goal of the current NCBI effort is to prepare a framework for those plants currently with only genetic maps but where chromosome sequence or transcriptome sequence is anticipated with the next five years.

10

ElucidateIt: A Bioinformatics Workflow and Analysis System

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Biologists now have access to vast amounts of DNA sequence and annotation data, but we do not yet have tool sets that make it easy for a novice to ask biological questions about these large sequence data sets. We are creating a visual user interface that will enable users to easily set up and answer questions like ìIs there an amino acid sequence pattern that is present in significant excess?î. This system will allow users to ask about frequency, correlation, and significance, queries typical of data mining methods, without writing programs. Analysis modules will also include clustering methods, pattern detection, and new statistical tools. Our system will be especially useful for crop plant EST collections and other frequently updated sequence sets, as complex queries and analyses can be logged and thus easily rerun when the databases are updated.

11

Stalking an A-Maize-ing Plant. Corn In Culture And Science; an Integrated Curriculum; Science Enhancement for K-5 Teachers

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The Wisconsin Teacher Enhancement Program, UW-Madison, is a national professional development program for K-14 science and health educators. Courses in the program are designed to enhance teachersí understanding of biology and provide curriculum directly applicable to the classroom. A key aspect of this program is the teaming of professors with master teachers for course development and instruction. In 1993, we developed 'Corn in Culture and Science; An Integrated Curriculum.' Usually it is taught in the summer as a one credit, one-week module. Focusing on plant biology, agriculture, and environmental science, we provide an integrated curriculum in which corn is used to teach all subjects. We focus on inquiry based science activities and provide information and curriculum for using corn in the class. We introduce 'corny' activities and strategies to challenge students in designing their own experiments and investigations using the scientific method. We provide information on growth systems for corn in the classroom. Participants gain knowledge in corn physiology, history, geography, economics, and genetics. Field trips, art activities, and projects, are a part of the course curriculum.

12

Plant Ontologies and the Plant Ontology™ Consortium (POC)

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Plant genomic databases need to accurately and consistently document features (e.g. gene products, functions, phenotypes, traits, developmental stages, anatomy [incl. 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) (www.plantontology.org) is applying and extending the Gene Ontology™ (GO) (www.geneontology.org) paradigm to knowledge domains pertinent to plant taxa. The POC aims at providing ontologies and controlled vocabularies for monocot and dicot plant taxa - currently *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: further representation of other plant taxa, numerical growth of participants, ongoing and extended collaboration with the research of the GO Consortium. 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 *Zea mays* (maize/corn) is presented. *Funded by NSF Plant Genome Grant DBI 9872655*.

13

Comparative analysis of splicing related proteins in plants

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Although the splicing machinery is generally conserved among eukaryotic organisms, plant pre-mRNA splicing has several distinct features that may correlate with plant specific splicing factors. In this study, the maize EST database was searched using 262 Arabidopsis splicing related proteins and their human homologs. A recursive BLAST strategy was employed to retrieve maize splicing related proteins. In addition, the spliced alignment program GeneSeqer was used to map the query proteins onto the rice genome to retrieve rice orthologs. For most of the query proteins, homologs in maize and rice could be identified. This fact further proves the conservation of the splicing mechanism between animals and plants. However, compared with animal homologs, plant splicing related proteins have more duplications. More than half of the splicing related proteins were duplicated. Some proteins such as SAP90 have high copy number in Arabidopsis but low copy number in maize. Interestingly, many of the duplications happened after the divergence of monocot and dicot plants, indicating that the ancestor of plants only have one copy of the duplicated gene. It seems that plants may have developed special ways to regulate splicing during their adaptation to the environment

14

The maize DEK1 calpain sub-domain functions as a cystein proteinase.

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Maintenance of aleurone cell fate depends on the activity of the Defective kernel 1 (dek1) gene, encoding a predicted 240 kDa membrane anchored protein with a carboxyl-terminus encoding a protein with high similarity to animal calpain domains II and II. In order to characterize the function of DEK1 in cell signaling, we used protein 3D modeling to show that DEK1 domain II contains a conserved catalytic site similar to animal calpains. Bacterially expressed DEK1 domain II display weak casinolytic activity that is enhanced by Ca++ , similar to domain II of animal m-calpain. DEK1 domain II & III exhibited a high level of the activity toward casein as compared to domain II alone, indicating that domain III plays important role for activation of DEK1. Mutation of Cys1796 to serine, part of the calpain cartalytic triad, led to a loss of caseinolytic activity, supporting the conclusion that the DEK1 calpain domain function as a cysteine protease. In situ hybridization analysis shows that the Dek1 transcript is present in all cell types in developing maize seeds, suggesting that the activity of the DEK1 cystein proteinase is not regulated at the transcription level. The potential role of DEK1 in aleurone signaling will be discussed.

15

Comparative Physical Maps of Maize and Rice in Gramene.

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{1}Cold Spring Harbor Laboratory

Gramene (www.gramene.org) is a comparative mapping database for rice and other cereals. The public resource leverages the rice genomic sequence to allow researchers working in maize and other monocots to apply knowledge derived from the study of the rice genome to corresponding syntenic region in their species of interest. In this abstract we present preliminary results of our comparative physical maps of maize and rice based upon finger print contig (FPC) physical maps of maize and rice available from the Arizona Genomic Institute and the MMP project, and from the public rice sequence draft available from the International Rice Genome Sequencing project (IRGSP). The analysis and displays are based upon the use of the rice genome as a scaffold to align maize sequences used for genetic and physical mapping studies. Using our mapping protocol (http://www.gramene.org/documentation/) 61% of the maize sequences were aligned, with 86% aligning to single rice BACs or PACs. Of the sequences aligned, 98.8% of the sequences fall within a single rice FPC contig. Of the cornsensus clones mapped to the rice genome over 5,000 have been hybridized to BACs used in the maize FPC maps as overgo probes, providing reference points to the rice genome. The sequence-based similarities become map-to-map correspondences within Gramene's comparative map viewer CMap, an open source application available at www.gmod.org. CMap dynamically generates maps based upon correspondences, in this case, to the expressed maize genes. The CMap displays are interactive, and provide links to known maize genes, in addition to rice sequence annotation available for the region of similarity. We welcome suggestions from the maize community on innovative displays that will provide maize researchers with the functionality to mine the rice genome for gene sequences of agronomic importance.

Genome Resources

16

The Maize Genome Sequencing Project at the Donald Danforth Plant Science Center

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Sequencing the maize genome will greatly influence our understanding of the molecular basis of important agronomic traits, gene regulation, transposable element function, genome evolution, plant development and biology. However, the large size of the maize genome and the expectation that upwards of 80 % of the genome is represented by repetitive elements has prompted the examination of sequencing technologies expected to target gene rich regions as an alternative to whole genome sequencing. A consortium consisting of the Donald Danforth Plant Science Center, TIGR, Purdue University and Orion Genomics has been awarded an NSF plant genome grant to develop and evaluate high-throughput and robust strategies to isolate and sequence maize genes. The project objectives are to examine the maize gene space by analyzing sequence obtained from a methyl-filtered

subclone library produced by Orion, and one high Cot library produced by Purdue University. The methyl-filtered libraries are clones with inserts composed of non-methylated maize genomic DNA. Methyl-filtered libraries have been demonstrated to be gene rich. High Cot selection exploits the relatively low abundance of the gene sequences, which are present in a small number of copies in the genome. Clone sequencing and sequence processing is being performed at TIGR, while the Danforth Center will provide an overall analysis of the gene hit rate/coverage of each method. A detailed overview of the projectís methods and analysis goals, as well as current progress and results of the analysis to date will be presented.

17

HIGH RESOLUTION PHYSICAL MAPPING OF THE MAIZE GENOME

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Maize is one of the most important crops worldwide. Its genome is composed of 2,500 Mb of DNA spread over 10 chromosomes. An effort has been funded through NSF (The National Science Foundation) to develop resources and infrastructure for sequencing the maize genome (http://mips.gsf.de/proj/maize), which would provide invaluable data for refining molecular breeding strategies. A pre-requisite to genome sequencing is a high-resolution genetic and physical map. The HindIII (ZMMBBb), EcoRI and MboI (ZMMBBc) BAC libraries (constructed at CUGI and CHORI, respectively) of Zea mays ssp. mays cv B73 form the main basis for the maize physical map. Together they consist of 449,989 clones (average insert size 155 kb). As of now, >256,000 BACs have been fingerprinted by HindIII digest and agarose gel electrophoresis at AGI (http://www.genome.arizona.edu/fpc/maize) into 4,518 contigs. In an effort to further bring down this number, a high-resolution fluorescent fingerprinting method capable of identifying smaller overlaps is being carried out. To have a deeper coverage (28X), all 449,989 BACs are being subjected to HICF (High-Information-Content Fingerprinting) using type IIS restriction enzyme and resolved on ABI 3700 capillary sequencers. The fingerprints would be assembled using FPC. It is expected that resolution of the HICF-generated contigs would be high enough to select the entire Minimum Tiling Path (MTP) at once as opposed to the clone-by-clone approach. To evaluate the MTP generated by HICF, 14 BACs would be sequenced from each chromosome. In addition, end sequencing would be carried out with more than half of the BACs from both ends. These STCs (Sequence Tagged Connectors) would serve as an in-silico mapping tool. Collinearity of STCs with the rice genomic sequence can also be used to order FPCs on the genetic map. Furthermore the library of sequence repeats thereby created would provide invaluable data on the sequence organization of the maize genome.

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***Activator* (*Ac*) Mutagenesis in Maize**

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The utility of *Ac* as a mutagenesis tool has been hindered by the propensity of *Ac* to move to linked sites and the limited number of *Ac* donor sites in the maize genome. As part of an NSF-funded Plant Genome Program 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 near-isogenic W22 lines, each containing a single *Ac* element at approximately 20 cM intervals. In the initial phase of this project, approximately 200 unlinked transpositions were generated from two separate locations in the genome. Several methods are being employed to facilitate the cloning of sequences flanking these *Ac* insertions. We are examining the efficiency of both short-range and long-range inverse-PCR protocols to amplify *Ac*-flanking sequences from 300bp to 8 kb away from the site of *Ac* insertion. To date, 53 anchor *Ac* insertions have been cloned and verified as active and 41 *Ac* insertions have been mapped. *Ac* elements have been placed on each of the 10 maize chromosomes and on most chromosome arms. In the second phase of this project, approximately 1800 transpositions have been generated from anchor *Ac*'s positioned on 8 of the maize chromosomes. We have initiated two-point testcross linkage analysis to map approximately 1000 of these transpositions relative to the donor *Ac's*. Insertions that fall within 10 to 30 cM of the donor *Ac* will be further characterized. A subset of these insertion sites will be cloned and placed on the IBM94 map. Together, these lines and methodologies to rapidly sequence *Ac* insertion sites will provide unique and valuable resources to the maize genetics community.

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Finishing of the publicly-funded physical map of ëB73í at the Arizona Genomics Institute and Arizona Genomics Computational Laboratory

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As a member of the Maize Mapping Project (www.maizemap.org), we are nearing completion of a physical map for maize, constructed by FPC assembly of DNA fingerprints from HindIII, EcoRI and MboI BAC libraries of the inbred line B73. More than 291,000 BAC clones have been fingerprinted by HindIII digestion and agarose gel electrophoresis. The resulting assembly provides a15x coverage of the maize genome. Over 13,600 molecular markers have been placed onto the map by hybridization. Manual editing of the map and contig merging are in progress and expected to be completed in late spring of 2003. The unedited assembly can be viewed via WebFPC at www.genome.arizona.edu/fpc/maize; the data set and FPC may be downloaded, as well. Approximate chromosomal locations of genetic markers and contigs may be viewed at this site using the WebChrom tool. In addition to the production of a high resolution map of B73 based upon agarose fingerprinting, AGI is also collaborating in a re-analysis of the same BAC libraries using capillary electrophoresis-based high information content fingerprinting (HICF). This re-analysis is part of an NSF-supported project to increase the resolution of the maize physical map and create an enhanced sequence-ready platform for maize. The BAC libraries (ZMMBBb and ZMMBBc) may be obtained from Clemson University Genomics Institute (http://www.genome.clemson.edu/orders/). This work was supported by NSF award #9872655

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FTIR and NIR spectroscopy to identify mutants in cell wall biogenesis

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The plant cell wall is of major importance because of its role in providing support to tissues, defense against pests and pathogens, and as a source of food, fibers, and fuels. Plant cell walls are composed of independent but interacting networks of carbohydrates, proteins, and aromatic substances. Interacting with this complex matrix are several hundred enzymes and other proteins that carry out many functions, including wall assembly and disassembly. As part of a multi-institutional project funded by the NSF Plant Genome Program (No. 0217552) we are targeting genes involved in cell wall biogenesis in both maize and Arabidopsis, two species that represent very different types of cell wall. In maize, we are screening the UniformMu transposon-tagging population . The UniformMu families share a common genetic background (W22) and genetic mechanism for Mu inactivation. We are screening coleoptiles using Fourier-transform infrared (FTIR) spectroscopy to identify changes in the primary cell wall. Near-infrared (NIR) spectroscopy is used to screen field-grown plants to identify mutants affected in primary and secondary cell wall composition. Putative mutants are self-pollinated and backcrossed to W22. After the heritability of the mutation has been confirmed, the mutated gene will be cloned using the Mu-TAIL protocol. In parallel, a reverse genetics approach is implemented in order to observe the mutant phenotype resulting from insertional mutagenesis in genes that impact cell wall biogenesis. Confirmed mutants will be subjected to detailed chemical and cytological analyses in order to define the function of the targeted genes. Resulting data will be available on the following web site: http://cellwall.genomics.purdue.edu These combined efforts will eventually result in a detailed picture of the many aspects of cell wall biogenesis. This in turn is expected to contribute significantly to the society at large and agriculture in particular.

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The Zea mays microarray resource

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At the University of Arizona, we have been providing a microarray resource for the academic and not-for-profit community for the least three years, as part of the NSF Plant Genome project entitled: Maize Gene Discovery, Sequencing and Phenotypic Analysis (Virginia Walbot, P.I.). The microarrays comprise DNA elements that are robotically deposited onto glass microscope slides. The elements are produced as amplicons from cDNA clones, previously sequenced at Stanford University, using universal primers. To date, a total of 128,787 ESTs have been sequenced, from 20 different libraries. Sequence analysis, done at Iowa State University (Volker Brendel), has allowed contig identification and assembly, and the definition of a Unigene set. We have provided microarrays for

four of the individual libraries (endosperm, ear tissue, root, and leaf primordia) as well as for the unigene set (~18,000 unigenes), which has been released in subsets over the last year. To date over 1,100 microarrays have been distributed to 80 researchers world-wide at a discounted cost relative to production. This project is funded through September 2003. Immediate future plans are amplification of another 7,000 ESTs generating a total of 25,000 amplified unigenes and consolidation of these onto 1 slide, which will be offered at $100/slide. Details on the performance of the slides and the project timetable will be presented on the poster.

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Functional Genomics of Maize Centromeres

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The poster will describe results from our collaborative work to better understand maize centromeres. It will be broken down as follows: 1) Objective: To sequence 300 kb of maize centromeric DNA from the A centromeres, and develop a database of repetitive DNA elements associated with maize centromeres. Status: The sequencing is complete and provide further support for the idea that centromeres are composed of satellite repeats and retrotransposons, many of which are centromere-specific. 2) Objective: To fully sequence the centromeres from two derivatives of the maize B chromosome that have suffered a series of centromere deletions. Status: We have constructred libraries and carried out about half of the sequencing necessary. Fiber-FISH approaches are helping in this effort. 3) Objective: Identify putative functional repeats by chromatin immunoprecipitation using antibodies to maize CENH3 (Centromeric Histone 3). Status: We demonstrated that CentC and the centromeric retrotranspson CRM interact strongly with CENH3, providing the first evidence in plants that centromere-localized DNAs are indeed functional components of the centromere/kinetochore complex. 4) Objective: Transform maize with promising centromeric BACs to determine whether they organize a kinetochore and generate chromosome movement. We hope to use the information to develop first-generation artificial chromosomes. Status: Transformation efforts with maize have been slow. We have turned to rice transformation to speed the process, and have now successfully incorporated rice centromeric DNA into rice with very encouraging results.

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Evolutionary genomics of maize

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The complete sequence of crop genomes or EST libraries for a crop species is changing the landscape in which plant breeders, biotechnologists, and biologists operate. Among the wealth of new opportunities will be one to exploit genomic sequence data to better utilize naturally occurring variation for important traits in crops. As new genes of agronomic importance are identified, we need to identify the amount, distribution and nature of functional variation in these genes that exist in the germplasm pools of crop species. Our project is creating the necessary infrastructure to do this in maize. We will define where in the maize germplasm pool and where in the maize genome useful variation is most apt to be found. We are examining nucleotide diversity in a set of candidate genes for agronomic traits and testing whether specific DNA sequence polymorphisms can be associated with phenotypic variation for these traits. Our overall goals are to better understand the distribution of genetic diversity within the maize genome and to facilitate the identification of polymorphisms at the nucleic acid level in candidate genes that control variation at the phenotypic level in agronomic traits.

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AN INTEGRATED GENETIC AND PHYSICAL MAP FOR MAIZE

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The goals of the Maize Mapping Project (MMP) are to develop genetic, physical, and database resources for the maize research community. The centerpiece of this effort is a comprehensively integrated genetic and physical map. To develop a deep-coverage physical map, 460,000 B73 BACs from HindIII, EcoRI, and MboI libraries have been fingerprinted to generate approximately 300,000 usable HindIII fingerprints that have been assembled

into contigs using FPC. The final FPC build, producing 4518 contigs, has been completed, and manual editing is underway. Manual editing is expected to reduce the number of contigs further. Concurrently, a high-resolution genetic map containing over 1800 RFLP and SSR markers has been generated. Currently, the task at hand is to integrate these two maps using hybridization, PCR, and in silico approaches. Over 9300 EST unigenes have been anchored to the physical map via 40 bp overgos, which are gene-specific hybridization probes. Selective SNP development and genetic mapping is focused on EST unigenes, which will allow unambiguous anchoring of contigs in a maximally efficient manner. BAC pools are being utilized to allow genetically mapped SSRs to be anchored to the BAC physical map. In silico approaches have been fruitful in identifying previously mapped SSRs and RFLPs in the EST unigene set that have been anchored to the BAC physical map via overgo probes. See http://www.maizemap.org/ and http://genome.arizona.edu/fpc/maize/

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Full-length cDNA sequencing for the functional genomics of endosperm development

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Maize (Zea mays L.) is one of the most important cereal crops in the world. Central to this productivity is the endosperm of the maize kernel. Endosperm not only makes up 85% of the seed weight, but also is a unique triploid tissue that results from double fertilization. To identify specifically the genes involved in endosperm development, we made cDNA libraries from tissue of a specific genetic background that has been micro-dissected from different developmental stages, 4 to 23 days after pollination. The tissue is derived from the W22 inbred used to generate UniformMu population so that tagged genes can be linked to cDNA sequences. The libraries have been normalized by hybridizing the EST colonies with two probes 1) cDNA prepared from endosperm mRNA and 2) a pool of already sequenced cDNA clones. In the first batch, both 3' and 5' ends of about 8,000 clones have been sequenced. Assembling the sequences from the 3' end showed a redundancy of roughly 50%. Out of 3,861 tentative unigenes from the first batch, we identified 1,378 (35%) unique sequences that are not yet present in the maize EST database. Initial assembly of the 5' and 3' end sequences showed that over 50% of the clones are longer than 1.5 kb. Comparative analysis is being used to identify full length cDNAís and to estimate remaining gap sizes.

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Sequence and phylogenetic analysis of the fie-orp intervals in the two subgenomes of maize with sorghum and rice as a reference

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We have used chromosome walking to connect two closely mapped loci in maize based on deep-coverage BAC libraries of maize inbred B73. Both loci are duplicate factors and known as fie1/fie2 and orp1/orp2. The two intervals are on chromosome 4 and 10 and have a length of 358 kb and 285 kb, respectively. Although these intervals have arisen from tretraploidization, their sequences differ significantly. As a reference we isolated and sequenced the same interval from sorghum (220 kb); the interval from rice (136kb) was available from the International Rice Genome Sequencing Project. Changes in these intervals affect gene density, gene content, gene order, gene orientation, and retrotransposon density. There appears to be greater gene conservation of the same interval of maize chromosome10, sorghum, and rice than maize chromosome 4. Relative to maize chromosome 4, the other intervals not only have a dramatically lower retrotransposon density and additional genes but some of the genes are also amplified. Furthermore, there are also non-collinear genes in the same interval of maize chromosome 10, sorghum, and rice. An interesting aspect is that the fie homolog is tandemly duplicated in sorghum and rice, but their upstream copies are conserved on maize chromosome 10, while the downstream copies are conserved on maize chromosome 4. Phylogenetic analysis of the duplicate factors in maize and their counterparts in sorghum and rice provide new insights in the evolution of the maize genome.

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Molecular analysis of Mu-insertions derived from smk mutants.

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The Robertsonís Mutator transposon system is a powerful tool for functional genomics. Mu elements preferentially insert in low-copy regions of the maize genome and transposon activity can be controlled genetically. The UniformMu transposon-tagging population was created by introgressing Robertsonís Mutator into color-converted W22. By purging visible seed mutations from the Mu-active parents in each generation, we ensured independence of the large collection of seed mutants generated by this population. For this report, fourteen smk mutants from the fourth and fifth backcross generations were selected for molecular analysis. MuTAIL-PCR products were amplified, and these flanking sequences were cloned to generate 14 microlibraries for single-pass sequencing. We describe the population of Mu-flanking sequences recovered from these lines and present progress towards identifying candidate genes for the smk loci. In addition to aiding identification of mutant genes, our database of sequence-tagged insertion mutations provides an important reverse genetics resource. These sequences will be available in the microlibrary database at www.endosperm.org.

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Functional Genomics of Chromatin Genes in Maize

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Chromatin level control of gene expression is an important regulatory component for growth and development in all living organisms. To better understand this regulation in plants, we are studying the function of the entire known complement of chromatin proteins in maize. The goal of the NSF-funded Plant Genome Research Project on Functional Genomics of Plant Chromatin Genes is to provide information and tools to the research community to enhance the study of the role of chromatin structure and modification. Using known chromatin genes as queries we have searched maize ESTs, identifying to date 239 genes that have been assigned to 42 functional classes on the basis of homology or domain structure. With the ultimate goal of acquiring additional mRNA sequence for each targeted gene, EST clones were obtained and sequenced, and, in some cases, RACE has been performed. Additional sequence is being submitted to Genbank and is posted on the project website (http://chromdb.org). For three functional classes, the additional sequence information has been utilized to analyze the phylogenetic relationships of these genes in maize, Arabidopsis and other model organisms. The chromosome location of each gene is being mapped relative to known markers in the maize genome. Constructs have been made to target 128 chromatin genes for silencing using a plasmid that will generate dsRNA, which is expected to interfere with the expression of the target homologous mRNA. These constructs have been introduced into HiII embryo tissue using microprojectile bombardment, herbicide selection of callus, and regeneration. The resulting dominant negative mutants are being assayed to confirm silencing of the targeted gene, and to determine if expression of the dsRNA targeted to chromatin genes impacts other epigenetic phenomena, such as DNA methylation, epimutations, paramutation and transgene silencing. Gene sequences, phylogenetic trees, mapping information, assay data and dominant negative mutants will be made available to the research community through the project website (http://chromdb.org). This poster presents an update on the progress of this project.

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SEQUENCE COMPARISONS OF MAIZE AND RICE MITOCHONDRIAL GENOMES AND THE REARRANGEMENTS WITHIN THREE MAIZE MITOCHONDRIAL STRAINS

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To date very few plant mitochondrial genomes have been completely sequenced. Those sequenced include a liverwort, Marchantia polymorpha, and two dicots, Arabidopsis thaliana and Beta vulgaris. Now the mitochondrial genomes of two monocots, Zea mays and Oryza sativa have been sequenced. The sequencing of maize includes three mitochondrial strains: fertile strains NB (B37) and NA (A188), as well as cytoplasmic male sterile strains CMS-C. The strains provide the first analysis of gene rearrangements in closely-related plant mitochondria. Sequences of maize strains are compared and illustrated to reveal major rearrangements utilizing known genes as markers. Gross rearrangements occur between maize strains, but sequences are otherwise highly conserved. Sequence homologies within maize mitochondrial genomes and rice are also examined. The major duplications within each genome are presented.

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A RADIATION HYBRID SYSTEM FOR THE GENETIC AND PHYSICAL MAPPING OF THE MAIZE GENOME

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The goal of our project is to generate and use chromosome-specific radiation hybrid (RH) maps with high physical resolution for each of the ten different maize chromosomes. We are developing the RH maps from DNA panels of oat plants that carry single maize chromosome segments in the form of added maize deletion chromosomes or oat-maize translocations. The maize chromosome aberrations are generated by gamma-irradiating monosomic oat-maize chromosome addition seeds. Monosomic additions are produced by backcrossing specific disomic oat-maize chromosome addition plants to their parental oats. From a large number of oat x maize crosses, fertile disomic oat-maize addition lines have been recovered for all maize chromosomes with the exception of chromosome 10. A ditelosomic for the short arm of chromosome 10 has recently been recovered. To date, we are finalizing RH low-resolution panels allowing marker assignment to one of 10 to 20 segments along each of maize chromosomes 2, 4 and 9. Seed and DNA is being produced for distribution. Such panels serve high-throughput mapping of large marker numbers. We are beginning studies with the 30 and 50 additional chromosome 2 and chromosome 9 RH lines available to determine the eventual number of RH lines to produce a high resolution, 5 Mbp or less, panel. A panel of five RH lines has been produced that divides chromosome 6 into five segments for low resolution mapping; additional lines are being isolated. Induced chromosome breaks in maize chromosome 3 RH lines tend to occur in a small genetic region that represents approximately half of the short arm. This material is based upon work supported by the National Science Foundation under Grant No. 0110134. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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Methylation-Filtration results in gene enrichment in plants but not in animals because plant genes are hypomethylated and animal genes are often methylated

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The maize and other crop genomes are much larger and repetitive than those of rice and Arabidopsis, which have been fully sequenced. For this reason full sequencing of complex genomes is currently unaffordable and alternative approaches are being developed to sequence and map their genic fraction or gene space. One of these approaches, called Methylation-Filtration, has been developed in our lab and due to its preliminary success, Methylation-Filtration became one of the two technologies selected to carry out a large scale NSF-funded, gene-targeted maize genomic sequencing project. The technology is based on the fact that the repetitive (intergenic) DNA is methylated in plants, while low copy DNA (i.e. genes) is hypomethylated. This differential methylation can be used to select genes using E. coli methylation-restriction systems, in particular McrBC. It has been reported that genes can also be methylated. Such genes would be excluded from methylation-filtered libraries. However, although gene methylation is frequent in animals, the methylation pattern of few genes has been studied in plants and methylation was usually found towards the ends of the genes. Using McrPCR, a new approach to study DNA methylation, we showed that most exons are unmethylated in plants while most of animal exons are methylated. These results show that most plant genes can be isolated using Methylation-Filtration. Furthermore, they support the idea that methylation is involved in the control of transposon activity in plants while in animals may be related to other biological functions.

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Regulation of Inflorescence Architecture in Maize

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Our project is focused on identifying genes that regulate tassel and ear inflorescence architecture in maize. We hope to use these genes to understand the same developmental processes in other grasses. A number of different approaches are being employed to find these genes and determine their function. EMS mutagenized populations have been made in two different inbreds and are being screened. Populations made by other NSF funded projects, such as RescueMu and UniformMu, are being examined for new inflorescence mutations. We are identifying QTL that regulate inflorescence architecture in mapping populations and relating QTL regions to map position of known mutants to identify candidate genes. We have initiated collaborative associative genetic studies for some candidate genes by evaluating allelic variation in a diverse set of inbreds. These inbreds are being studied by Major Goodman and Ed Buckler as part of the NSF funded Evolutionary Genomics project. cDNA libraries enriched in genes expressed at early stages of inflorescence development have been prepared and subtracted. These genes have been arrayed with the Maize Gene Discovery project for expression analysis. We have also synthesized gene specific oligos for spotting on slides and are in the process of testing this method for expression analysis. We are identifying genes in other grasses that are orthologous to genes whose function that have already been characterized in maize.

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MaizeGDB: A Next Generation Maize Database

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MaizeGDB (Maize Genetics/Genomics DataBase) is an on-going project initiated by the USDA-ARS. The goals of this project are (1) to build a next generation maize database providing curated and integrated data such as sequences, maps, genetic markers, phenotypes; and (2) to provide a comprehensive online workbench for maize biologists to analyze the data. Since our development work started on April 1, 2002, we have begun to integrate the two existing major maize databases, ZmDB (http://www.zmdb.iastate.edu) and MaizeDB (http://www.agron.missouri.edu). Data sets from ZmDB and MaizeDB have gone through a process of evaluation and excavation before being added into MaizeGDB's re-designed schema. We have ported MaizeDB and ZmDB data into the Oracle-based MaizeGDB. A new intuitive web interface has been developed that provides easy access to the data and related information. Analytical tools are embedded within data display to facilitate in-depth study. Other developments include protocols and standards for data sharing, i.e., XML specifications for various data types and text downloads. We are developing web-based curation tools for both designated experts and general researchers. We work closely with a nation-wide MaizeGDB Steering Committee on both the scientific and technical aspects of the database. In addition, the Steering Committee members have been serving as beta testers of MaizeGDB as well as guiding the site development to meet the needs of the maize research community. MaizeGDB is publicly available for testing at http://www.maizegdb.org.

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Applications of oligonucleotide microarrays for maize functional genomics

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Two prototype oligonucleotide microarrays have been synthesized using a NimbleGen maskless array synthesizer (MAS). The first array was designed to test the utility of oligonucleotide microarrays for SNP detection in maize mRNA or genomic DNA samples. We report the effects of oligonucleotide length, position of the SNP within the oligonucleotide, and nucleic acid source on signal intensity and on the level of discrimination between SNPs. The second prototype array was designed to test the utility of oligonucleotide microarrays for expression analysis in maize. Specifically, we designed this array to gain an understanding of the ability to separate the expression pattern of closely related genes and to compare the results with those obtained using cDNA spotted arrays. Preliminary analysis of the results obtained using both prototype arrays will be presented along with a discussion of potential future applications of oligonucleotide microarrays such as EMS mutation detection and custom-made expression arrays.

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Maize Gene Discovery Project -- Update 2003

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Assembly of maize ESTs from the MGDP and other sources at ZmDB (zmdb.iastate.edu) defines ~32,000 genes, and most are in contigs. There is nearly complete coverage of the maize life cycle of maize. We have started 5' and 3' end sequencing from a commercially prepared, normalized 'full length' library with diverse tissue sources; the Gateway compatible vector permits later manipulations without restriction enzymes. Primer walking will be used to close a subset of the cDNAs. cDNA microarrays using collections from four libraries (immature ear, seedling root, leaf primordia, endosperm) and Unigenes (eventually totaling ~25,000 cDNAs) can be ordered through ZmDB. A second aspect of functional genomics is recovery of mutants and RescueMu insertion alleles. Grids of up to 48 rows x 48 columns are used for Mu tagging and construction of individual row and column E. coli libraries of RescueMu insertions in library plates. These plates are screened by PCR to identify which plants have insertions into genes of interest; order the corresponding seed from the Maize Coop. We have sequenced either the rows or column libraries of Grids G, H, I, K, M, P, and S with AA and Q partway completed, identifying thousands of likely germinal insertions (project analysis picked 25 'putatives' and all were transmitted to the next generation). >30 MB of genomic sequence has been coassembled with ESTs showing that 2/3 of likely germinal RescueMu sites match a maize EST; this confirms the strong preference of Mu insertion into or near genes. Most mutations in the selfed progeny of tagging grids are caused by standard Mu elements; as a community resource we are scoring the seed, seedling, and adult segregating phenotypes, which are found in a searchable PhenotypeDB at ZmDB. By project completion we expect data from ~48,000 ears, ~15,000 seedling, and 10,000 adult families.

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Functional Genomics of Chloroplast Biogenesis: The Photosythetic Mutant Library.

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The study of mutants deficient in chloroplast biogenesis can elucidate chloroplast gene regulation, protein targeting, membrane assembly, and organellar cross talk, as well as aspects of prokaryotic biology that have been conserved during chloroplast evolution. To this end, we have generated a large collection of *Mutator*-induced maize mutants. The library currently consists of ~2100 mutants with visible chlorophyll deficiencies (from subtle to severe), phenotypes known to result from mutations that disrupt the expression, targeting, assembly, stability, or function of components of the photosynthetic apparatus. The collection has been made available to the scientific community via a searchable database which describes the defects associated with each mutation at the pigment, chloroplast protein, and chloroplast RNA levels http://chloroplast.uoregon.edu. The collection is also the basis of a reverse-genetic service for genes potentially involved in chloroplast function; users can request we screen the library for *Mu* insertions in their gene of interest. Multiple alleles are generally identified in successful reverse genetic screens indicating that the collection is nearing saturation.

The major photosynthetic enzyme complexes in each mutant are cataloged via immunoblot, and chloroplast mRNAs encoding missing proteins are subsequently analyzed on Northern blots. Protein data for 1100 mutants and RNA data for ~500 mutants are currently available online, providing comprehensive phenotypic information to help users select mutants of interest. Protein defects include; the specific loss of each single photosynthetic enzyme complex, global protein losses, protein losses diagnostic for defects in the cpSec and DpH thylakoid targeting pathways, and the loss of subsets of photosynthetic complexes in virtually every combination. This latter class of mutants is essentially unexplored and may point to unanticipated circuitry coordinating the synthesis or assembly of components of the photosynthetic apparatus. Of the 500 mutants thus far examined at the RNA level, 44 show defects in chloroplast RNAs metabolism. Many of the RNA phenotypes have not been described previously, revealing the benefit of this large scale analysis of nuclear genes involved in chloroplast RNA metabolism. Seed from mutants with phenotypes of interest can be requested for detailed study.

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Physical localization of single copy sequences on 2-D maize pachytene chromosomes by fluorescence in situ hybridization

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Fluorescence in situ hybridization (FISH) is a powerful technique for physical mapping. However, the sensitivity of FISH in plant chromosomes has been low because of limited probe penetration and high levels of background staining in the cytoplasm. Here we demonstrate that single copy sequences can be mapped on ethanol:acetic acid

fixed maize pachytene chromosome squashes by FISH using directly labeled fluorescent DNA probes. A 13.4 kb genomic clone, pHF10 generously provided by Fu and Dooner, which contains single copy sequences from the bronze (bz) region is localized on the short arm of chromosome 9, corresponding to its genetic locus. Another 8.5 kb genomic clone, pAMY2, which contains a beta-amylase gene shows 2 signals on chromosome 2 and 7, respectively, whereas genetic mapping data suggests that there is only one gene each on chromosome 2 and 7. This is the first report of physical mapping of single copy DNA sequences by FISH in plants using directly labeled fluorescent probes. Our attempts to localize single copy sequences on 3-D maize pachytene chromosomes fixed to preserve nuclear architecture have been less successful. In order to compare the morphology of 2-D squashes of ethanol:acetic acid fixed chromosomes related to 3-D formaldehyde fixed chromosomes, we have measured chromosome lengths, arm ratios, and placement of 5S rDNA on 2-D and 3-D chromosomes in two different inbred lines using these different FISH techniques. The development of a technology for localizing single copy sequences will be useful to the maize community for mapping sequences, ordering clones, and for correlating the genetic and cytological maps.

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Establishment of Robust Maize Transformation Systems for the Public Sector

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The long-term goal of this research is to establish a robust maize transformation system to enable the maize research community for future functional genomic research as well as crop improvement. Our specific objectives are: 1) Developing a routine Agrobacterium-mediated transformation system. We will conduct experiments to systematically optimize transformation and regeneration parameters for Agrobacterium-mediated maize transformation using non-super binary vector systems. 2) Enhancing transgene integration and expression. We will transfer and express the Arabidopsis and maize rat (resistant to Agrobacterium transformation) genes/proteins in maize to evaluate their effects in enhancing transgene integration. We will also evaluate the effect of tobacco and maize MARs (Matrix Attachment Regions) on the efficiency of our transformation protocols and on the frequency of somatic silencing events in maize. 3) Investigating germline transformation protocols. We will investigate tissue culture-independent transformation protocols such as meristem transformation and female gametophyte transformation. 4) Exploring inbred line transformation. We will conduct research to improve inbred line transformation on B73, H99, Oh43 and W22. In addition, we will facilitate transfer of improved protocols to the public sector by providing a more efficient transformation service at the Plant Transformation Facility at Iowa State University and organizing transformation workshops during the course of this program. It is also our intention that any vector systems, reliable transformation protocols and information generated from this research program will be made available for the maize community upon request. Deliverables: 1) Both Biolistic-mediated and Agrobacterium-mediated maize transformation services are available at the Plant Transformation Facility of Iowa State University(http://www.agron.iastate.edu/ptf/Web/mainframe.htm) 2) A Maize Transformation Workshop is scheduled at Madison, WI, on March 10-13, 2003

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Gene Discovery and Mapping in Maize

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More than 32,000 ESTs have been submitted to Genbank. Most were generated by sequencing the 3í ends of cDNAs derived from the inbred line B73. Hence, it is possible for these ESTs to be assembled into a set of unique genes with a very high degree of confidence. 28,500 3í B73 ESTs from two normalized libraries defined 3,794 singletons and 3,679 contigs (7,473 unique genes), resulting in an overall rate of gene discovery of 26%. IDPs (InDel Polymorphisms) are a class of PCR-based, genetic markers that detect the insertions and deletions (indels) that exist among maize alleles. Because they can be detected using agarose gel electrophoresis, these markers are suitable for use by almost all maize genetics laboratories. Over 1,000 IDP markers that distinguish B73 and Mo17 have been developed. On average any given IDP marker has an approximately 50% chance of being polymorphic in any pair of 22 tested inbred lines. Hence, it is likely that approximately 500 of the IDP markers will prove informative in any experiment that requires genetic markers. Over 1,000 ESTs have been genetically mapped using the IBM mapping population and another ~700 have been mapped using other populations involving B73 or Mo17. By substantially increasing the number of sequence-based connections between the maize genetic map and the rice physical map, these data will enhance the value of the rice genome sequence to the maize genetics

community. Over half of the genetically mapped ESTs exhibit a high degree of sequence similarity (a stringent >e-75) to the EST contigs used to design the overgo probes being used by the NSF Project led by Ed Coe to physically map ESTs to BACs. Hence, in combination these experiments promise to yield hundreds of cross-linkages between the genetic and physical maps of maize.

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CONSORTIUM FOR MAIZE GENOMICS - APPROACH EVALUATION FOR TARGETED SEQUENCING OF MAIZE GENES

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Maize is both a classical genetic model for plant research and an economically important crop; however, the size and complexity of the maize genome deem it recalcitrant to whole genome sequencing. Current estimates indicate that genes constitute a mere 15-20% of the maize genome with the remainder consisting of highly repetitive DNA. The initial objective of the Consortium for Maize Genomics is to evaluate two approaches to sequencing the maize ëgenespaceí (methylation filtration and high Cot selection) in order to provide the most rapid and cost-effective alternative to sequencing the whole genome. At TIGR, we are generating paired end sequence reads from 250,000 methylation filtered clones and 250,000 high Cot clones. The sequences are clustered and assembled, both independently and in combination, at quarterly intervals. The resulting maize genomic assemblies and singletons are annotated based on homology searches, with subsequent development of improved methods for annotation. The results of these analyses, a BLAST-searchable database and Maize Assembly Annotator are presented in the TIGR Maize Database (http://www.tigr.org/tdb/tgi/maize). The results of the latest assemblies will be presented and discussed.

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Gene Enrichment Technologies for Selective Sequence Analysis of the Maize Genome

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Like other grass genomes, the nuclear DNA of maize is mostly composed of highly repetitive sequences. Over 60% of the maize genome is composed of LTR retrotransposons that are often found as nested blocks of 10-100 kb intermixed with short genic regions. The large size and the similarity of these retrotransposons will make it very difficult to assemble shotgun sequence data from the maize genome into long contiguous sequences (contigs). In order to avoid the inefficiency of sequencing the unwanted repetitive DNA, we and others have developed various gene enrichment technologies based on methylation pattern differences and copy number differences between repetitive DNAs and genes. These techniques will allow a shotgun approach for relatively efficient sequencing of gene-rich regions in the maize genome and other higher plant genomes. We will discuss and compare these technologies, indicating their respective strengths and weaknesses, especially with regard to the genic components that might be missed and their representation of the boundaries between repetitive DNAs and genic regions. Several of these techniques appear to be highly complementary. If used in a combined approach, these gene enrichment techniques should produce almost all genes in sequence contigs, and will also locate these contigs on genetic and physical maps.

Biochemical Genetics

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Correlation between SU1 isoamylase expression level and amylopectin structure

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Genetic analysis of maize *sugary1* (*su1*) mutants indicates that isoamylase-type starch debranching enzymes (DBE) are required for normal starch levels, although whether or not DBEs are directly involved in starch biosynthesis is unknown. This study utilized a transgenic approach to test the hypothesis that DBEs provide a molecular editing function that directly influences the structure of the amylopectin (Ap) component of starch. Expression of *Su1*, which codes for one of the three isoamylase-type DBEs conserved in maize and other plants, was modified in transgenic maize in an attempt to correlate increased or reduced expression of SU1 isoamylase-

type DBE with alterations in the distribution of chain lengths and/or branch linkages in Ap. Thirteen transgenic maize lines are now in at least their fifth generation and have been introgressed into the B73 inbred background. Reduced expression of *Su1* has been achieved to various degrees in antisense transformants and in two gene-silenced lines. Chain length profiles of endosperm Ap from these lines by fluorophore assisted carbohydrate electrophoresis (FACE) revealed that Ap molecules have more short chains relative to wild type, similar to *su1-* mutants. Unlike the genetic mutants, however, most of the transgenic plants do not accumulate the water-soluble glucan phytoglycogen. Thus, phytoglycogen production is not a necessary condition for modification of Ap structure as a result of SU1 isoamylase deficiency. Three lines have been identified that over-express *Su1*. FACE analysis of endosperm Ap shows that increased *Su1* expression results in fewer short chains and more intermediate length and long chains, which is the opposite effect of the *su1-* mutations and transgenically-induced deficiency in SU1 isoamylase-type DBE. The reciprocal effects of increased and decreased *Su1* activity on Ap structure suggest that the SU1 isoamylase-type DBE directly catalyzes hydrolysis of branch linkages in a pre-Ap substrate.

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Identification of a novel starch hydrolytic enzyme in maize

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Starch is mobilized by a combination of phosphorolytic and hydrolytic enzyme activities. Despite the critical functions of starch degradation in plant metabolism, the molecular details of the process remain to be described. In plants, it is known that debranching enzymes, disproportionating enzymes, and starch phosphorylases are potential degradative enzymes, as are the _-1,4 specific hydrolases of the _-amylase and _-amylase classes. In this study a novel starch degradative enzyme activity was identified, beginning with the observation of a low mobility band in native activity gels (zymograms) containing starch as the substrate. The novel enzyme (termed SHE for starch hydrolyzing enzyme) was partially purified from developing maize endosperm by a combination of anion exchange chromatography and gel permeation chromatography. Proteins from fractions in which SHE activity was enriched were separated by native PAGE and transferred to a series of gels containing different glucan polymers. Dye staining of each glucan substrate revealed that SHE hydrolyzes amylopectin, the _-limit dextrin of amylopectin, amylose, and glycogen, but does not hydrolyze pullulan, a linear polymer of isomaltotriosyl units. Separation of the hydrolysis products by fluorophore-assisted carbohydrate electrophoresis (FACE) indicated that maltose was the sole hydrolysis product in all instances. Although _-amylases are known to release maltose from the non-reducing ends of glucan polymers, acting in an exo-specific manner to cleave _-1,4 linkages, they have limited activity toward amylose and are unable to further hydrolyze _-limit dextrin. Because SHE readily hydrolyzes these substrates, we predict that this enzyme acts endo-specifically, cleaving internal _-1,4 linkages to release maltose. Maltogenic amylases have been reported in bacteria, although these enzymes also have activity toward pullulan. This is the first description of an endo-acting maltogenic glucan hydrolase in a plant. Further experiments are underway to define SHE enzymatic activity and to discern its function in maize starch metabolism.

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Comparison of cell wall proteins in drought tolerant and susceptible maize lines

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Drought is a major limitation to global crop production. Many factors affect drought response including root architecture, root growth and dimension. Expansin expression increases in roots of water-stressed compared to well-watered, indicating changes in wall structure or chemistry Wu et al. (1996, 2001). Our goal is to identify root cell-wall specific proteins that play a role in drought tolerance. Our objectives are: 1) to evaluate the Bio-Rad Sequential Extraction Kit II for its ability to separate cytosolic and cell wall proteins using 2-dimensional isoelectric focusing gels; 2) to compare the cell wall proteins from root and shoot of seedlings at the 6-leaf stage, and; 3) to evaluate genotypic differences in root cell wall proteins. For this experiment inbreds Mo17, B73, B97, and Fr697 were used. Four replicates of five plants were planted in a completely random design in the greenhouse. All plants were well watered and root and shoot tissue was harvested at the 6-leaf stage. Proteins were extracted using a Sequential Extraction Kit from Bio-Rad. The proteins were separated on 11 cm pH 3-10 strips and 4-20% Tris HCl gels, and then stained with coomassie blue. Gel images were compared and analyzed using PD Quest to identify differences in position and relative intensity of spots. Subsequent experiments will compare cell wall proteins in water stressed vs. well watered roots to identify candidate cell wall proteins associated with drought tolerance.

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A Comprehensive Real-time PCR Expression Analysis of the Maize Starch Debranching Enzyme Gene Family

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This study describes the full complement in maize of starch debranching enzymes (_(1 _ 6) glucan hydrolase, or DBE), and their expression pattern. Plants contain two conserved types of DBE, the isoamylase- and pullulanase-type enzymes. Genetic analysis of *sugary1* (*su1*) mutations in maize and other cereals indicates that at least one isoamylase-type DBE is required for normal starch biosynthesis. Genetic analysis of maize *zpu1*, coding for the pullulanase-type DBE, suggests functions both in starch degradation and starch biosynthesis, and that the biosynthetic function overlaps partially with that of the isoamylase-type DBE coded for by *Su1*. Here we report the cloning of two additional isoamylase-type DBE cDNAs, *Iso2* and *Iso3*. Like *su1* and *zpu1*, these sequences have been conserved throughout the evolution of plants, i.e. Arabidopsis, potato, barley, wheat, sorghum, and rice all possess three isoamylase-like sequences that have been independently conserved to a high degree. A comprehensive analysis of the expression of all four DBE transcripts was conducted in metabolically active tissues. Debranching enzymes are expressed ubiquitously throughout the plant, although to varying levels. *Su1* is the most highly expressed mRNA, and is present during times of net starch synthesis. The pattern of *Su1* expression follows closely that of *Iso2*, consistent with the idea that these isoforms form a heteromultimer. The expression of *Iso3* is most abundant in germinating seed, consistent with a role for this isoform in starch degradation. Thus, individual DBE isoforms appear to be dedicated to specific catabolic and/or anabolic functions.

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Functional Significance of the Evolutionary Steps that Shaped the *R2R3 Myb* Gene Family

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Plants express hundreds of R2R3 MYB transcription factors. The expansion of the *R2R3 Myb* gene family occurred in plants 250-450 million years ago. R2R3 Myb transcription factors are derived from widely distributed *R1R2R3 Myb* genes, represented in animals by the *c-myb* proto-oncogene and in the plants by the members of the small *pc-myb* gene family. Discrete evolutionary steps involving the loss of the R1 MYB repeat, the insertion of a Leu residue in the R2 MYB repeat and the replacement of the first Trp in R3 by a hydrophobic residue shaped the R2R3 MYB family that dramatically expanded in plants. In addition, recent duplications of R2R3 Myb genes have characteristic changes in the MYB domains. One example is provided by the P-to-A clade formed in maize by at least 10 members and characterized by the change of a very conserved Pro to Ala in the hinge region that joins R2 and R3. Here, we investigated how each of these changes influenced the activity of R2R3 Myb proteins, starting from the P1 gene, a member of the P-to-A group of maize R2R3 Myb factors. Our results indicate that, while DNA-binding has clearly been influenced by the changes that forged the *R2R3 Myb* gene family, some changes must have other functions unrelated to DNA binding, and what those functions might be will be discussed

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Photosynthetic Mutant Library (PML): A Reverse Genetics Resource for Chloroplast Biogenesis Genes

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The goal of this project is to develop, use, and disseminate a set of resources for the genetic and biochemical dissection of chloroplast biogenesis. We have developed a core resource consisting of ~2100 transposon-induced chloroplast-defective maize Mutants. Mutants were selected from *Mutator* (*Mu*) maize lines based upon their chlorophyll-deficient leaves and/or increased level of chlorophyll fluorescence. Previous studies support the notion that a disruption of many aspects of chloroplast biogenesis (e.g. import of proteins into the organelle, lipid, pigment, and prosthetic group synthesis, chloroplast gene expression, intra-chloroplast protein sorting, assembly of the photosynthetic apparatus) will result in one of these easily scored phenotypes. One application of the Mutant collection is as a reverse genetics resource that can be used to determine the roles of genes of known sequence with suspected roles in chloroplast biogenesis. Pooled Mutant DNA samples are screened by PCR for *Mu* insertions in genes of interest. Mutants are identified and seed from the Mutant line is provided as a service. The current collection of ~2100 Mutants appears to be nearing saturation because successful reverse genetic screens generally yield multiple Mutant alleles. This resource has proven useful for testing the *in vivo* relevance of

yeast two-hybrid interactions, for identifying new Mutant alleles of previously cloned genes, for testing the functions of proteins identified through biochemical study of chloroplast import, and for testing functions of plant homologs of bacterial secretory proteins.

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The Protein Disulfide Isomerase Multigene Family in Maize

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In immature seed, storage protein synthesis and subsequent packaging into protein bodies is a major metabolic activity. Perturbing protein folding can have dramatic phenotypic effects, such as the lower amounts of storage proteins and abnormally shaped protein bodies of the maize endosperm mutants floury-2 (fl2), Defective Endosperm B-30 (De-B30), and Mucronate (Mc). The biological response to accumulation of non-native protein domains is the induction of a battery of molecular chaperones, including protein disulfide isomerase (PDI). PDI provides a key role in aiding misfolded protein to achieve their native conformation by catalyzing disulfide bond redox/isomerization. The enzyme is encoded by a multigene family within a subgroup of the thioredoxin superfamily. PDIs are characterized by their domain structures that generally contain two active thioredoxin-like domains minimally composed of a CXXC tetrapeptide and a distinct fold pattern. Although a number of PDIs have been described, the evolution of plant PDIs is poorly understood. Using BLAST analysis, we performed iterative searches with published plant sequences for PDIs in the Arabidopsis genome and subsequently removed putative thioredoxins. We then searched maize and rice genomes to obtain a data matrix of thirty-one putative plant PDIs for phylogenetic analysis. Both Bayesian and maximum likelihood phylogenetic analyses of PDIs suggest that at least five duplication events occurred before the divergence of monocots and dicots. The phylogeny also implies that there were frequent duplications within different lineages of flowering plants. We have initiated gene and protein expression analyses of PDIs in maize. The major PDI, which has homologues in most higher eukaryotes, has increased mRNA and protein expression levels in endosperm of the fl2, De-B30 and Mc mutants compared to the normal inbred. A second putative PDI appears to be plant specific and has a single thioredoxin-like domain. Immunoblot analysis shows induction of this unusual PDI in fl2 but not in De-B30 or Mc.

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Evidence for physical interactions among specific starch metabolizing enzymes

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Multiple isoforms of starch synthases (SS), starch branching enzymes (BE), and starch debranching enzymes (DBE) are evolutionarily conserved, suggesting each has a distinct function in starch metabolism. Reduced expression of a particular isoform can trigger pleiotropic effects on other enzymes in the pathway, indicating that some enzyme activities are inter-dependent and coordinately regulated. A likely molecular explanation involves protein-protein interactions between components of the starch biosynthetic system. This study investigated the potential for protein complex formation among maize SS, BE, and DBE isoforms by three different approaches: the yeast two-hybrid interaction system, affinity chromatography, and co-immunoprecipitation. Reciprocal pair-wise tests for protein interactions in yeast indicate that physical associations occur between SU1 (the isoamylase-type DBE that is the product of the *sugary1* gene) and BEIIb (the product of the *amylose extender* gene), and between BEIIa and DU1 (the SSIII isoform that is the product of the *dull1* gene). Positive unilateral results were obtained for a number of isoforms when SU1, BEIIa, and DU1 were expressed as *GAL*4 binding domain fusions, including evidence for a BEIIa/SSI association. Affinity column chromatography provides further support for this interaction. Immunoblot analysis revealed that BEIIa was tightly bound to a SSI affinity column, and the reciprocal experiment showed that SSI was tightly bound to a BEIIa affinity column. These results are supported by the co-immunoprecipitation of BEIIa and SSI from protein extracts of developing maize endosperm, using antibodies to both proteins. Similar experiments with anti-DU1 antibodies reveal that both BEIIa and SSI immunoprecipitate with DU1. The discoveries of these interactions provide strong support for the existence of protein complexes comprised of specific SS, BE, and DBE isoforms. We hypothesize that such protein complex formation serves to coordinate and regulate the activities of the enzymes involved, and provide for the architectural specificity of glucosyl linkages inherent in starch granules.

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Utility of 2 Dimensional Isoelectric Focusing in Identifying Biochemical Networks.

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There are two distinct phases in *Zea mays* L. leaf development, juvenile and adult phase. The first leaves are juvenile and have a dull green appearance with thick bluish wax rich in fatty alcohols. Adult leaves have a distinctive glossy appearance. Their epicuticular wax is high in long chain wax esters. The number of juvenile leaves is correlated to insect damage from the fall armyworm, (*Spodoptera frugiperda* (J.E. Smith), and the southwestern corn borer, (*Diatraea grandiosella* Dyar). The larvae of both species cause significant whorl stage damage. *Glossy15 (Gl15)* is an apetala-2 type transcription factor. Mutations at this locus cause an abbreviated juvenile phase. This shortened juvenile stage plays a key role in insect resistance. Studies indicate that the juvenile-to-adult phase change occurs earlier in resistant than in susceptible corn lines. Twenty-two glossy mutants have been identified to date. Our objective is to use regulatory and structural genes from the epicuticular wax biosynthesis (*Glossy15*) pathway to evaluate the potential for identifying biochemical networks using single gene mutants and 2-dimensional isoelectric focusing. Leaves of seven families segregating for glossy genes in different genetic backgrounds were collected by phenotype. Glossy phenotype and wild-type for each structural gene were compared against *Gl15* protein profiles to identify putative proteins involved in epicuticular wax biosynthesis.

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Characterization of a putative maize ERAD protein

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Accumulation of mutant or foreign polypeptides in the endoplasmic reticulum (ER) can lead to channeling of the polypeptides for degradation through the ER-associated protein degradation (ERAD) pathway. Several of the polypeptides of this multi-subunit degradation machinery have been described in yeast but little is known about the phenomenon in plants. We used the amino acid sequence of Der1p, a ~24 kDa component of the yeast ERAD pathway in a homology search of maize ESTs. We identified two maize sequences that encode putative Der1 proteins and have initiated studies to determine whether they are involved in ERAD in maize. 'Digital northern' analysis of EST representation among ~480,000 cDNA clones in the Pioneer database showed widespread representation in different organs although the relative abundance within a given organ differed for the two clones. Yeast Der1p is highly hydrophobic and is localized to the ER where it is predicted to span the membrane four times. Both maize proteins show similar in silico transmembrane domains and localization. Subcellular fractionation followed by immunoblotting showed Der1-1 in both protein bodies and ER. Further fractionation showed tight association of Der1-1 with the ER membrane where it remained in the membrane fraction following treatment with TritonX-100, taurichолate, and deoxycholate but was released into the supernatant in the presence of digitonin. Prior to ERAD, proteins that fail to fold or assemble properly often induce an ER stress response which is accompanied by increases in accumulation of both molecular chaperones and polypeptides of the ERAD machinery. We assayed Der1 expression in maize endosperm mutants which we have previously shown to exhibit an endosperm-specific unfolded protein response (UPR). RNA profiling and immunoblot analyses showed overexpression of both Der1 genes in endosperm of the mutants relative to the normal inbred.

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Function of the nitrilases ZmNIT1 and ZmNIT2 of Zea mays in auxin biosynthesis

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Nitrilases are suggested to convert indole-3-acetonitrile (IAN) to the auxin indole-3-acetic acid (IAA). Maize seedlings grown in water containing IAN showed typical auxin-like effects, e.g. decreased root length, and increased nitrilase expression. In maize kernel we detected highest nitrilase expression and activity in protein extracts from aleurone/pericarp and embryo. Heterologeously expressed ZmNIT2 hydrolyses IAN to IAA 7 to 20fold more efficiently than Arabidopsis thaliana AtNIT1-3 and showed also high activity towards different nitrile compounds like 3-phenylpropionitrile and 4-phenylbutyronitrile. In spite of a 69% identity in amino acid sequence between ZmNIT2 and AtNIT4, ZmNIT2 did not accept the AtNIT4 substrate fl-cyanoalanine, which is discussed as an intermediate in cyanide detoxification. The fact that ZmNIT2 is expressed in maize kernel, the high turnover rate compared to Arabidopsis thaliana and the finding of the substrate IAN indicate an involvement of nitrilases in auxin biosynthesis.

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Color complementation in *E. coli* for the functional testing of a cDNA required for maize carotenoid biosynthesis

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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. On the basis of GenBank homology searching, we identified a cDNA predicted to encode betacarotene hydroxylase (HYD), which catalyzes the addition of hydroxyl groups at position three on the cyclic rings of betacarotene to form the xanthophyll zeaxanthin. This maize EST was sequenced and found to encode a protein with 56% identity to a homolog from the dicot, *Arabidopsis thaliana*. The cDNA, cloned into *Eco*RI/*Xho*I sites of pET23c, encodes a 359 amino acid polypeptide. Since sequence homology implies, but does not always indicate enzyme specificity and activity, we used a heterologous bacterial system to demonstrate function of the putative maize HYD. The cloned cDNA was tested by co-transformation with a second plasmid conferring accumulation, of the HYD substrate, betacarotene, in *Escherichia coli*. Expression of a functional cDNA product altered colony pigmentation of the betacarotene accumulating strain. HPLC analysis of the carotenoid products confirmed the accumulation of zeaxanthin and the HYD intermediate, betacryptoxanthin. This research is funded in part by the National Institutes of Health.

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Enhancing Essential Amino Acids in Crop Plants

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Several amino acids are essential to animals because they are required for growth and maintenance, but cannot be synthesized de novo. Both cereals and oil seed crops make up an important part of livestock feed. However, both types of seed proteins do not contain optimal amounts of several essential amino acids including shikimate derived (Phenylalanine and tryptophan) and aspartate derived (lysine, threonine, methionine and isoleucine) amino acids. One way to provide a more nutritionally balanced meal is to produce and accumulate these essential amino acids in the seed. We are utilizing a transgenic approach to increase the accumulation of free amino acids by changing the allosteric regulation of key enzymes in essential amino acid biosynthetic pathways. This presentation focuses on anthranilate synthase for the production of tryptophan.

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A maize ribosome-associated membrane protein is overexpressed in the floury-2 mutant

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Synthesis of functional secretory proteins requires the coordinated interaction of several multi-subunit protein complexes: ribosomes that associate with the ER, the translocation apparatus for transport of the nascent protein into the ER, and molecular chaperones for protein folding and assembly. Synthesis of both molecular chaperones (called the ER stress response) and ER associated degradation (ERAD) proteins is induced upon accumulation of mutant or foreign proteins in the ER. Recently, ribosome-associated membrane protein 4 (RAMP4) was found to be induced by environmental stress and pharmacological agents that cause an ER stress response in rats(Yamaguchi et al.,1999). We used the rat RAMP4 amino acid sequence to screen maize ESTs and isolated two putative homologs, RAMP4-1 and RAMP4-2. Both encode 69 amino acid polypeptides with a single predicted membrane spanning domain. RNA profiling experiments showed expression of both genes in a variety of tissues. RAMP4-1 was at least 10-fold more abundant in endosperm than in any other tissue while RAMP4-2 showed little variation in expression from tissue to tissue. Polyclonal antibodies were raised against synthetic peptides for both RAMP4 polypeptides. No cross-reacting material was detected in crude endosperm extracts of either normal or fl2 maize. However, immunoblots of proteins from a ribosome fraction containing associated membrane proteins showed weak cross-reactivity in normal endosperm and much stronger signal in the fl2 mutant. The higher levels of RAMP4 in the fl2 mutant are suggestive of cross talk among molecular chaperones, ERAD associated proteins and the translocation machinery. Yamaguchi, A., O. Hori, D.M. Stern, E. Hartmann, S.

Ogawa, M. Tohyama (1999) 147:1195-1204.

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From Proplastid to Chloroplast: Understanding Plastid Differentiation in Maize through Microarray and Proteome Analysis (NSF Award - #0211935).

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Although best known for their role in photosynthesis, plastids perform many other important functions. These include the synthesis of hormones, amino acids, fatty acids, lipids, vitamins, nucleotides and secondary metabolites. Despite their essential role, little is known about the developmental signals governing plastid differentiation. To elucidate the mechanisms of plastid biogenesis in maize, we will utilize genomic and proteomic tools in conjunction with genetic analysis. Bioinfomatic approaches have enabled us to identify a set of approximately 3500 maize EST sequences that are predicted to encode chloroplast targeted proteins. We intend to use these sequences as the basis for building a DNA microarray. We are currently confirming the identities of clones corresponding to approximately 1500 EST sequences selected for the Phase I array. This array will also fully represent the maize chloroplast and mitochondrial genomes. In parallel, we have begun a broad survey of proteins that accumulate in maize bundle sheath and mesophyll plastids using mass spectromerty. We have separated and visualized proteins from purified plastids by high resolution 2-Dimensional Electrophoresis (2-DE). These proteins will then be identified using both Matrix-Assisted-Laser-Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and on-line LC-Electrospray Ionisation Tandem Mass Spectrometry (LC-ESI-MS/MS). Preliminary data will be presented. We will create reference protein profiles of plastid membrane proteins using solvent-based fractionations, 1-dimensional electrophoresis (1-DE) gels and mass spectrometry. As a further tool to aid in the characterization of photosynthetic development, transgenic plants carrying bundle sheath and mesophyll cell-specific markers will be constructed. Work has begun on constructs to express GFP and GUS in plastids, the cytosol or in mitochondria of both bundle sheath and mesophyll cells. The bioinformatic integration of proteomic and expression profiling data will provide a detailed picture of the transcriptional and translational control mechanisms utilized by maize plastids. The approaches described above will result in an improved understanding of the biochemical functions of different plastid types and novel insights into the differentiation process. Chloroplast function is directly related to agronomic performance, and therefore these studies will greatly improve our understanding and ability to engineer important plant traits.

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A Functional Genomics Program for the Illinois Long Term Protein Selection Strains

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The Illinois Protein Strains (Illinois High Protein and Illinois Low Protein) represent the known genetic extremes for endosperm storage product accumulation and nitrogen metabolism in vegetative tissues. They are also the product of the worldís longest-running breeding and selection experiment. The Illinois Protein Strains are thus an excellent experimental system to investigate genome responses to selection and the genetic control of whole plant nitrogen metabolism. We are employing a number of functional genomics strategies to address several important questions about the Illinois Protein Strains. We are comparing genotypes/haplotypes for candidate genes and molecular markers from generations 65 through 100 of the experiment to assess changes in allele frequencies during the last 35 cycles of selection. mRNA expression profiling is being used to survey the global transcriptional responses of the genome to 100 years of divergent selection for grain protein concentration, with particular emphasis on genes associated with zein accumulation and nitrogen metabolism. Zein profiles will also reveal the relative contribution of transcriptional and post-transcriptional regulation to the dramatic differences in zein accumulation observed among the Illinois Protein Strains. To complement these profiling and gene discovery efforts, mutations with known effects on grain composition are being introgressed into the Illinois Protein Strains and EMS mutagenized populations have been generated for Illinois High Protein and Illinois Low Protein. These populations are being screened for novel mutant phenotypes in these phenotypically extreme genetic backgrounds. This poster will present our progress to date in each of the above research areas.

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A genetic approach to dissecting the maize cell wall

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The presence of lignin in the plant cell wall has evolved to serve multiple purposes, including water transport, provision of rigidity, and defense against pests and pathogens. It is likely that the composition of the cell wall as we know it, is a compromise that provides all functions in an adequate manner as long as the environmental conditions stay within certain boundaries. With the current agricultural practice and the prospect of global climatic change, however, a different cell wall composition may be optimal. In order to design the optimal cell wall, a thorough understanding of the relationship between cell wall structure and function is required. We are using genetics, in combination with detailed chemical, morphological and physiological analyses to address this challenge. Three different strategies are employed: (**a**) we are developing near-isogenic lines in which different mutations that affect the cell wall are combined, (**b**) we use forward and reverse genetics approaches to obtain novel cell wall mutants (as part of an NSF Plant Genome project), and (**c**) we perform molecular and chemical analyses on different lines that display significant variation in a trait of interest, such as forage quality. Recent results include the finding that (**1**) there is an evolutionarily conserved interaction between cell wall composition and flowering time, (**2**) the *Brown midrib1* (*Bm1*) locus affects cell wall composition and plant development in a dose-dependent manner, (**3**) the *Bm2*gene is important for the establishment of tissue-specific patterns of lignification, (**4**) the cell wall of the *bm3* mutant contains benzodioxanes, representing a novel structural feature of lignin, (**5**) combining certain *bm* mutations has dramatic effects on plant growth and development, and (**6**) there is a distinct but complex chemical basis underlying variation in forage quality of maize.

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Inhibition of *Aspergillus flavus* growth by genistin and diosmin

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Aflatoxin is produced by *Aspergillus flavus* and *A. paraciticus*. Maize, figs, peanuts, cotton, and rice are all hosts of *A. flavus*. Aflatoxin is the most potent natural carcinogen identified. 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, very few detoxification and utilization options are available. Some flavanoid compounds and derivatives can alter the growth rate of fungi *in vitro* including *A. flavus* or the amount of toxin produced. Flavanoids from soybeans such as genistein, genistin, daidzein, and daidzin have been shown to reduce plasma cholesterol levels. Studies have also shown that a healthy diet that includes flavanoids reduces the risk of cancer. 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. This indicates that flavanoids may also have an *in vivo* role in reducing aflatoxin. *A. flavus* strain NRRL3357 was grown on Czepak's media plus 10g/L NaCl. The media was supplemented with diosmin, daidzein, daidzin, genistein, and genistin at concentrations of 50, 100, 150, 200, and 250 mM. Fungal growth was measured every 2 days for 14 days after inoculation. Diosmin significantly reduced growth at all time points compared to both the unsupplemented and DMSO supplemented media. Genistin at concentrations > 150mM reduced growth from 4 to14 days after inoculation. This information will aid in the identification of the enzymatic reactions that are critical in reducing aflatoxin production and in the identification of naturally occurring maize alleles that can be used to produce low toxin accumulating maize lines.

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Center for Eukaryotic Stuctural Genomics: Facility for Structure Determination of Proteins from Arabidopsis thaliana and Other Model Eukaryotes.

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The Center for Eukaryotic Structural Genomics (CESG) was founded as a collaborative effort to develop critical technologies for determining three-dimensional structures of proteins rapidly and economically. CESG's initial focus is on the genome of the model plant Arabidopsis thaliana. CESG has developed its own laboratory information management system ('Sesame') designed to track, evaluate, and eventually direct all steps in the process leading from gene to published structure. CESG's software periodically analyzes and ranks the entire Arabidopsis proteome, consisting of ~29,000 open reading frames, to determine the targets to be carried forward through a defined strategy leading to structure determination by either X-ray crystallography or NMR spectroscopy. Priority is given to targets likely to open up important regions of conformational space or to elucidate novel fold-function relationships. CESG also considers proteins of structural interest suggested by the plant science community. Progress at CESG is tracked at the Structural Genomics web site at the PDB. Technology and products developed by CESG, including clones, expression vectors, and excess protein, are being made available to the scientific community. Initially, CESG has concentrated on bottlenecks associated with cloning eukaryotic targets and the large-scale production of soluble protein. Gene chips produced by maskless array DNA synthesis are being used to determine the presence of targets in cDNA pools generated by RT-PCR of RNA isolated from an Arabidopsis callus cell line. CESG's standard protocol utilizes Invitrogens's 'Gateway' plasmid construction system. Preliminary results on the steps leading to structure determinations will be presented. Also presented will be specific steps that members of the maize community can use to tap into and help fill CESG's pipeline.

CESG (http://uwstructuralgenomics.org/) is supported as a Pilot Project in the NIH Protein Structure Initiative under Grant P50 GM64598 from the National Institute of General Medical Sciences.

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The maize gene *sugary2* codes for starch synthase IIa

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The *sugary2* (*su2*) gene of maize is of industrial interest owing to phenotypic effects of mutations on starch qualities, including reduced gelatinization temperature. The product of *Su2* was not known previously, although the gene that codes for starch synthase IIa (SSIIa) is tightly linked to the *su2* locus. Here we used transposon tagging in a forward genetic strategy to demonstrate that *su2* does in fact code for SSIIa. Starting with the published cDNA sequence for SSIIa, the sequence of the complete genomic locus that codes for this enzyme was obtained from the wild type W64A background. The gene contains 9 introns in a transcription unit that codes for an mRNA of approximately 2.2 Kb. The translational start site is located in exon 2 and is preceded by a large 5' UTR. Two independent mutations, *su2-2279* and *su2-5178*, were isolated from populations with an active *Mutator* transposon system. Each mutation was introgressed into W64A by at least four backcrosses. Both mutations failed to complement the reference mutation *su2-Ref*. The SSIIa coding region was examined by PCR amplification for the presence of transposon insertions in homozygous *su2-2279* or *su2-5178* plants. Mutant *su2-2279* was found to contain a *Mu1* element in the third exon of the SSIIa gene, and mutant *su2-5178* contained a small retrotransposon-like insertion in exon 10. Because two independent *su2-* mutations are coincident with physical alterations of the gene that codes for SSIIa, we conclude that *su2* and the SSIIa locus are the same genetic element. Further support for this conclusion comes from the fact that *su2-2279* endosperm lacks both SSIIa protein and enzymatic activity, as shown in native activity gels and immunoblot analyses that definitively identified SSIIa among all other starch synthase enzymes of maize. Chain length distributions in the amylopectin component of endosperm starch from five different *su2-* mutant lines also are reported. We conclude that the phenotypic effects of *su2-* mutations on maize starch result from a deficiency in SSIIa activity.

Cytogenetics

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Single-locus cytogenetic mapping in maize with marker-selected sorghum BACs as FISH probes on pachytene spreads from maize-chromosome-addition lines of oat.

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We have achieved a major technological advance towards the development of a cytogenetic map for maize (*Zea mays*). The use of a maize chromosome-addition line of oat, OMAd9.2 (Kynast et al., 2001; *Plant Physiol.*

125:1216), provides a distinct advantage for obtaining clear images of optically-isolated chromosomes using a chromosome spread and painting technique. We designed a direct-labeled oligonucleotide FISH probe, MCCY (Maize CentC labeled with Cy5), to detect the centromere. The MCCY probe reproducibly hybridizes to a small discrete region in the centromere of maize-9 (Koumbaris and Bass, 2002; Maize Newsletter, vol.76). Using 3D deconvolution microscopy and computer-assisted image analysis, we verified that the maize-9 arm ratio (Long/Short; 1.6 to 2.1 from prior studies) is preserved in the oat genome background (1.7 in our system). Next we have determined the cytogenetic map position for three test case core bin marker loci; CBM91 (Core Bin Marker 9.01, umc109), CBM92 (9.02, bz1), and CBM98 (9.08, csu54b). Marker-selected sorghum BAC clones were identified by library hybridization screening, purified, and labeled for FISH. We detected FISH signals at 9S.79, 9S.65, and 9L.95+.99 for sCBM91, sCBM92, and sCBM98 (two spots). We also mapped a sorghum GISH locus (SorGS1) to ~9L.975. The CBM positions are consistent with their distribution on the linkage map and with previous single-copy FISH-mapped loci on maize-9 from Sadder and Weber (Theor. Appl. Genet., 2002). This work represents a new approach for efficient FISH-mapping of maize genetic markers. Given the relative ease with which the first three loci were mapped using standard commercial FISH kits, this method could be extended to develop a comprehensive cytogenetic map for maize. Extension of this approach could contribute to resource development for chromosome painting, genome assembly efforts, comparative cytogenetics in grasses, and placement of translocations and centromeres on the new IBM linkage maps.

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absence of the first division1 (afd1) is a maize rec8-cohesin

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Prominent features of the afd1 mutant phenotype is the bypass of the early prophase I stages, leptotene, zygotene, and pachytene; and equational instead of reductional segregation of sister chromatids during the first meiotic division due to sister chromatid cohesion (SSC) failure. Recently we have shown that afd1 mutants fail to properly reorganize meiotic chromatin during prophase I. After interphase, which is not distinguishable from normal interphase, afd1 prophase chromosomes fail to form a telomere bouquet. Telomeres do not attach to the nuclear envelope during prophase I and remain scattered throughout the nucleus. afd1 prophase chromosomes do not look like normal meiotic chromosomes; the two chromatids of each chromosome can be recognized during the prophase I stage and even 80 telomeres (one for each chromatid on both chromosome ends) can be counted. Neither homologous pairing nor synapsis occurs in afd1 meiocytes as monitored by following the behavior of whole chromosomes or specific (5S rDNA) loci. The synaptonemal complex never forms in afd1 mutants. We made substantial progress in understanding the nature of the afd1 mutation during the past year, owing to finding a novel afd1 allele tagged with a Mu1 transposon. Finding this new allele was a result of a collaborative effort to fine map afd1 and a leaf mutant tiedyed1 (tdy1) using SSR markers and classical genetics. The two genes are very closely linked on the long arm of chromosome 6. The new allele, afd1 : Mu1, came from a directed tagging experiment of the tdy1 gene. One tdy1-Reference/tdy : Mu1 mutant found by screening 30,000 plants showed a unique pattern of inheritance. In a F2 progeny from a cross of the mutant plant to B73, 3/4 plants were fertile and had normal leaves and 1/4 plants were both tiedyed and sterile. All tiedyed, sterile plants (19 examined) showed a typical afd1 pattern of chromosome behavior during meiosis. Molecular analyses indicated that a Mu1 insertion caused a small deletion encompassing both afd1 and tdy1. Cloning the deleted DNA region and sequence analysis indicated that afd1 is likely a homolog of the yeast meiosis specific cohesin rec8. rec8 mutants in yeast are defective in SSC and meiotic chromosome morphology and this phenotype is compatible with the mutant phenotype of afd1.

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Genetic and Cytogenetic Analysis of Abnormal Chromosome 10 Indicates that at Least Four Loci are Required for Meiotic Drive

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In the presence of a rare version of chromosome 10, Abnormal chromosome 10 (Ab10), specific chromosome regions are preferentially segregated to the megaspore. Neocentromeric activity of knobs (heterochromatic regions containing specific tandem repeats) is required for meiotic drive. Two regions of Ab10 are indicated in the trans-activation of neocentromeres. Deletion analysis presented here indicates that only one of the neocentromere-activating regions is necessary for the activation of many knobs. Recombination in structural heterozygotes is increased when Ab10 is present in the genome. Deletion analysis has localized the region of Ab10 responsible for this function. A third function identified by deletion analysis is localized to the tip of the Ab10 chromosome and results in the complete loss of meiotic drive while maintaining typical Ab10 levels of neocentromeric activity and recombination. A meiotic drive mutation, suppressor of meiotic drive 3 (smd3) was identified that also abolishes meiotic drive while maintaining neocentromeric activity and recombination. The mutation, smd3, does not map to

the tip of Ab10, indicating the presence of a fourth function required for meiotic drive. All four functions have been localized to specific regions of the Ab10 chromosome.

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Chromosome doubling in maize

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Chromosome doubling methods are important for obtaining homozygous lines in a short period or the establishment of polyploid inbred series to investigate gene dosage effects. During the last several years the authors have developed three new chromosome doubling techniques in maize. 1) Doubled haploid induction from parthenogenetically induced haploids. Kernels with a haploid embryo were induced by the stock 6 method (Coe 1963) with the combination of the R1-scm2 embryo color marker. The haploid kernels were germinated in small pots and treated with nitrous oxide gas (600kPa) at the 6 leaf stage for two days in an air tight iron chamber (Kato 2002). The treated plants were grown in large pots and pollinated in a greenhouse. After self pollination, 44% of the haploid plants produced doubled haploid kernels. 2) Tetraploid induction from diploid inbred lines. Maize inbred lines just after pollination (30-36 hrs) were treated with nitrous oxide gas (800-900kPa) for 20 hrs. The ears were harvested after maturity and chromosome numbers were determined on the germinated seedlings by the method described by Kato (1999). From the treated ears, kernels with tetraploid embryos were obtained at 2-16% level. Those tetraploid inbred lines were highly sterile in field conditions but moderately fertile in the winter greenhouse. Six tetraploid inbred derivatives were established (A188, Oh43, W22, H99, stock 6, and B73). 3) Triploid induction from diploid inbred lines. Tassels 7-9 days before flowering were treated with trifluralin solution. By the dual pollination method (Kato 1997) kernels with a triploid embryo were detected as kernels with white embryo and purple aleurone. Five - ten triploid kernels per ear can be obtained by this method.

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The maize B chromosome contains multiple centromeric elements located away from the functional centromere

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Various DNA elements have been identified that are located preferentially or specifically at the centromeres of maize chromosomes. This fact has suggested that these elements play a role in centromere function. FISH analysis of the maize B chromosome using these various centromeric elements shows that they are distributed outside the functional centromere. A B-specific repeat shown to reside at the functional B centromere is also located near the tip of the long arm. The centromere specific Ty3/gypsy-like retroelement CRM hybridizes at the B centromere as well as near the B-specific repeat on the long arm. The Cent-C repeat, tandemly arrayed only at the centromeres of the A chromosomes, is distributed in the heterochromatic regions of the long arm of the B-chromosomes in several clusters. Probing with the Prem1 and Huck retroelements, which are enriched near the centromeres of A chromosomes, shows that these sequences are enriched in the heterochromatin of the long arm of the B chromosome. Co-localization of the CENH3 centromeric histone and these elements is being examined. Our results to date indicate that CENH3 is located only at the functional B centromere. These data contradict a simple model where centromeric DNA elements alone are sufficient to recruit centromeric proteins and form a functional centromere. Instead, particular combinations of elements, greater quantities of these elements or an irrelevance of underlying DNA sequence is involved with centromere formation.

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Increased Meiotic Recombination in Maize Genotypes After Chronic Water-Deficit Stress

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The effect of water stress on meiotic recombination, first reported by our group in 2001, has been investigated in greater detail and with larger sample sizes for genotypes B73/Mo17 and Mo17/H99. F1 plants were exposed to either stress or non-stress treatments. For each genotype, 6 populations were created by backcrossing 6 F1 plants (3 F1 plants from each treatment) as males to B73 or H99, respectively. 93 seedlings of each population were genotyped at microsatellite loci to create genetic maps for chromosomes 1 and 10. For B73/Mo17, the maps of chromosomes 1 and 10 were larger for stressed plants (172 vs 149 cM for ch. 1; 83 vs 72 cM for ch. 10). In the 6 B73/Mo17 populations, crossovers were not detected in 19 and 44% of the gametes for chromosomes 1 and 10, respectively. For Mo17/H99, the maps of chromosomes 1 and 10 were also larger for stressed plants (177 vs 161 cM for ch. 1; 84 vs 65 cM for ch. 10). In the 6 Mo17/H99 populations, crossovers were not detected in 17 and 48% of the gametes for chromosomes 1 and 10, respectively. In both genotypes, 1 interval of chromosome 10

exhibited significantly more recombination.

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'Master switches' of meiosis

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The switch from the somatic to the meiotic cell cycle in maize is regulated by ameiotic1. The am1 gene acts downstream from genes determining meiocyte identity and upstream from all other meiotic genes. In most mutant alleles of am1, meiocytes undergo mitosis instead of meiosis or arrest in pre-division interphase. In contrast, in the am1-praI allele, the meiocytes enter meiosis and arrest in zygotene. It has been previously shown that the am1 gene regulates establishment of a meiosis-specific cytoskeleton. We now demonstrate that am1 also regulates installation of the meiotic recombination machinery, as meiocytes in all am1 mutants lack chromosomal foci of the recombination protein RAD51 involved in repairing meiotic double-strand breaks. We cloned the am1 gene using a new Mutator-tagged allele. It encodes a novel protein with two conserved domains of unknown function and a coiled-coil domain, frequently associated with protein-protein interactions. Interestingly, the am1 transcript is constitutively expressed in all plant parts and not limited to meiocytes. These data suggest that am1 acts as a switch activated by cell cycle-related proteins and triggers a number of downstream events during the meiotic division. Another key meiotic protein, encoded by the poor homologous synapsis1 gene, is required for the homology search, which ensures that homologous chromosomes find their proper partners during the meiotic prophase. phs1 mutants show almost complete chromosome pairing and subsequent synapsis but homologous pairing is replaced by non-homologous pairing. The mutants are severely defective in meiotic recombination: they do not install RAD51 foci on chromosomes and show unrepaired double-strand breaks. The phs1 gene encodes a novel meiosis-specific protein. We hypothesis that the PHS1 protein may be involved in loading recombination complexes, containing RAD51, onto chromosomes. Unlike most recombination-related proteins, PHS1 shows a low level of evolutionary conservation but nevertheless has two domains that are conserved among monocots and dicots.

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Cytological crossover maps for all maize bivalents using recombination nodules

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Cytological markers such as chiasmata, MLH1 foci, and recombination nodules (RNs) are useful for defining the frequency and distribution of crossovers along the length of chromosomes. Of these, RNs provide the highest resolution currently available because they are observed by electron microscopy of synaptonemal complexes (SCs) in extended pachytene chromosomes. The most useful cytological crossover maps are those in which each bivalent can be unequivocally identified and related to a specific linkage group. To date, such maps have been generated only for tomato (using RNs) and mouse (using MLH1 foci). To achieve this goal for maize, we have prepared an SC karyotype for the maize inbred line KYS, in which each SC can be identified based on its relative length and arm ratio. Each SC was related to the proper chromosome and linkage group using inversion heterozygotes. Using this karyotype, we mapped RNs on more than 2000 SCs to produce high resolution maps of RN frequency and distribution on each bivalent. The average RN frequency per bivalent is closely correlated with SC length. The crossover frequency using RNs is about 10% higher than estimated using chiasmata. While the total length of the RN map is about two-fold shorter than current linkage maps, the correlations between the RN and linkage maps are good ($r2 > 0.63$) when the cM lengths of the bivalents are compared. Each bivalent has a unique distribution of crossing over, but all bivalents share certain general characteristics such as a high frequency of distal RNs and a severe reduction of RNs at and immediately adjacent to kinetochores. The frequency of RNs at knobs is either similar to or higher than the average frequency of RNs along the SCs. These RN maps represent an independent measure of crossing over along maize bivalents and provide a means to integrate genetic linkage maps with chromosome structure.

Developmental Genetics

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A screen for non-reduction mutants in maize

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Development of normal kernels 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 plump kernels when pollinated with a 4n pollen-donor. Three 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. Flow cytometry revealed that two of the mutants produced unreduced embryo sacs. In order to analyse the altered female gametophyte development in the mutants, a protocol for confocal laser scanning microscopy was developed. To determine the viability of the protocol, embryo sac formation of the maize mutant elongate (which produces both reduced and unreduced embryo sacs) was analysed. In addition, transposon display will be used to identify the genes disrupted by Mu.

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Differential expression of a new HSP70-like gene and of the transposable elements Pong and PREM during in vitro androgenesis in maize NILs underlines new aspects of induction mechanisms

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Extensive studies have been conducted to understand the genetic control of in vitro androgenesis, but little is know about the genes and the mechanisms involved in the switch that allows an immature pollen grain to start embryo development. We have developed two maize isogenic lines, named AH5-44 and AH5-49, with high androgenetic aptitude through backcross and selection from a high responsive DH229 line on the non-responding A188 line genetic background. The genomic structure of these two lines was precisely described with microsatellites. Five regions issued from the parent DH229 highly responsive to androgenesis were localised in both AH5-44 and AH5-49. Sequences expressed on microspores extracted from the four lines were amplified using a cDNA AFLP protocol. In addition, for each line, eight culture conditions were compared: microspores extracted after tassel recovery, after 7 or 14 days in cold room, and after 1, 2, 3 and 4 days of in vitro culture. This genetic and developmental screening allowed us to identify a new HSP 70 like candidate gene, a retrotransposon PREM-1 analogue strongly expressed after microspore culture only in responsive lines, and a Pong-like transposon recently described in rice, with expression restricted to the non responsive A188 line. Possible implication of the HSP70-like sequence and of transposable elements in androgenesis response is discussed.

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Quantitative analyses and transgenic overexpression support the maize FLORICAULA/LEAFY homologs as QTL candidates.

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One of the many morphological differences between maize and its wild ancestor teosinte, is a drastic increase in kernel row number and length of the ear of maize. QTL for this morphological change have been mapped, and a large effect QTL maps to the same chromosomal location as one of two maize FLORICAULA/LEAFY homologs, zfl2. The orthologous genes are known in dicots to control inflorescence architecture by affecting the identity of lateral structures produced by inflorescence meristems. We have previously supported the candidacy of zfl2 for this QTL by analyzing its role in development through mutant analysis. We showed that plants carrying mutations in the two maize FLORICAULA/LEAFY homologs, are indeed associated with lower kernel row number and shorter ears than wild type siblings. To further test the candidacy of these genes, we analyzed quantitative effects of mutations in the zfl genes on inflorescence architecture in more detail, and used transgenic plants ectopically expressing zfl1 to ask whether increasing zfl activity in the inflorescence apex can specifically lead to higher kernel row number and ear length (more cupules per row) in maize ears. The transgenic method allows us to test that associated quantitative trends observed are specific to zfl activity, rather than the effects of linked genes. Preliminary results suggest that transgenic plants ectopically expressing the zfl1 cDNA show an increase in kernel

row number compared to non-transgenic siblings. This suggests that increasing zfl activity in the inflorescence promotes higher row number, and supports our hypothesis that increased zfl activity in the ear was selected during the domestication of maize to increase ear size.

73

tie-dyed1 promotes the sink/source transition in developing leaves

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We are interested in the signals and genetic programs coordinating leaf cell differentiation and development. To identify genes controlling these processes we are characterizing mutations with altered pattern formation during the final stages of leaf development. We focused initially on studying the tie-dyed1 (tdy1) mutant due to its nonclonal variegation. tdy1 is a recessive mutation that develops yellow and green sectors in leaves. Sectoring requires high light, is restricted to a narrow developmental time and results in the yellow tissue hyperaccumulating starch. We hypothesize that tdy1 integrates developmental, environmental and metabolic signals to promote the switch from a young, carbon importing tissue (sink) to a mature, carbon exporting tissue (source).

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An analysis of CNS-lg3-i2 in non-ligule forming plants and plants with altered ligules

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Development occurs through the coordinated temporal and spatial regulation of genetic networks. Genetic networks are integrated through many mechanisms including binding sites for regulatory proteins. Cross species comparisons have proven a useful tool for identifying conserved elements in orthologous genes. These sequences have been termed Conserved Non-Coding Sequences (CNSs). Through maize-rice comparisons the Freeling Lab has identified a Conserved Non-Coding sequence in the second intron of liguleless3 and liguleless4 knotted1-like homeobox (KNOX) genes. This family of genes is thought to encode DNA binding proteins that are involved in lateral organ development. The CNS identified in liguleless3 and liguleless4 has been named CNSlg3-i2. An analysis of lg3 orthologs from grasses representing each subfamily demonstrated that CNSlg3-i2 is most likely conserved in position, sequence, and polarity in all grasses (Lubkowitz and Freeling, unpublished). This high degree of conservation in a non-coding sequence suggests functionality. We are attempting to determine if the absence or alteration of CNSlg3-2 will be observed in plants that fail to make a ligule. To this end, we have cloned and sequenced the second intron of Lg3-O mutants and are attempting to sequence the second intron from grass relatives such as rushes, sedges, cattails, and bur reeds.

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Maize Sprouts Polarity as Factor Defining Exogenetic Phytohormones Influence

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The general morphogenesis theory, the most important side of which is systemic functions such as polarity, gradients, correlation, etc., has been actively developed for the last years. These functions correlation with hormone regulation processes and sex expression have been studied in this work by marking of gibberellic acid (–ê3 - 2.9 10-1 m/l) and benzylaminopurine (6-BAP - 4.4 10-2 m/l) the various parts of plant sprouts of two maize sorts (Zea mays Imdurata) early ripe ‚Äì Podolskaya-98 and late ripe Krasnodarskaya 382. Phytohormones‚Äô treatment has discovered that –ê3 has the greatest effect when marking apical or middle sprout parts, but 6-BAP does when treating lower or middle ones (phytohormones distribution polarity is changing in 3-4 times). Initial stages of development and flowering phases proved to be the most sensitive to the above treatment. Exogenetic –ê3 is able to accelerate late ripe sorts earing for 5-7 days and early ripe ones for 4-6 days. The cytokinin has had the opposite effect, particularly, by injecting 6-BAP into basal sprout part, which has caused delay of earing for 5-6 days in early ripe sorts. 6-BAP rather delayed coming next development phases, but later development phases of corncob went faster. In general, the cytokinin aided the earlier formation of ears and the increase of their amount. The –ê3 usually caused decrease of the ears amount, particularly in early development phases. It has been displayed that the accurate correlation between sprout parts, treated by the 6-BAP or –ê3, and the ears location height. It is obvious that ear placing and development (possibly and sprout node ones) require specific phytohormone balance in definite sprout part. To describe growth processes the mathematical models have been developed (approximation accuracy R2 is never less than 0,999). Character of the plants growth speed of the both sorts is evidence of A3 accelerating plants development. The model, describing 6-BAP influences, proved to be the mirror reflection of the model describing A3 but with less effect. Furthermore, 6-BAP is removing critical

point of dependences change for 1,5-2 metameter higher, but for all that critical point is located at the same height as in model using A3.

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Pangloss1 and Pangloss2 are Required for Polarization of Subsidiary Mother Cells in the Formation of Maize Stomata.

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One of the most important events in plant development is the formation of stomata, which control the exchange of gases and moisture between the plant and its environment. In maize, stomata are composed of four cells--a central guard cell pair flanked by a pair of triangular subsidiary cells. These subsidiary cells are formed through the asymmetric divisions of subsidiary mother cells (SMCs). We have isolated mutations in two genes that are required for the polarized divisions of subsidiary mother cells. In pangloss1 and pangloss2 (pan1 and pan2) mutants, SMCs often fail to polarize correctly, and frequently invade neighboring cell files. Cytoskeletal analysis of pan1 and pan2 has revealed abnormalities in both actin and microtubule organization during subsidiary mother cell division. Preliminary analysis of pan1;pan2 double mutants indicates these genes function independently of one another to polarize SMCs and specify subsidiary cell fate. In brick1 (brk1) mutants, subsidiary cells often fail to divide and specify correctly. Preliminary analysis of pan1;brk1 double mutants shows an additive phenotype, indicating pan1 and brk1 likely act in separate pathways to specify subsidiary cell fate.

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TRAITMILL TM :AN APPLIED GENOMICS PLATFORM FOR THE IMPROVEMENT OF CEREALS.

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CropDesign's TraitMillTM platform aims at closing the application gap between classical genomics and the development of crops with improved or novel traits. TraitMill allows high-throughput and high-resolution testing of the effect of plant-based transgenes on agronomically valuable traits in crop plants. The focus of the TraitMill platform is on rice, a globally important crop, which is also a good model for other important cereals such as corn and wheat. The Traitmill involves the following key components: i) Using a high throughput proprietary gene cloning system, expression levels of transgenes and transgene combinations can be modulated throughout entire plants or in selected tissues. ii) An industrialized plant transformation system generates the tens of thousands of transgenic plants required annually to analyse the transgene constructs. iii) Several automated plant evaluation technologies, including digital image analysis of plants, are used to detect alterations brought about in important traits such as biomass production, seed yield, thousand kernel weight, and stress tolerance. At the input side of the TraitMill, CropDesign's research team focuses on genes that modulate the size, shape and growth of specific plant tissues, as well as the plant as a whole. Plant organ growth, as well as overall plant growth and development, are also linked to interactions with the environment via multiple signal exchange networks, and by the production, uptake, translocation and sensing of nutrients. Many of the genes controlling these complex processes remain functionally undefined and are the target of functional genomics research at CropDesign. The TraitMill has generated a range interesting product leads over the last year. These leads include transgenic rice lines showing increased seed yield, increased green biomass, or increased tillering. A phenotypic description of these transgenic lines will be presented.

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Maize mutants arrested in early embryogenesis disclose an irregular pattern of cell divisions and altered programmed cell death

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Embryo-specific (emb) mutants exhibit defects in the development of the embryo, while the endosperm is essentially normal. Thus they may define genes whose products are specifically required for embryo morphogenesis. The maize emb mutants analyzed in this work are arrested at the proembryo or early transition stage. The block in embryo development is associated with abnormal suspensor proliferation, and disorganized tissue proliferation persisting during seed maturation. The program of cell death appears impaired in these mutants. In wild-type immature embryos, 14 days old, TUNEL positive nuclei are detected in the scutellar region surrounding the shoot primordium and in the coleoptile whereas in the mutants no positive cells are detectable on the embryo proper or on the long suspensor. An unexpected result is obtained when mutant embryos rescue is attempted. Immature mutant embryos transferred to a basal medium germinate and produce single seedlings that

appear morphologically normal although retarded in their growth. The culture of these embryos may cause the induction of the shoot by promoting the formation of a single SAM in an undifferentiated region of the embryo. The analysis of emb mutants appears a promising tool to elucidate crucial points of embryo development such as the coupling of cell division with morphogenesis, the cell to cell interactions, the relationship between embryo and endosperm development and the interaction between embryo proper and suspensor.

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Functional analysis of the maize FT/TFL homologs reveals potential players in the floral transition.

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The transition from vegetative to reproductive growth is a critical point in plant development. Despite the importance of flowering time as a developmental process and agronomic trait, only one maize flowering gene, id1 (indeterminate-1), has been cloned. However, genetic pathways leading to the initiation of flowering are well defined in Arabidopsis. The Arabidopsis flowering genes FT (flowering locus T) and TFL (terminal flower) encode 23-kD proteins with similar structures, but antagonistic functions. FT promotes flowering, whereas TFL acts as a repressor. The FT/TFL proteins are evolutionarily conserved between dicots and monocots. Eight maize FT/TFL homologs were found in the Pioneer/DuPont maize EST (Expressed Sequencing Tags) database. Phylogenetic analysis placed 6 maize genes into the FT clade and 2 into the TFL clade. Electronic Northern and RT-PCR revealed different expression patterns of maize FT/TFL homologs. Both ZmTFL1 and ZmTFL2 are expressed in the shoot apical meristem during vegetative growth but not expressed in the reproductive meristem. This result is consistent with their possible roles as repressors of flowering. Conversely, only one maize FT homolog, ZmFT1, is expressed in the apical meristem just after the floral transition. Five other FT homologs show expression in different tissues. ZmFT1 transcripts are absent in the id1-m1 mutant, suggesting that ZmFT1 activation in the apical meristem during the floral transition requires Id1 function. ZmFT1 maps to the flowering QTLs on chromosome 8. Our results suggest that ZmFT1 and ZmTFL1- ZmTFL2 are candidates for maize flowering genes with possible antagonistic functions like the Arabidopsis genes.

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Biochemical characterization of the FEA2 protein

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The maize mutant fasciated ear2 (fea2) displays a massive over-proliferation of the ear inflorescence meristem. The modestly affected floral meristem gives rise to a small increase in floral organ number. This suggests that FEA2 acts to limit the growth of these meristems. Cloning of the FEA2 gene via transposon tagging showed it encodes a leucine-rich repeat receptor-like protein, predicted to be plasma membrane targeted. The most closely related protein is CLAVATA2 (CLV2) from Arabidopsis, which also negatively controls the proliferation of pluripotent stem cells in shoot apical meristems. This indicates that the CLAVATA pathway has been functionally preserved beyond the monocot to dicot split in evolution. Genetic and molecular data suggest CLV1and CLV2 form part of a receptor complex, to which a ligand encoded by CLV3 binds. Protein extracts from young ears were size fractionated to see whether FEA2 is part of a higher molecular weight complex. Preliminary data shows FEA2 is part of a membrane protein complex eluting between 400-500kDa. The FEA2 profile overlaps with that of CLV1, which forms a complex of around 450kDa, though CLV1 also forms a 185kDa complex. In addition, it was found that under denaturing conditions, the FEA2 monomer migrated at a higher molecular weight of 70 kDa than the predicted 64kDa. Specific removal of oligosaccharides from N-linked glycoproteins accounted for this size difference.

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Molecular Genetic Analysis of rgh Endosperm Mutants

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Much effort has been directed to maize endosperm improvement for its importance in feed and food making as well as its use for secondary products such as oils. The maize endosperm shows many similar developmental processes of other plant organs in addition to being an important storage reserve for the seed. An understanding of endosperm developmental processes could provide a basis for develop more efficient approaches to seed improvement as well as generate a model for organ development in maize. The rough endosperm (rgh) class of seed mutants disrupts normal endosperm and embryo development and is characterized by seeds with a pitted or etched surface. The development of a mutagenic inbred (UniformMu) (McCarty et al., NSF award 007676)

provides a unique tool for efficient and rapid characterization of transposon-tagged mutants. UniformMu is a Robertsonís Mutator transposon-active population that it is introgressed into the W22 color-converted inbred. Robertonís Mutator elements typically have 50-200 copies/genome which creates challenges for molecular genetic analysis. The UniformMu population is carefully pedigreed with known non-mutant progenitors and carries a somatic transposon active marker to select against further transposition once mutants are identified. We have identified ~80 rgh mutant isolates from the UniformMu population. Because of the high number of isolates (~180 estimated) required to uncover all rgh loci (~30 rgh loci) and the fact that most rgh homozygous mutants are not viable, a traditional genetic analysis is not reasonable. Instead, we are using phenotypic, molecular and genetic map data to sort rgh mutants into allelic groups. Once loci are identified we will concentrate on detail genetic and morphological analysis of 3-5 rgh loci that represent the range of seed phenotypes within this class of mutants.

82

Three Brick Genes Are Required for Polarized Growth and Division in Maize Leaf Epidermal Cells

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Cells of the maize leaf epidermis assume a wide variety of shapes, and cell polarity plays an important role in determining these diverse shapes. To gain new insights into the mechanisms governing cell polarity, we isolated mutations in three Brick genes, which affect maize leaf epidermal cell morphogenesis. 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, pavement cells expand normally without forming these marginal lobes, resulting in rectangular 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. Also, when wheat mesophyll cells were treated with an actin-disrupting drug, these cells failed to form lobes, and microtubules did not form bands, suggesting 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 in expanding brk cells, suggesting an additional role for actin in lobe formation. In addition to the effect on pavement cell shape, in brk leaves, stomatal subsidiary cells form abnormally, and epidermal hairs are shorter and blunter than wild type hairs. Double mutant analysis indicated that all three Brk genes are involved in the same pathway or process affecting epidermal cell morphogenesis. The Brk1 gene was cloned and shown to encode a small, novel protein, which is highly conserved in both plants and animals. Recent work on HSPC300, the BRK1 mammalian homolog, has shown that HSPC300 forms a complex with four other proteins including WAVE1, an activator of the Arp2/3 complex. These observations suggest that BRK proteins are involved in the local regulation of actin polymerization in plant cells.

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Functional analyses of EMPTY PERICARP2, an essential regulator of the heat shock response that is required for maize embyogenesis.

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The heat shock response (HSR) is an evolutionarily conserved molecular/biochemical reaction to thermal stress that is essential to the survival of eukaryotic organisms. Previous analyses reveal that recessive, null mutations at the maize empty pericarp2(emp2) locus lead to dramatically increased expression of heat shock genes, retarded embryo development, and early-stage abortion of embryogenesis. Cloning and sequence analyses revealed that emp2 encodes a predicted protein with high similarity to HEAT SHOCK BINDING PROTEIN1, which was first described in animals as a negative regulator of the HSR. The emp2 locus is duplicated in maize (Zmhsbp2) and other grass species, although only one hsbp is identified in animals and eudicots. However, the embryo-lethal emp2 mutant phenotype reveals that the hsbp homologues emp2 and Zmhsbp2 are functionally divergent during kernel development. Clonal analyses further reveal that emp2 and hsbp2 perform non-redundant functions in adult shoot tissues. Surprisingly, emp2/-sectors condition phenocopies of maize mutants affecting phyllotaxy, ligule development and leaf development. Likewise, the differential interaction of EMP2 and ZmHSBP2 with maize HEAT SHOCK FACTORS as revealed by yeast two-hybrid analyses further supports the model that EMP2 and HSBP2 are functionally divergent. Moreover, the developmental retardation of emp2 mutant kernels before they are competent to invoke the HSR suggests an additional role for EMP2 during embryo development, beyond regulation of the HSR. We are currently investigating this model by yeast two-hybrid screening and immunoprecipitation analyses of developing maize kernels.

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The Protein Secretory Pathway is Upregulated in Several *opaque* Mutants

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To better understand the mechanisms that lead to an opaque or starchy endosperm phenotype, we performed a thorough analysis of mRNA transcript profiles in several opaque mutants: *opaque1*, *opaque2* (*o2*), *opaque5*, *opaque9*, *opaque11*, *floury2*, *defective endosperm B-30*, and *mucronate*. The mutations were introgressed into the inbred line W64A, backcrossed six generations, and transcript profiles of mRNAs present at 23 DAP were obtained using Curagenís Gene Calling technology. Genes that showed changes in expression for a majority of the mutants revealed information about disrupted metabolic processes. One generally altered metabolic pathway was protein synthesis. The expression of several ribosomal protein genes was reduced approximately two-fold. However, there was no evidence for a reduction in the abundance of protein synthesis initiation or elongation factors. The most surprising change was a substantial increase in expression of genes related to vesicle trafficking and organelle biogenesis. These included cytoskeletal genes, such as actin and katanin. Additionally, mRNAs encoding Sec31 and t-SNARE homologs, factors involved in vesicle budding and fusion, were increased. Furthermore, vacuolar sorting receptors, tonoplast intrinsic proteins, and proteins predicted to localize to vacuolar compartments were increased in expression. These changes indicate that protein storage may be redirected from ER-bound zein protein bodies to protein storage vacuoles. Proteomic analysis of CM105, CM105 *o2*, and modified CM105 *o2* endosperm proteins revealed that globulin 1 and globulin 2 are increased significantly, as is a Rab GTPase, further supporting the hypothesis that the protein secretory pathway is altered in opaque mutants.

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GENOMIC CLONING THROUGH APOMIXIS RESULTS IN EXTENSIVE EPIGENETIC VARIATION

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Apomixis in higher plants is defined as the process of cloning through seeds. Apomictic plants bypass both female meiosis and fertilization, resulting in progenies that are genetic replica of their mother plant. This method of genetic cloning, which does not exist as yet in any major crop is regarded as a revolutionary tool in agriculture; it could allow fixing indefinitely the best allelic combinations, including those of high yielding hybrids. Here, we studied the genetic consequences of apomictic cloning in hybrid plants between maize and its apomictic wild relatives, Tripsacum dactyloides. We show that apomixis, while allowing faithful reproduction of the genome of the maternal plant, also results in extensive phenotypic variation. This variation results from inaccurate reproduction of the epigenetic state prevailing in the mother genome.

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Clonal analysis of Wavy auricle in blade (Wab)

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Maize leaves are composed of two distinct parts, the distal blade, which is flat and leans away from the plant, and the sheath, which wraps around the culm. At the junction of the blade and sheath is the ligule region which contains the ligule itself, a flap of epidermal fringe, and the wedge-shaped auricles on either side of the leaf. The auricles function as hinges for the blade. The liguleless mutants, lg1 and lg2, lack auricle and ligule, but still have a clearly distinct blade and sheath. Wavy auricle in blade (Wab) mutants are characterized by two distinct features, the leaves are more narrow than wild-type, and proximal tissues, such as sheath and auricle, are found in the blade. The leaf blade of double mutants between lg1 or lg2 and Wab is very narrow. The sheath extends into the blade such that a distinction between the two tissues is lost except at the midrib. We have carried out a clonal analysis, in the presence and in the absence of a functional lg1 gene, to determine how Wab regulates leaf shape. Our results suggest that the two defects may be separable, white sectors in the blade (indicating the absence of Wab) lack sheath and auricle characteristics, but are still narrow. The data also allow us to produce a fate map of the narrow lg; Wab leaves.

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ragged seedling2 fails to maintain the dorsoventral axes of leaf tissues in maize

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Unlike previously described dorsoventral mutants in various plant species, radial leaves of ragged seedling2 (rgd2) mutants retain dorsoventral polarity. Less-severe mutants exhibit planar leaves with defects in the

dorsoventral orientation of vascular and epidermal tissue: vascular bundles are rotated out of their normal positions and the lamina of mildly affected leaves display patches of epidermal tissue that have an inverted dorsiventral identity. rgd2 is a novel, recessive mutation that maps to chromosome 1S, proximal to the translocation breakpoint and near roughsheath2. Evaluation of rgd2 mutants provides important insights into characteristics of adaxial and abaxial leaf tissue, dorsoventral patterning of maize leaves, and leaf development in general.

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PARAMUTATION AND PLANT DEVELOPMENT REQUIRE rmr12 FUNCTIONS

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Genetic and molecular analyses of paramutation at the purple plant 1(pl1) locus are ongoing to gain insights into general chromosome processes used in gene control. The PL1 R2R3-Myb protein is a transcriptional activator of the genes encoding enzymes required for anthocyanin biosynthesis. Heritable epigenetic changes occurring at pl1 are thus reflected by visual differences of anthocyanin pigment levels and tissue-specific pigment patterns. 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. Stable maintenance of the silenced Plí state requires undefined interactions with a second Pl-Rh or Plí allele and at least eight distinct trans-acting factors, mop1 (mediator of paramutation; Dorweiler et al 2000), rmr1 and rmr2 (required to maintain repression; Hollick and Chandler 2001), rmr6, rmr7, rmr8, rmr9, and rmr11 (J. Hollick, unpublished). Genetic and phenotypic analyses of two mutations isolated from an EMS pollen mutagensis define one additional trans-acting factor encoded by a novel locus, rmr12. Both mutations behave as stable recessive alleles that are unlinked to the pl1 locus and fail to genetically complement each other. Homozygous mutant plants that are genotypically Plí/Plí display a strong Pl-like phenotype while the expression of other pl1 alleles appears unaffected. Unlike most rmr mutations, homozygous rmr12 mutants have strong defects in plant growth and development. These defects are distinct from those associated with mop1 and rmr6 mutations, thus indicating that individual components required for paramutation-based epigenetic repression play selective, rather than simply general, roles in the control of developmental genetic programs. Hollick, J.B. and Chandler, V.L. (2001) Genetics 157: 369-378. Dorweiler, J.E. et al. (2000) The Plant Cell 12 (11): 2101-2118.

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The characterization of pleiotropic shoot phenotype in *leafy coleoptile(lco)1-R*

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In the novel maize coleoptile mutants *leafy coleoptile(lco)*, the coleoptile is converted into leafy structure. The *lco1-R* coleoptile has multiple veins with blade/sheath and ligule development at the margin. It originally splits in the embryo and lacks mesocotyl. Besides this coleoptile phenotype, *lco1-R* exhibits various shoot defects. The dwarf plant lacks any reproductive organs. The leaves are narrower and shorter than normal leaves and without midrib. Not only the midrib, but the vasculature is also affected, and epidermal structure is disorganized. The ligule displacement is often found in the seedling leaves. Immunolocalization has given a pattern of RS1/GN1 knox genes expression in leaves that indicates some extopic expression, which is now being confirmed.

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Hypomethylation at Pl-blotched is reset by shoot meristem culture-induced rejuvenation

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Phase change is the transition from one epigenetic state, the non-flowering, juvenile phase, to another, the adult phase where flowering can occur. Methylation status of the DNA at the blotched allele of Pl isolated from leaves is correlated with phase of the plant at the time the leaf differentiated (1), and the juvenile pattern of methylation is restored with each new generation. To test whether methylation status can be modified independently of passage through a sexual generation, we used shoot meristem culture (2) to rejuvenate Pl-blotched plants. We found that of 22 plants rejuvenated by culture, all showed initial reductions in methylation, followed by a return to adult patterns of methylation upon acquisition of the adult phase morphology. Some 13 of these plants had methylation patterns identical to seedlings in their basal leaves. Thus, methylation status can be reversed upon rejuvenation of the shoot. This result supports the view that methylation provides a mechanism of maintaining an epigenetic state. 1. Hoekenga, Muszynski, and Cone (2000), Genetics 155, 1889-1902. 2. Irish and Karlen (1998), Int. J. Pl. Sci.159, 695-701.

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Inbred modifier effects on adaxial patterning of the Maize leaf

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We have identified three mutant loci that affect adaxial patterning in the developing maize leaf. All mutant loci have been introgressed five times into several different inbred lines. Dramatic differences in the severity of the mutant phenotypes were observed in the various inbred backgrounds. Interestingly, each inbred line modifies the severity of the set of mutations in a similar way. This suggests that all three mutations affect the same process, which is modified in a consistent manner in all inbreds analyzed. One exception to the general pattern is the *ragged seedling (lbl1-rgd1)* allele of *leafbladeless1 (lbl1)* in the W22 background. In contrast to the extremely mild phenotypes of the other adaxial patterning mutants, the phenotype of *lbl1-rgd1* is most severe in W22. This suggests the presence of a *lbl1-rgd1* allele specific modifier in W22. Intercrosses were made with *lbl1-rgd1* in the W22 and B73 inbred backgrounds to determine the action of a W22 inbred specific modifier. The phenotypes observed are consistent with the presence of a single dominant modifier in W22.

In the W22 inbred the *lbl1-rgd1* mutation is embryo lethal. The *lbl1-rgd1* 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 maintance defect. In wild type vegetative SAM the maize *yabby* gene *(zyb)* is expressed in the adaxial domain of the young leaf primordia. We are currently analyzing the expression pattern of *zyb* in the scutellum. Which will provide more insight into the nature of the polarity defect in the *lbl1-rgd1* mutant embryo.

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Interactions between XCL1 and KNOX genes

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An important question in plant developmental biology is how division and differentiation are balanced in shoot apical meristems (SAMs) in order to maintain meristematic cells and to allow for the production of leaf primordia from the flanks of the meristem. KNOX genes play an important role in the maintenance of meristem identity. In simple-leafed model species such as maize and Arabidopsis, class 1 KNOX genes are downregulated in the P0 and remain turned off throughout leaf development. Dominant, neomorphic mutations in maize KNOX genes such as KNOTTED1 (KN1), lead to the production of knots of tissue over veins and ectopic ligules which require periclinal cell divisions in epidermal cells (Gelinas et al., 1969). In addition, the severity of kn1 loss-of-function phenotypes is worsened when the mutation is introgressed into inbred backgrounds with shorter meristems (Vollbrecht et al., 2000), indicating that KN1 plays a role in maintaining meristem size. Extra cell layers1 (Xcl1) is a semi-dominant, hypermorphic mutation that causes the overproduction of a normal gene product and affects cell division and differentiation patterns in developing leaves and kernels. Oblique, periclinal divisions in the protoderm give rise to multiple epidermal layers in leaves. Leaves are twice as thick but only half as wide as normal sibling leaves. In addition, Xcl1 meristems are 60% shorter than normal sibling meristems, and recruit more cells into P0 (Kessler et al., 2002). Thus, Xcl1 shares some phenotypic similarities with KNOX mutants. The short meristems and increased recruitment of cells into P0 seen in Xcl1 mutants may reflect compromised KNOX activity. Double mutant analysis between Xcl1 and dominant KNOX mutants indicate that Xcl1 is a suppressor of the Kn1 phenotype, but may be an enhancer of Gnarly1 and Rough sheath1. The developmental implications of these regulatory relationships will be discussed.

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Spatial and temporal control of programmed cell death (PCD) in developing caryopses of maize.

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We show here that a developing caryopsis is chimeric in spatial and temporal control of PCD that occur throughout the duration of kernel development. PCD was visualized at a cellular level in both maternal (placento-chalazal layer in pedicel and nucellus) and filial endosperm tissues (regions surrounding the embryo and starchy cells). PCD in the placento-chalazal layer was fertilization-dependent and was marked by loss of nuclei and all subcellular membranous organization in all cell layers during 6 ñ 12 days after pollination. These ëemptyí cells, however, remained intact throughout kernel development. Because enucleation was initiated early, coincident with endosperm cellularization, and was completed rapidly prior to the beginning of the storage phase in

endosperm, we suggest that PCD may be causal to functional activation of the placento-chalazal layer. Remarkably, a second wave of PCD, apoptotic in nature as diagnosed by TUNEL stain, was seen in only a subset of cells underneath the enucleated placento-chalazal cells. Furthermore, such TUNEL staining was seen in the wild type, *Mn1*, but not in the invertase-deficient *miniature seed1* (*mn1*) seed mutant that leads to reduced size and weight of the endosperm. Overall, the two pathways to PCD in these maternal cells appeared to be signaled by various developmental changes in filial endosperm and its metabolic sink strength. By contrast, the enucleation and PCD in autolysing nucellar tissue and endosperm cells surrounding embryo, and in starchy endosperm that showed only the enucleation in otherwise well-packed cells that remain intact until germination, preceded and/or coincided with the TUNEL stain.

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Sucrose synthase isozyme SUS1 in the maize root cap is preferentially localized in the endopolyploid outer cells

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Spatial distribution of sucrose synthase isozyme SUS1 was studied in the maize root cap in relation to the level of endopolyploidy. Median longitudinal root cap sections were analyzed using immunolocalization, quantitative DNA staining and image cytometry. High immunolocalization signal for the SUS1 protein was detected in the cells of the outer root cap, while the signal was low in the meristematic and the central root cap region. The meristematic cells had ploidy levels of 2C and 4C corresponding to the G1 and G2 phases of the cell cycle, respectively (1C is the DNA content of the unreplicated haploid genome). With respect to spatial distribution, both the SUS1 signal and the level of endopolyploidy increased from calyptrogen towards the root cap periphery, and were thus the highest in the outer root cap cells. About 90% of cells with ploidy level 16C or higher exhibited a high SUS1 signal, compared to only 5% of the 2C cells. The high amount of the SUS1 protein in the outer, endopolyploid cells suggests an association between endoreduplication and the abundance of this enzyme. Because the outer root cap cells are involved in mucilage production, sucrose synthase located in these cells may provide monosaccharide precursors for mucilage synthesis.

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Mu-Tagged Empty Pericarp Mutants in the UniformMu Maize Population

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A phenotypic analysis was undertaken to further define the extent of variation among the Mu-tagged empty pericarp (ep) mutants isolated from the UniformMu population. The ep mutants identified thus far (ca 25% of total Mu-tagged seed mutants) were initially classified on the basis of external visual analysis that indicated little or no internal seed structure was present. However, examination via longitudinal sections showed significant endosperm and/or embryo formation in many 'ep' kernels. The range in degree of mutant severity revealed corresponding differences in timing of developmental termination for embryo and/or endosperm. The ep mutants of intermediate severity in this population often showed evidence of initial endosperm or nucellar development resulting in hollow, translucent, paper-like structures within kernels. Further analysis will be directed toward identifying the sites and timing of developmental cessation in the different ep classes observed in the UniformMu population, and on molecular isolation of the relevant genes.

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The indeterminate floral apex1 (ifa1) mutant phenotype is associated with a mutation in the Zea mays MADS box 14 (ZMM14) gene.

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Maize inflorescence architecture is controlled by the activity of inflorescence meristems during development. Both the identity and determinacy of inflorescence meristems are afffected in the indeterminate floral apex1 (ifa1) mutant. Ifa1 mutants have a loss of determinacy in the Floral Meristem (FM), Spikelet Meristem(SM), and Spikelet Pair Meristem (SPM) of the tassel, displaying extra floral organs, extra florets and and extra spikelets in the male inflorescence. In the female inflorescence, the FM?s are affected, with nucellar-like proliferative tissue growing at the floral apex between rudimentary silks. MADS box genes are involved in floral development in a wide range of plants, affecting both organ identity and meristem determinacy. Many lines of evidence indicate

that the ifa1 phenotype is caused by a mutation of the MADS box gene, ZMM14. Mapping data and expression studies show that ZMM14?s map position and expression are linked to the mutant phenotype. A Mu insertion 150bp 5?of ATG shows a weak ifa1-like phenotype when homozygous, and complementation crosses between this insertion and the ifa1-Reference allele are ongoing. A strong ifa1-like phenotype is observed when the duplicate gene, ZMM8, is interrupted by a Mu insertion. Taken together, this evidence suggests that the ifa1 phenotype is probably caused by a mutation at the ZMM14 locus.

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The maize *Disorgal 1* and *Disorgal 2* genes restrict mitotic division plane in the aleurone layer and is necessary for normal epidermal cell development and plant stature.

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The aleurone layer of maize endosperm consists of a single layer of highly regular epidermal like cells. As part of our effort to identify genes controlling the cell division plane in the aleurone layer, we are characterizing two mutant loci, *disorgal 1* (*dal1*) (three alleles) and *disorgal 2* (*dal2*) (one allele) which display a highly irregular organization of the aleurone layer. Both *dal1* and *dal2* are inherited as simple mendelian traits. In *dal1* and *dal2* homozygous endosperms, the control of mitotic division plane is relaxed or missing, resulting in grains with disorganized aleurone layers. The cells of the aleurone layer are highly irregular, both with respect to cell size and shape, and often more than one layer of aleurone cells are present. Homozygous *dal1* and *dal2* grains are shrunken due to reduced size of the starchy endosperm and developmental arrest of the embryo at premature stages. Mature mutant grains germinate at a very low rate and fail to develop into plants. However, homozygous mutant plants can be obtained through embryo rescue. *dal1* plants have broad leaves that become necrotic towards the tip, whereas *dal2* plants are extreme dwarfs. Both *dal1* and *dal2* plants display irregular leaf epidermis as well as reduced number of root hairs. Taken together, these results indicate that *dal1* and *dal2* are involved in mitotic control and cell patterning in epidermal like tissues. Efforts aiming to clone the *dal1* and *dal2* genes are underway.

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Genetic Analysis of the Maize Rop2 Gene

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Rop family GTPases have been implicated in the regulation of plant signal transduction and cell morphogenesis. In collaboration with Pioneer Hi-Bred, we have isolated a collection of mutant rop2 alleles to explore ROP2 function in maize. In genetic crosses, three of five rop2 mutant alleles (rop2-m1, rop2-m2, and rop2-m5) transmit at a lower frequency through the pollen than the wild-type allele. Transmission through the female gametophyte was not affected by the rop2 mutation. The male-specific transmission defect associated with the rop2 mutations strongly supports an important role for rop2 in pollen function. We have been investigating the effects of the rop2 mutation on pollen germination, pollen tube growth and guidance, and fertilization. Measurement of pollen tube germination and growth rate in vitro indicated that both are unaffected by the rop2 mutation. In vivo pollen tube growth patterns, as well as pollen tube penetration of the style, did not appear significantly different between wild-type and rop2 mutant pollen. We are now focusing on the final stages of pollen function: pollen tube growth patterns near the ovule and fertilization of the egg sac.

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Comparative Analysis of MADS-Box Sequences in Arabidopsis and Rice

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MADS-box genes are important transcription factors involved in various biological processes in plants, especially in floral development. The availability of the whole genomes of Arabidopsis and rice makes it possible to conduct comparative analysis of this gene family in the two model plants of dicots and monocots respectively. We compared 62 rice MADS-box sequences with 72 Arabidopsis MADS-box sequences. Among nine phylogenetic groups containing both rice and Arabidopsis MADS-box sequences, six were involved in flower development or flowering time; one group represented by AGL12 works in root development, and the other two groups have not

been functionally studied. There are nine groups containing only Arabidopsis MADS-box sequences with more than two members in each group, including the MADS-box genes responsible for vernalization represented by FLOWER LOCUS C. Five groups contain only rice MADS-box sequences, including the groups composed by OsMADS2 and OsMADS4 that are potential orthologues of Arabidopsis PISTILATA (PI). A consensus sequence was generated using MADS-box sequences from Arabidopsis, rice and tomato. Comparison of MADS-box sequences with this consensus sequence showed that functionally known MADS-box sequences were most similar to the consensus sequence, whereas nearly half of the MADS-box sequences that have not been studied functionally were highly diversified. Our results showed that, although rice and Arabidopsis have respectively developed MADS-box genes to meet their special developmental needs such as flowering time, the ABC model that was firstly established in dicots could almost fully extend to monocotelydonous plants such as rice.

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Developmental Analysis of Transgenic Maize Lines that Overexpress Glossy15

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The glossy15 (gl15) gene activates juvenile and suppresses adult leaf epidermal cell identity in response to upstream factors that regulate vegetative phase change. Previous genetic and molecular analyses suggested that the expression of juvenile leaf epidermal traits during shoot development is correlated with gl15 activity, which is stimulated by the Corngrass1 (Cg1) and Teopod (Tp) mutations but downregulated by factors such as gibberellins and Viviparous8 (Vp8) that promote the switch to adult vegetative development. To test this hypothesis, we generated transgenic maize lines that overexpress gl15 in an appropriate developmental context, due to the integration of additional functional copies of a gl15 genomic fragment that fully complements gl15 mutants. The phenotypic effects of gl15 overexpression were characterized in a number of different genetic backgrounds, including inbred lines that differ in their proportion of juvenile leaves as well as the Cg1, Tp1, Tp2, dwarf1 and vp8 mutations that enhance the expression of juvenile traits during shoot development. The results from these analyses support a model where gl15 operates via a threshold activity mechanism to regulate leaf epidermal cell identity, with gl15 activity levels being determined by the antagonistic effects of upstream factors that promote either juvenile or adult shoot development. High gl15 activity is sufficient to specify juvenile and suppress adult leaf epidermal cell differentiation, resulting in leaves with only juvenile traits. Intermediate levels of gl15 activity result in the expression of both juvenile and adult epidermal cell traits (e.g. transition leaves), whereas only adult traits are expressed once gl15 activity falls below a minimum threshold required to activate juvenile traits.

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Functional genomics of rice grain development : Establishement of a database of mutant phenotypes and enhancer trap gus expression in mature seeds.

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In the framework of the French national initiative GÈnoplante, a T-DNA/Tos17 insertion lines collection in rice is being developed in Montpellier (See communication 'Creation of a genome-wide library of insertion lines in rice', Sallaud et al.). This mutants library gives the very interesting opportunity to collect knowledge on grain development for rice and cereals in general. We are screening the insertion lines for seed mutant phenotypes and for expression of a reporter gene in mature seeds. In most cases, the collection allows to directly observe some seed phenotypes on the primary transformantsí progenies without additional sowing. Indeed, we can easily observe a mendelian segregation where a quarter of the seeds presents a mutant phenotype in a case of a recessive mutation. We have already evaluated several thousand lines mainly for seed and endosperm morphology. We will present the different types of mutations found and their frequencies. Using the gusA gene enhancer trap located in the T-DNA, we have also evaluated the gus expression pattern in dry seeds. Examples and frequencies of gus expression in seeds will be presented. Our first results in rice and the results obtained in well-characterised Arabidopsis mutants collections will be compared. Moreover, FST sequences available for a large proportion of the library will give us access to putative key genes involved in seed development and filling. Cytological and molecular aspects of key developmental stages of rice seed are also investigated in order to further characterise more precisely interesting mutants. Finally we will discuss how the development of such rice kernel phenotype database will allow in silico reverse genetics for any rice and cereals genes and will serve most of the Genoplante Seed and Grain projects.

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extended auricle (eta), an Essential Component in the Developmental Network Controlling Maize Leaf Development

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We have characterized a novel mutant eta, extended auricle, which is involved in proximodisal patterning in the maize leaf. The most noticeable phenotype is an extension of auricle tissue in to blade and a distal displacement of the blade/sheath boundary. While the eta phenotype is very similar to the suite of dominant Knotted1-like homeobox (knox) mutants, it is a recessive mutation and does not ectopically express knox genes. However, eta acts as an enhancer in combination with proximodistal patterning mutants like Kn1, Lg3, Gn1, Rs1and rs2. There is a precedent for this type of interaction in Arabidopsis. The Arabidopsis ortholog of rs2, asymmetric1shows a genetic interaction with the chromatin remodeling factor pickle (pkl). Although pkl itself does not ectopically express knat1 and knat2, it is thought to act as a general repressor of knox genes in the leaf based on its enhancement of as1. eta also has a synergistic interaction with lg1 and a dosage effect with lg2. Neither lg1 nor lg2 are misexpressed in the eta mutant. SEM analysis reveals Eta+ functions early in leaf development to properly establish the blade/sheath boundary. In addition, we describe results from a mosaic analysis showing Eta+ functions cell autonomously and is required in all three internal cell layers for wildtype phenotype.

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A transient expression system for maize silks

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From the moment they emerge from the husks, maize silks provide an entryway into the ear for pollen and various pathogens. As part of an effort to engineer plants resistant to earmold, we developed a transient expression system for the analysis of novel gene expression in silks. Silks are bombarded while still attached to cob explants, and are then cultured on a sucrose/ascorbic acid-containing media for up to several days. We have used this system to study silk gene expression both before and after pollination. Experiments with the maize ubiquitin promoter driving GUS or a reef coral fluorescent protein reveal pollination-triggered changes in expression patterns. This transient system serves as a tool for screening transgenes in silks prior to larger-scale, stable analysis.

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Developmental analysis of the ramosa3-fasciated ear 1 mutant

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To understand what kind of genetic and molecular mechanisms regulate maize inflorescence architecture, we have analyzed the mutants that show fasciated phenotypes, in our lab. Interestingly, the fasciated ear 1 (fea1) mutant which is allelic to ramosa 3 mutant displays a fasciated ear phenotype in some genetic backgrounds (A619 and W23) and a branched ear phenotype in another background (B73). That is, in A619 and W23, fea1 ears abnormally flatten with irregular rows of seeds, and in B73 some spikelet pair meristems at the bottom of the ears go on to form indeterminate branch meristems. It seems the genetic modifier in A619 is dominant to the one in B73. We will present scanning electron microscopy analysis, histological analysis and in situ hybridization with the KNOTTED 1 (KN1) gene marker. Preliminary results of double mutant analysis with kn1, and mapping data using simple sequence repeat polymorphisms will also be presented.

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Inhibition of polar auxin transport disrupts KNOX protein regulation, founder cell recruitment, and elaboration of leaf margins in maize shoots.

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Maize leaves develop basipetally (Poethig and Szymkowiak, 1995); the upper blade emerges first from the shoot apical meristem (SAM) whereas the sheath expands later in leaf development. Founder cells, maize leaf initials located in the peripheral zone of the SAM, are distinguished from cells in the SAM proper by the differential accumulation of KNOX proteins (Smith et al., 1992). KNOX proteins accumulate in the SAM, but are excluded from maize leaf primordia and leaf founder cells. As in Arabidopsis and tomato (Reinhardt et al., 2000), maize shoots containing five extant leaf primordia fail to initiate new leaves when cultured in the polar auxin transport inhibitor NPA. We demonstrate that NPA-induced arrest of leaf initiation in maize is correlated with the failure to downregulate KNOX accumulation in the peripheral zone. Moreover, inhibition of polar auxin transport in

cultured apices results in the formation of tubular leaf bases, in which the margins fail to emerge and separate in the lower leaf zone. The tubular leaf bases always formed in the fourth leaf from the meristem (P4). Invariably, the unseparated margin regions of the tubular leaves accumulate high levels of ectopic KNOX proteins. These data suggest that the lower, sheath margins emerge from the disc of insertion (Sharman, 1942) late in maize leaf development, and that the elaboration of leaf margins is correlated with auxin transport and downregulation of KNOX proteins. In addition, these results suggest that the regulation of KNOX accumulation is not upstream of polar auxin transport; a more complex feedback relationship is implied. A model for L1-derived margin development in maize leaves is presented.

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RAB2 contributes to orderly cell division and expansion during leaf development

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The maize leaf is composed of an orderly array of cells that grow by coordinated cell division and expansion. Cell pattern mutations, however, cause general disorder when cells expand and divide abnormally, disrupting the balance of cell size and shape. It is likely that similar cellular defects are due to mutations in many different classes of genes. One of these mutations identifies a gene, warty1 (wty1, also rli1) that encodes RAB2, a small GTP binding protein involved in vesicle transport in plants and yeast. We speculate that disrupted RAB2 alters vesicle transport sufficiently to prevent cells from receiving signals to stop expanding. To examine the effect of various disruptions in Rab2 on cellular phenotype, we isolate cell clusters from leaves and use a modification of in situ PCR to identify somatic Mu insertions in Rab2. Leaves showing late somatic Mu activity are used. Clusters composed of 10 to15 abnormal wart-like cells and adjacent normal cells are removed by microdissection, amplified using Mu and gene-specific primers, and the products sequenced. A majority of isolated warts are due to insertions of Mu into Rab2 based on statistical analysis of up to 100 wart and non-wart clusters. Furthermore, Mu inserts throughout the gene, but at statistically higher frequency into intron 2 of Rab2. The remaining warts not caused by Rab2 insertion could be due to insertions in candidate genes that encode proteins in the RAB signaling pathway. Similar analyses could help to identify these myriad interacting genes as sequence data become available from maize, rice and other organisms.

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Adaxial/Abaxial polarity specification in Maize leaf development

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The first steps in lateral organ formation include the recruitment of founder cells from the meristem and the establishment of new developmental axes relative to the main body axis. Characterization of the *leafbladeless1(lbl1)* mutant phenotype suggested that *lbl1* is required to establish adaxial cell identity in leaves and leaf-like lateral organs. In the absence of LBL1 activity, cells obtain an abaxial identity that results in the formation of radially symmetric, abaxialized leaves.

The semi-dominant *Rolled1-O (Rld1-O)* mutant of maize also affects adaxial/abaxial patterning in that the polarity of the leaf is inverted. Double mutants between *Rld1-O* and *lbl1* resulted in a mutual suppression of both phenotypes. These results suggest that *lbl1* and *rld1* act in an opposing fashion on the same pathway, or that *lbl1* and *rld1* negatively regulate each other.

In order to further characterize the *lbl1, Rld1* and *lbl1;Rld1* double mutant phenotypes, we isolated the maize homologs of the *Arabidopsis YABBY* genes which are expressed in the incipient primordium and in the abaxial domain of developing lateral organs. The maize *yabby* genes *(zyb)* are also expressed in the incipient and young leaf primordia, but interestingly expression is restricted to the adaxial side. In situ hybridization and RT-PCR data both suggest that *zyb* is down-regulated in *lbl1* mutants and up-regulated in *Rld1* mutants. The *zyb* expression pattern further suggests they function during lateral outgrowth. Our genetic and *zyb* expression data suggest *lbl1* and *Rld1* act in the same pathway and upstream of the *zyb* genes. We have preliminary data on the cloning of *rld1*. Experiments with further analysis will be presented.

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Vegetative Phase Change and Response to Puccinia sorghi in Sweet Corn

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We performed three cycles of divergent full sib recurrent selection to develop early (adult earlier in development) and late (adult later in development) phase change populations. Divergent selection for vegetative phase change was successful with last leaf with juvenile wax exhibiting a strong linear response to selection. The average last leaf with juvenile wax in C0 was 9.7. Average leaf number for C3L (late) was 11.7 while that for C3E (early) was 8.2. We evaluated the populations for resistance to common rust (Puccinia sorghi) by inoculating them at three different developmental stages, v5, v10, and v15. Rust damage was evaluated on leaves seven through thirteen, individually. The differences among cycles were greater in v10 than in v5 or v15. In the late direction, in v5 there were significant linear trends over cycles for leaves 7, 8, 9, and 10; in v10 there were significant linear trends on leaves 7, 8, and 9; and in v15 there were significant linear trends in leaves 9 and 10. In all these cases C3L had more rust than either C0 or C3E. There were no linear trends in the early direction with C3E not different from C0. Close examination, revealed that within leaves, rust pustules were more abundant on juvenile tissues, as indicated by epicuticular wax. Fewer pustules per leaf and fewer leaves with pustules below the ear should reduce the amount of inoculum for allo-infection and could reduce the total amount of damage.

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Role of Pollen Allergens and Beta-Expansin Zea m 1 in Pollen Development and Fertilization

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Group-1 grass pollen allergens are glycoproteins 28-35 kDa in size and are specifically expressed in grass pollen. Group-1 pollen allergens make up a sub class of beta-expansins, which are cell wall loosening proteins that loosen plant cell walls in characteristic ways. Group-1 pollen allergens are proposed to loosen the cell walls of the silks to aid in the penetration and growth of the pollen tube through the silks. In maize, the group-1 pollen allergens are called Zea m 1 and there are two divergent forms of Zea m 1. Each of the two forms is encoded by two genes, making a total of four Zea m 1 genes in maize. To assess the function of Zea m 1, maize plants were transformed with an RNAi construct for RNA silencing, driven by a pollen specific promoter. The first generation of transgenic plants have the following unusual traits: low pollen production, shriveled anthers, and continued silk growth post pollination. These phenotypes are more extensive than that found previously for a maize line carrying an Mu insertion in one of the Zea m 1 genes. The first generation RNAi plants are being analyzed by northern blot and immunoblot blot assays. Immunohistostaining, using monoclonal antibodies specific to group-1 pollen allergens, is also being performed on male and female flowers at various developmental stages to examine the localization of allergens during development.

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Transgenic analyses of a duplicate pair of maize MADS-box genes, Zag1 and Zmm2, suggest protein subfunctionalization of the C class in the traditional ABC model of flower development.

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In the ABC model of flower development as defined by Arabidospsis, the C class is controlled by a single MADS-box gene, AGAMOUS (AG). In ag mutants, stamens are converted to petals, and in the place of carpels a new flower arises, such that ag flowers reiterate a patten of speal, petal, petal. In a search for maize genes similar to AG, two genes were originally identified as potential AG orthologs: Zea AGamous1 (ZAG1) and Zea Mays Mads2 (ZMM2). ZAG1 and ZMM2 have overlapping but non-identical expression patterns, such that ZAG1 is more highly expressed in carpels, and ZMM2 more highly expressed in stamens. Maize plants homozygous for a null zag1-mum1 allele show a phenotype consistent with a partial loss of C-function. However, attempts to isolate ZMM2 loss of function have shown no phenotype. These results are consistent with the hypothesis that gene duplication was followed by subfunctionalization via different expression domains. In order to test the possibility that during evolution the ZAG1 and ZMM2 proteins themselves have subfunctionalized C class gene activity, we have tested their ability specify stamen and carpel development in an Arabidopsis ag mutant. Our results indicate that ZAG1 is better than ZMM2 at promoting carpel identity, while ZMM2 is better than ZAG1at promoting stamen identity. This suggests that subfunctionalization has occurred at the protein level in addition to that seen in the expression pattern. Recently we have cloned a duplicate locus of ZMM2, which we call ZMM2b. The sequence and expression of ZMM2b suggest it also contributes to the maize C class activity, which could explain previous difficulties in identifying a ZMM2 knockout. Work is underway to isolate a ZMM2/ZMM2b loss of function, as well as to determine the region(s) of ZAG1 and ZMM2 that have evolved to result in differential organ-identity function.

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Functional analysis of the maize root specific gene ZmGrp3

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Plant roots play an important role in water and nutrition uptake and anchoring the plant in the soil. In maize there are four different types of roots: the embryonic primary- and seminal-roots and the postembryonic crown- and brace-roots. In addition, lateral roots originate from all root types. So far, only little is known about the molecular and genetic mechanisms underlying root formation in monocotyledonous plants. In an effort to isolate root-specific genes, a differential screening of a coleoptilar node cDNA-library of maize was conducted in Dr. Feix lab (University of Freiburg, Germany) which revealed a full-length cDNA clone designated ZmGrp3. ZmGrp3 represents a glycine rich, putative cell wall protein which is specifically expressed in roots. No homologues of this gene are found in the completely sequenced rice and Arabidopsis-genomes. A functional characterization of the ZmGrp3 gene expression and protein accumulation was now initiated. Gene expression was studied in RNA-gel-blot and in situ -hybridization experiments. Northern-blot experiments with expression data from various aboveground organs of the plants supported the notion that ZmGrp3 is exclusively expressed in roots. Further, experiments including the root initiation-mutant lrt1, that fails to initiate lateral roots on the embryonic primary- and seminal-roots (Hochholdinger et al., 1998, Plant J., 848- 855) and the mutant rtcs, that lacks the formation of crown- and seminal- roots (Hetz et al., 1996, Plant J., 247-255) led to the hypothesis that the gene ZmGrp3 is exclusively expressed in root-tips. In situ- hybridization experiments were performed to analyze the cell-type specific expression of ZmGrp3 in root-tips. Sections of embryos, crown roots and lateral roots of different developmental stages of the maize inbred-line B73 were hybridized using a DIG-labeled ZmGrp3-RNA probe. Hybridization signals showed that ZmGrp3 is exclusively expressed in root-tips, confining the expression to the epidermis and the columella. Further experiments on the protein-level including western-blot analyses and immunolocalization experiments as well as screening for a knock out mutant with a reverse genetic gene machine are in progress.

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The Ac-tagged aberrant pollen transmission 1 (apt1) is a homologue of the Arabidopsis gene SABRE required for root cell expansion

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The Ac-linked apt1 mutation was detected by the aberrant pollen transmission of Ac. A cross between an apt1/+ heterozygous female and a wild-type male gives, as expected, a 1:1 ratio for Ac, but the reciprocal cross gives a 1:12 ratio for Ac. Thus, Ac transmission is normal in the female germline, but aberrant in the pollen. Heterozygous apt1/+ plants segregate pollen with normal tubes and shorter tubes. The DNA adjacent to Ac was isolated and shown by Southern blot analysis to cosegregate with the apt1 mutation. A revertant with normal pollen transmission was recovered. It left an 8-bp excision footprint at the prior Ac insertion site. Southern blot analysis of Ac-carrying progeny from the cross between apt1(Ac)/+ males and wild-type females shows that apt1(Ac) is rarely, if ever, male-transmitted . All the bz-m spotted progeny from the cross carry a transposed Ac (trAc). trAcs are recovered at a frequency of 8%. The APT1 protein is homologous to SABRE, a novel protein involved in root cortex cell elongation in Arabidopsis . Northern blots show that apt1 is expressed in pollen. The apt1 transcript is about 4 kb in size, unusually large for plants. We are currently working on isolation and characterization of the apt1 gene.

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NAC genes in maize: highly cell type specific expression patterns in embryogenesis

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In monocot plants like Zea mays little is known about pattern formation processes organizing the developing embryo. In contrast to the early stereotypic cell division pattern in Arabidopsis, cell division planes in the early maize embryo are unpredictable. Developmental studies may be improved by molecular markers which monitor cellular differentiation. An essential aspect of early maize embryogenesis is the specification of the root/shoot axis in the transition stage. This decision results in a separation of the globular embryo proper into the scutellum, the single maize cotyledon, and cells that will contribute to the future plant body. A similar developmental decision is taken in the globular stage Arabidopsis embryo where the prospective shoot apical meristem (SAM) forms between the two cotyledons. Arabidopsis seedlings which are mutant for CUC1 and CUC2 (CUP-SHAPED COTYLEDON) lack the anlage of a SAM which and show a characteristic fusion of cotyledons to a cup-shaped structure (Aida et al.,1997). Recently, a closely related gene, CUC3, was cloned and characterized (Casper Vroemen, pers. communication). The embryonic expression patterns for CUC1, CUC2 and CUC3 suggest that all three genes are redundantly required for meristem initiation and organ separation. The CUC-genes are members of the plant-specific NAC gene family which is thought to encode transcription factors. Based on the high

sequence conservation of the NAC domain, we screened for CUC-homologous genes in maize to establish developmental markers with crucial functions in SAM-initiation. Several candidate genes of the maize NAC gene family were isolated. The expression pattern of a putative CUC3 homologue suggests that it may set a boundary between the SAM and leaf primordia and between the SAM and the coleoptile. All isolated NAC members show cell-type specific expression patterns during embryogenesis e.g. in the vascular system, the coleorhiza or the developing endosperm.

Genome Structure/Synteny

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MAPPING MAIZE MUTANTS WITH SSR MARKERS

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Mutants that can be identified visually are a powerful source of biological information. They can be utilized for both gene discovery and gene function studies. We have been working on a project designed to create and evaluate a system to gain map information for a large set of mutants. We have analyzed over 476 mutants and have mapped more than 384 using SSR markers. Further, we have produced F2 families for 596 placed kernel, seedling and adult plant mutants from B-A translocation tested EMS stocks, and 234 seedling, viviparous, and adult plant mutants from the Maize Genetics Cooperation-Stock Center. The mutants analyzed by this project represent nearly every class and phenotype found in maize. F2 families are derived from crosses between mutant stocks and the inbred parents A619, A632, B73, and Mo17. Predicted mutants appear in more than 80% of our F2 families. Some placed mutants (16%) were not located on the chromosome arm predicted. In turn, we are characterizing many new mutants that we identified in some of these families. Our mapping strategy begins with bulked-segregant analysis using SSR markers that span either the whole genome for unplaced mutants or specific genomic segments for placed mutants. The phenotypes of the individuals in mutant families allow for the identification of homozygous and segregating samples. These are pooled separately and subjected to PCR with SSR primers. Polymorphic band patterns are compared between the two pools for reciprocal differences that indicate linkage between a SSR marker allele and the homozygous allele of the mutant. Most BSA results were tested by examining the individual homozygous samples with the putative linked SSR markers. New loci have been identified, and many more have been accurately targeted for future experiments between tightly linked loci having the same phenotype. NSF Plant Genome Grant DBI 9872655.

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Duplication and Divergence of the *R2R3 Myb* Gene Family in the Grasses

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R2R3 Myb genes are widely distributed in higher plants and comprise one of the largest known families of regulatory proteins. The dramatic expansion of this family of transcription factors provides a striking example of how gene amplifications followed by divergence may have impacted plant evolution. A group of recently duplicated maize *R2R3 Myb* genes characterized by a change Pro 63 to Ala (Myb^{PtoA}) provides a unique opportunity to understand the role of amplification and divergence of regulatory genes in the development of novel cellular functions. Myb^{PtoA} comprises at least 10 members in maize, including the well characterized P gene, which controls a subset of flavonoid biosynthetic genes, a regulatory function acquired recently in the evolution of grasses. Members of the Myb^{PtoA} clade are also found in sorghum and rice, further supporting the ancient origin, yet recent expansion of this group. To identify the patterns of evolutionary divergence after duplication of *R2R3 Myb* genes, we have cloned and characterized several additional members of the Myb^{PtoA} clade from maize and other grasses. The patterns of sequence divergence of the Myb domains and the corresponding C-termini, the evolution and size expansion of the introns and their possible contributions to the evolution of function of these recently amplified *R2R3 Myb* genes in the grasses will be discussed.

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Large-scale Maize cDNA mapping for candidate gene approach

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One of the ways to look for genes controlling traits of agronomical importance is to pre-select in silico EST sequences with a putative interesting function, and to genetically map the corresponding cDNAs to look for co-localization with QTLs. This poster presents the maize cDNA mapping project that is being carried out within the Genoplante consortium. In this project, we use two Intermated Recombinant Inbred Lines mapping populations: the Intermated B73xMo17 (IBM) population developed by Mike Lee (ISU) and available from Missouri University, and the Intermated F2xF252 (LHRF) population developed by Alain Charcosset at INRA Le Moulon, France. Both populations were developed following the same scheme. Genetic mapping of the cDNAs is performed by RFLP, on a subset of 81 to 85 lines, whereas the framework maps were constructed with mostly SSRs on 94 lines. The IBM framework map has 237 RFLP and SSR markers, including 84 Coremarkers. The LHRF framework map has 250 RFLP and SSR markers, including 51 Coremarkers. 96 markers are common to both framework genetic maps. Linkage analysis to place the cDNAs on the framework maps is automated by using the ActionMap software (Albini, Falque & Joets, publication in prep.). 652 cDNAs, corresponding mostly to genes with no publicly available mapping information, revealed 1160 loci with an average of 1.8 locus per cDNA. Monomorphic genes on both populations represented 4% of the probes, and 6% of the genes could not be genotyped. Among the cDNAs successfully mapped, 18% were monomorphic in the IBM population but could be mapped with LHRF. This population provided also additional loci for some genes already mapped with IBM. The LHRF population will be made available publicly (after 2003 summer multiplication, request to Alain Charcosset: charcos@moulon.inra.fr). In addition to the framework map and the cDNAs, 1829 RFLP and SSR markers (including 1316 raw mapping data obtained from MaizeDB) have been placed on our IBM framework map for anchoring purposes with the QTL maps.

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MAIZE MITOCHONDRIAL GENOMICS

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Plant mitochondrial genomes are unusual in their diversity of structure and rapidity of change. A comparison of the complete sequences of mitochondrial DNA from two dicot species showed that, although most of the known coding regions are very conserved, large intergenic regions show no sequence similarities. The regions between genes have important roles in the rapid structural evolution seen for plant mitochondrial genomes. Moreover, these 'non-coding' regions can become functional as components of chimeric genes that have been described as being responsible for several cases of cytoplasmic male sterility (CMS). In order to understand how non-coding regions of mitochondrial genomes contribute to their structural and functional diversity, it is necessary to examine closely related species. Our NSF-funded Plant Genome Project is focused on the organization, evolution and expression of the mitochondrial genomes of maize and its relatives, Currently, six mitochondrial genomes within the genus Zea are being sequenced, including fertile (NB and NA genotypes) and male sterile (CMS-T and CMS-C genotypes) maize, Zea parviglumis (the probable progenitor of maize) and Zea perennis. In addition, the mtDNA sequences of related grasses, Tripsacum dactyloides and Sorghum bicolor, are being determined. The sequencing of the Zea mays mays NB mitochondrial genome is now complete. The final assembly generated a circular map of 569,630 base pairs with an average G+C content of 43.9%. The size compares well with the previous restriction enzyme analyses (Fauron and Havlik, NAR 16:10395, 1988), which generated a map of 570kb. Annotation data and comparative analysis with other plant mitochondrial genomes will be presented.

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Conserved Noncoding Sequences (CNSs) in Grasses

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Space surrounding and between exons carry discreet sequences that bind proteins or RNAs that wire any gene into a regulated network of expression. One way to identify such cis-acting sites is as conserved noncoding sequences (CNSs), these being phylogenetic footprints so large and exact that just two orthologous gene sequences are required. CNSs have been found in higher plant genes (Kaplinsky et al., 2002. PNAS 99: 6147) as well as mammalian genes, and presumably become discernable when orthologs are diverged for enough time to randomize functionless sequence (approximately 50 MY), but not for so long as to realize mutations in all but absolutely essential nucleotides. As with mice and man, maize and rice have diverged for a useful amount of time. We add data on 52 new maize-rice orthologs where exon identities are supported experimentally. The average

grass gene has about three CNSs (greater than or equal to a 15/15 exact bp match within the gene space), with a mode of 0 at 28%. The mean CNS size is about 25 bp, these being located about evenly in 5í, 3íand intron space. --CNSs make excellent pan-grass PCR primer sites. --Grasses have about 10X fewer and much shorter CNSs as compared to mammals. --There is a poor correlation between CNS and known transcription factor binding sites. --Some grass genes are CNS-rich, from 7-34. These tend to be upstream regulatory genes. --One such CNS-rich gene is homeobox gene knotted1. Here, an intron region shown by Greene and coworkers (1994. Genetics 138: 1275) to bind factors turning KN off in leaves is extraordinarily CNS-rich. By comparing higher plants and animals for gene space structure, morphological and regulatory complexity and the illusive ìplasticity,î useful inferences will be made as to what CNSs bind and to what end.

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Sequence diversity at the y1 locus of maize.

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Previous studies of various alleles of the y1 gene of maize have demonstrated that single nucleotide polymorphisms (SNP), insertion/deletion polymorphisms (InDel) and microsatellite length variability can be used to distinguish alleles. In order to further characterize the degree of sequence diversity at this locus, additional regions of the gene, including exons and introns, were cloned and sequenced from seventeen inbred lines, six open-pollinated landraces and four accessions of teosinte. The inbred lines investigated in this study were selected because they were used to establish the agronomically important BSSS and BSCB1 breeding populations. Multisequence alignments and phylogenetic trees of these y1 alleles established that 1 SNP occurred every 101 bp of exonic sequence and every 44 bp of intronic sequence. No InDels were found in exonic sequences while, on average, there was 1 InDel per 220 bp of intronic sequences. The inbred lines exhibit relatively little sequence diversity as compared to the open-pollinated landraces and teosinte. The teosinte alleles analyzed in this study had a degree of greater sequence similarity to the recessive than the dominant maize alleles.

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INTERGRATING A MAIZE PHYSICAL AND GENETIC MAP USING PCR BASED MARKERS CONVERTED FROM SINGLE COPY RFLP MARKERS ON BAC POOL DNAs

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The long-term goal of this project is to provide an anchored physical and genetic map of the maize genome. In order to meet this goal, PCR-based physical mapping of genetically mapped single copy RFLPs was performed on 6-dimensional pools from the HindIII BAC library. Single copy genes were chosen for anchoring because of the high amount of repetitive DNA and large size of the maize genome. The BAC pooling strategy is efficient and cost effective. Exactly 110,592 maize BAC clones were pooled into 6 different dimensions to create 288 pools of BAC DNA, 48 pools per dimension. Individual BAC clones containing the gene of interest can be determined from actual and predicted positives. The quality of the BAC DNA pools and their role in identifying BACs containing certain genomic sequences were initially tested using SSR primers. Single copy RFLP markers were converted into PCR-based markers to provide more anchoring points quickly. Primers were first tested for amplification on B73 genomic DNA, then amplified on B73 pool DNAs. Among the 176 primers designed for PstI RFLP clones, 32 primers (18%) did not amplify bands on genomic DNA. The remaining primers were analyzed on BAC pool DNAs. Amplification products from the pools were deconvoluted into individual BAC address using a program known as Resolve Script. The marker data will be integrated with the FPC fingerprint assembly generated by AGI to anchor the BAC contigs and contribute to the manual editing process.

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Hormonal, Light and Organospecific Regulation of Ribosomal Protein S14 Gene Expression

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Phytohormones, cytokinin and abscisic acid (AA) are most important endogenic regulators of protein synthesis in plants. It is shown participation in regulation of individual matrices accumulation, rRNA synthesis, ribosomes new formation, polysomes forming and degradation and also ribosomal protein phosphorylation. However, the effect of phytohormones on ribosomal protein gene expression, the most important components of translation

system, is not studied yet. The purpose of this work consisted in analysis of cytokinin and abscisic acid, white and red light effect on accumulation of ribosomal protein S14 and its matrix in isolated cotyledons of two maize sorts (Zea mays Imdurata) early ripe ñ Voronezhskaya-76 and late ripe Odesskaya-10. Based on the differential screening it was isolated full-size cDNA, corresponding to transcript of the gene rps14, which expression is being regulated by cytokinin (benzylaminopurine), abscisic acid and light in isolated cotyledons of maize. To examine hormone and light regulation of the gene rps14 expression, maize seeds have been germinated in the dark in humid filter paper for three days. Then cotyledons have been separated from the sprouts and have been incubated for 24 hours on the water to exhaust endogenic phytohormones content in it. Incubation has been continued in benzylaminopurine solution (2,2 x 10-5 M), abscisic acid (7,6 ı 10-5 Ã) and in the water in the dark and in the light for 1 ñ 120 hours. Analysis of the protein S14 distribution in various organs of 8-days sprouts, grown in the light, has shown that organs with mitotic active tissue prevalence (hypocotyl and new leaves) are characterised by the most protein S14 content. The minimum protein S14 content is available in exhausting cotyledons and finished growth root sections. Level comparison of mRNA and protein S14 displays important role of post-transcriptional regulation of this gene expression in various maize organs. S14 level reflects ribosomesí content, which is much more in cells of actively growing tissues. Therefore for the first time it is shown participation of cytokinin and abscisic acid on mRNA and protein level in regulation of expression of rps14 gene, coding one of ribosomal proteins. It is suggested that many ribosomal proteins genes are under co-ordinated hormone control, however it is not to except differential regulation of these genes expression. The full-size cDNA isolation and also obtaining of antibody to protein S14 significantly extend opportunities to study mechanisms of hormone regulation of transcription and translation processes in plants.

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An integrated physical map in maize

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An integrated genetic and physic map in maize serves as the foundation for positional cloning of genetically mapped genes. A whole-genome physical map was constructed by fluorescent fingerprinting of a corn BAC library. Manual curation has greatly improved the quality of the map. More than 140,000 BAC end sequences were obtained from the BAC clones. These sequences provide a rich source for genetic markers and automatically place several thousands genes on the map. Moreover, about 17,000 ESTs and genetic markers have been placed on the BAC contigs through overgo hybridization. A map-based cloning project will be given as an example to demonstrate the utility of the physical map.

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Genomic regions comprising the entire alpha zein gene family of Zea mays in a single inbred line

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Zeins are the major storage proteins in maize seeds. Based on sequence homology, the alpha zein gene family can be divided into four subfamilies: z1A, z1B, z1C and z1D. We have isolated BAC clones from each subfamily by specific probes and sequenced the genomic regions comprising all copies of alpha zein genes of maize inbred line B73. This yielded a total of more than 1.4 Mb genomic sequences, representing the most extensive genomic sequence analysis in maize to date. A total of 41 alpha zein genes were discovered from this analysis, with 12 genes of the z1A subfamily, 8 genes of the z1B subfamily, 16 genes of the z1C subfamily, and 5 genes of the z1D subfamily, respectively. Except for the z1D subfamily, which only occupies one genomic location, all other subfamilies occupy two genomic locations. Distance analysis using alpha zein gene coding regions indicated that alpha zein genes arose from a single ancestor gene around 35 mya. Gene duplication, followed by translocation and further amplification formed todayís genomic organization of this gene family. Less than half of the alpha zein genes still remain active in the B73 inbred line, and their expression level also varies greatly. Having now the positional information of all alpha zein genes, we can study in parallel the expression of each gene copy, the different haplotypes of this gene family in maize, and the combinatorial aspects of different haplotypes in gene expression. Such combinatorial studies could provide important insights into phenomena like heterosis.

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Haplotypes of a genomic region containing z1C gene family in maize

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Genomic regions of nearly every species diverged into different haplotypes, mostly based on point mutations, small deletions and insertions that do not affect the collinearity of genes within a species. However, when an

orthologous genomic regions of two inbred lines of Zea mays were compared, not only genes were lost from the entire genome but also the regulation of genes were different. As a consequence, the expression potential of inbreds and hybrids differed significantly. The orthologous regions also differed in length, 360 kb versus 263 kb, and a survey of 43 common maize inbred lines illustrated similar structural differences. However, the same orthologous region even between two subspecies rather than cultivars of rice was conserved in length and gene order. Changes between two maize inbreds were as drastic as inter-species changes of maize and sorghum. Intra-species changes were also enhanced by the insertion of different transposable elements. Changes in gene order affect not only the cluster of z1C storage protein genes but also three unrelated genes. Analysis of the expression of individual members of the z1C genes exhibited unexpected shifts of gene expression patterns in hybrid crosses, exemplifying allelic and non-allelic interactions of the two haplotypes. Given that chromosomes could conceivably consist of intervals of ìbinaryî haplotypes that are highly diverged, one could envision endless breeding opportunities because of their linear arrangement along a chromosome and their expression potential in hybrid combinations.

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Validation of in silico connection between maize physical map and genetic map by PCR-based screening on BAC pools.

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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, several strategies were used simultaneously. An overgo hybridization approach provided a large number of anchoring points to the maize BAC contig map. Our objective was to validate the in silico connections between the maize physical and genetic maps using a BAC pooling strategy combined with a high-throughput PCR-based screening method. The BAC DNAs were pooled from 6X haploid genome equivalents (110,592 maize BAC clones) of a maize HindIII BAC library in six different dimensions, producing 288 pools of BAC DNA (48 pools per dimension). A total of 95 PCR-based markers associated with overgo probe by in silico methods were screened on the pools. Amplified PCR products from the pools were deconvoluted into individual BAC addresses using Resolve script. On average, PCR screening on BAC pool and overgo hybridization identified 4.72 and 5.26 BAC clones per each probe analyzed. BAC: probe data shows 88% (eighty-four out of ninety-five probes) containing BAC clones identified by both approaches, strongly supporting the in silico associations. This data was integrated with the fingerprinting data generated by Arizona Genome Institute (AGI) to assemble the BAC contigs using FPC and contribute to the process anchoring physical map to the genetic map.

Molecular Genetics

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Keeping the babies healthy. Defence mechanisms operate at the base of the endosperm in developing seeds.

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Endosperm transfer cells, located at the basal part of the seed, are designed to facilitate nutrient import into the growing maize kernel. Specific genetic systems devised to improve transport capacity are therefore thought to be operative in this tissue. Nevertheless, in a series of screening experiments conducted to identify transfer cell specific genes, we could not find any gene putatively involved in the transport of metabolites. Many genes have been identified, however, apparently involved in defence mechanisms and in one case, the BAP family of proteins, antimicrobial activity has been previously demonstrated in vitro. We report here on the characterisation of another antimicrobial activity, in this case isolated from a different endosperm tissue also located at the base of the seed, the embryo surrounding region. We propose that, in addition to their role in nutrient transport, cells at the base of the endosperm are actively involved in the formation of a barrier to impede the entry of pathogens into the seed.

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Expression and inheritance patterns of a modified porcine a-lactalbumin transgene in maize kernels

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A codon-adjusted version of a gene encoding the porcine milk protein a-lactalbumin was synthesized to create maize with improved nutritional quality. Three expression vectors containing the synthetic gene were constructed and transformed into maize callus by particle bombardment. The constructs (P45, P64, P57) differ in their promoters and subcellular targeting domains. The objective of this study was to characterize the expression and inheritance patterns of these transgenes. a-La DNA sequences were detected by PCR analysis of F1 plants in 6 of 8 events of P64, 6 events of P45 and 4 of 5 events of P57, indicating that the a-La gene was integrated into the maize genome and transmitted to the F1 generation. Western blot analysis indicated that the a-La protein accumulated in the endosperm, showing that the transgenes were functional. Expression and inheritance of the a-La transgenes were assessed. In 5 of 6 events of P64, the a-La transgene was inherited as a single locus through the F3 generation. In P45, irregular expression and inheritance patterns were observed. In 3 events, the a-La transgene was inherited as a single locus in the BC1F1 and F2 generations. In the F3 generation, segregation distortion manifested by an increase in frequency of kernels with no detectable a-La protein was observed. PCR analysis of these kernels confirmed presence of the a-La transgene, indicating that the transgene was not functional. In 2 events that expressed a-La in the F1, the a-La protein was not detected in the BC1F1 and F2 generations. However, in F3 kernels derived from self-pollination of these F2 null plants, a-La was detected, suggesting that the transgene was reactivated. This phenomenon was also observed in the F3 generation in 2 events of P57 that manifested transgene inactivation in the BC1F1 and F2 generations. Possible causes of these irregular inheritance patterns are discussed.

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Class III Aldehyde Dehydrogenase Genes of Maize

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The maize *rf2a* gene, which is a restorer of T-cytoplasm male sterility, encodes a Class II aldehyde dehydrogenase (ALDH). The maize genome contains another gene that encodes a Class II ALDH (*rf2b*) and two that encode Class I ALDHs (*rf2c* and *rf2d*). Four maize genes that encode Class III ALDHs have been identified (*zmfaldh1*, *zmfaldh2*. *zmfaldh3* and *zmfaldh4*). The deduced amino acid sequences of the corresponding proteins exhibit ~60% similarity to human fatty ALDH (ALDH10), the deficiency of which causes the genetic disorder Sjogren-Larsson syndrome. These proteins are also >70% similar to Class III ALDHs from *Craterostigma plantagineum*, which are thought to be involved in ABA-dependent responses to dehydration stress. Each of the maize Class III ALDHs contains a putative ER targeting sequence at its C-terminal. Three of the four Class III ALDHs genes have been genetically mapped. Mutant alleles have been isolated for each gene. *Mu* transposon insertion alleles of *zmfaldh1*, *zmfaldh2* and *zmfaldh4* were obtained using the TUSC system. An EMS-induced mutant of *zmfaldh3*, identified via TILLING, contains a single nucleotide transition that causes a G to E change in the protein.

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Use of DNA pooling to assess diversity within and among maize populations. Application to the investigation of maize introduction into Europe.

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Large collections of traditional maize open pollinated varieties (OPVs) are maintained by the breeding programs and genetic resource centers of many institutions (e.g., CIMMYT, USDA, INRA). It is widely acknowledged that these collections contain as yet unexploited genetic diversity with a high potential value for genetic investigations and breeding. Their practical use in research programs is presently limited by a low level of information regarding the structure of genetic diversity, such as the level of redundancy among accessions, presence of groups of related accessions, and presence of unique diversity in a few, key populations. The use of neutral molecular markers has the potential to provide interesting new information regarding these topics. However, high levels of within population genetic diversity typical in maize OPVs call for the analysis of a representative sample of individuals for each accession, which makes analyses difficult and time-consuming. We have tested a method first using RFLP markers and then SSRs, which consists in analyzing bulks of leaves from different individuals, and estimating allelic frequencies using signal intensities of alleles present within a pool. The use of controlled pools of inbred lines shows that this approach is accurate for RFLPs (overall correlation between estimated and expected frequencies of 0.93). The same approach can be used for SSRs provided (i) loci with low amplification competition between alleles are selected and (ii) frequency estimation takes into account possible ìstutteringî phenomena (presence of additional fragments of predictable sizes due to errors of the Taq polymerase). Overall correlation between estimated and expected frequencies was 0.96 in this cases. Data were obtained for 29 RFLP

loci and 24 SSR loci on a set of 174 representative American OPVs (from South, Central, and North America) and a set of 131 representative European OPVs. Results obtained on American material show strong structure patterns associated with geographical origin, consistent with recent results of Matsuoka et al. (2002, PNAS 99:6080-6084). Comparison of European and American diversity (i) confirms the contribution of Caribbean material introduced by Colombus in Spain to the establishment of European diversity and (ii) shows a major contribution of Northern Flint populations cultivated by Native Americans in the North-Eastern United States. A detailed historical investigation shows that this introduction occurred very shortly after new world discovery (before 1539). This research program was supported by PROMAIS members: ASGROW France (Monsanto SAS), CARGILL SEMENCES (Monsanto SAS), CAUSSADE SEMENCES, CEBECO SEMENCES, EURALIS GENETIQUE, MAœSADOUR SEMENCES, NOVARTIS SEEDS (Syngenta), LIMAGRAIN GENETICS, PIONEER GENETIQUE, S.D.M.E. K.W.S. France, VERNEUIL RECHERCHE

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Certain Mutations Preventing Paramutation Heritably Reactivate 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-I, 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-I genomic transgene was activated in homozygous paramutation mutant plants (darkly purple), while it remained silent in heterozygous plants (green). Northern blots and nuclear run-on assays demonstrate that the transgene is transcriptionally silenced and that the 'absence' of the wild type gene products in each of the recessive mutants relieves this silencing. In the paramutation mutants, another transcriptionally silenced transgene (35S Bar) within the same transgene array is also activated. We have been investigating whether or not the reactivated transgene remains active when the paramutation mutants are segregated away. We have identified two classes of mutants. With the first class, represented by the rmr1-1 mutation, the transgene is efficiently resilenced in plants heterozygous for the wild type allele and the mutant. In contrast, when segregated away from the other mutations, the transgene can remain active for a number of generations in the absence of the mutation responsible for its initial reactivation.

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Comparative mapping of drought responsive genes in *Zea mays* L. and *Arabidopsis thaliana*

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Drought is a major limitation to productivity of *Zea mays* world wide. The search for drought responsive genes in maize is therefore a very important and valuable undertaking. Studies have shown that abscisic acid (ABA) concentration increases in response to drought stress. ABA application can also affect the expression of drought responsive genes suggesting that ABA acts as a signal for water deficit response (Leung and Giraudat, 1998). In *Arabidopsis thaliana*, numerous genes that respond to dehydration stress have been identified. These genes have been categorized as *rd (responsive to dehydration)* and *erd (early response to drought)*. Several of these genes have been found to be induced in the ABA pathways. The fact that maize and Arabidopsis have many common genes leads to the possibility that the *erd* and *rd* genes in Arabidopsis could be found in maize. The objectives of this experiment are to determine if the *rd* and *erd* gene sequences in Arabidopsis can be found in maize, to map these genes in maize and to sequence the maize PCR products to determine if they are the selected *rd* or *erd* gene from Arabidopsis. Six genes from Arabidopsis were selected and primers were designed from their sequence. DNA from maize inbreds, Mo17 and B73 was amplified to determine if the corresponding sequences were present in the maize genome. Primer products were obtained for all six genes. Five of the six primer pairs had mappable polymorphisms. Further research is needed to determine the function of these genes in maize.

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Screening phenotypic diversity in light-responsiveness to identify potential phytochrome mutants in maize

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Mesocotyl length is a phenotypically plastic trait in different light environments and provides a quantitative

measure of light responsiveness during early seedling development. Mesocotyl elongation is sensitive to several wavelengths of light, implicative of red/blue photoreceptor systems. Low light levels in growth chamber screens have revealed significant underlying genetic variation in this trait among 100 maize inbreds. We compared mean mesocotyl length in non-stiff and stiff stalk lines originating from the North American Corn Belt with those from semi-tropical lines and found that semitropical lines had mesocotyls that were on average 41-49 % shorter than the corn belt inbreds. This suggests that a significant loss of light responsiveness at the seedling emergence stage accompanied selection by breeders in northern temperate regions. Has diversity in phytochrome gene sequence or gene expression played a role in this process? To begin to answer this question, we have designed a molecular-genetic screen to identify large-scale changes in the highly conserved region of the duplicated phytochrome B genes, spanning Exons I and II. In our assay, no allelic diversity in phyB1 has been detected, but seven of the 88 inbreds screened appear to contain an approximately 6 Kb insertion in phyB2. A subset of these mutants will be targeted for sequence and expression analysis, as well as phenotypic characterization of other phytochrome-mediated traits.

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Is the maize C2-Idf mutation controlled by an RNA-based silencing mechanism?

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The maize Inhibitor-diffuse (C2-Idf) mutation is a dominant loss-of-function allele of c2. The c2 gene product is chalcone synthase, the enzyme catalyzing the first dedicated step in the anthocyanin and phlobaphene pigment pathways. Without a functional c2 product, no pigments are made. Plants that are heterozygous for wild-type functional C2 and a recessive non-functional c2 allele make half the normal amount of pigment. By contrast, plants that are heterozygous for wild-type C2 and C2-Idf make virtually no pigment. We are interested in understanding how C2-Idf inhibits the function of a normal C2 allele. Experimental observations suggest that C2-Idf inhibition involves regulation at the epigenetic level, perhaps through a RNA-based silencing mechanism. C2-Idf/C2-Idf homozygotes accumulate very little c2 mRNA. In C2-Idf/C2 heterozygotes, c2 mRNA is reduced to roughly 10% of wild-type. This implies both that there is a negative interaction between the inhibitor and wild-type alleles and that the loss of function associated with C2-Idf inhibition is controlled at the level of RNA accumulation. The promoter of the C2-Idf allele is hypermethylated relative to the wild type, further suggesting an epigenetic effect on regulation of this allele. As a means to test these ideas, we infected C2-Idf plants with maize dwarf mosaic virus, a potyvirus that carries an inhibitor of the RNA-based silencing mechanism. In contrast to the unpigmented phenotype of uninfected plants, infected plants were highly pigmented. The increased pigmentation was accompanied by increased c2 mRNA accumulation. These results suggest an RNA-based silencing mechanism as the means for C2-Idf inhibition, although it remains to be determined whether changes in RNA accumulation are modulated post-transcriptionally by RNA degradation or transcriptionally by chromatin-level regulation.

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Characterization of a Peptide Transport Gene from Oryza sativa

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Peptide transport is a common phenomenon in both prokaryotic and eukaryotic organisms. The obvious role of peptide transport is to translocate amino acids across cellular membranes for protein synthesis and for nitrogen acquisition. However, the abundance of peptide transport genes, particularly in plants, suggests that transport proteins may play other important roles. We have begun characterizing the PTR family (Peptide TRansporter) of peptide transport genes in both maize and rice. The recent discovery of 51 putative PTR transporters in Arabidopsis suggests this family may be playing a significant biological role in plants (Stacey et al., 2002). A peptide transport gene (OsPTR1) from Oryza sativa was identified through homology searches with an existing peptide transport gene from Arabidopsis. We are currently in the process of cloning the OsPTR1 open reading frame into a yeast expression vector for heterologous expression in a Saccharomyces PTR mutant. In a second experiment, we are investigating a possible connection between plant hormones and peptide transport by determining if OsPTR1 expression is affected by hormone levels in germinating embryos.

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One gene family that may influence maize endosperm betacarotene content.

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Carotenoids, synthesized by plants and other organisms, include over 600 different compounds, many of which

are essential in human health. Endosperms of major food crops, including maize, are low in carotenoid content and therefore potential targets for improvement via marker-assisted selection or transgenic metabolic engineering approaches. To provide the foundation needed to develop rational engineering strategies, we are investigating regulation of the endosperm biosynthetic pathway through identification of the genes and gene families that encode the pathway enzymes. We wish to determine the contribution of each family member to tissue specificity of carotenoid accumulation and more specifically to assembly of pathway enzymes with regard to plastid membrane localization. Here we focused on betacarotene hydroxylase (HYD), the enzyme that converts provitamin A carotenoids (betacarotene) to nonprovitamin A xanthophylls (zeaxanthin). A maize cDNA, demonstrated to encode a HYD enzyme, was used as a probe to screen a maize B73 BAC genomic DNA library. Characterization of the isolated clones revealed three gene groups. On the basis of sequence analysis, one family member (*HYD3*) appeared to be complete, while the other two (*HYD1* and *HYD2*), though homologous, appeared to share deletions that would likely interfere with expression of a functional enzyme. From database searching, it appeared that other varieties had normal *HYD1/2* sequences suggesting that allelic variations may be responsible for variations in endosperm carotene content. This research is funded in part by the National Institutes of Health.

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THE LETHAL LEAF-SPOT 1 (LLS1) PROTEIN IS LOCALIZED TO CHLOROPLAST MEMBRANES AND IS HIGHLY CONSERVED IN OXYGENIC PHOTOSYNTHETIC ORGANISMS.

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The LLS1 protein provides an important protection against light-dependent cell death in higher plants but its molecular function remains undetermined. The cell death that occurs in *lls1* plants is mediated by mature chloroplasts leading us to hypothesize that LLS1 provides a protective function in that cell compartment (Gray *et al,* 2002, Plant Physiology, 130:1894-1907). We provide evidence to show that the LLS1 protein is localized to the inner membrane of the chloroplast in both monocots and dicots. Firstly, the *acd-1* (*accelerated cell death 1*) mutation of *Arabidopsis* was complemented using the maize *Lls1* cDNA. Secondly, subcellular fractions of isolated pea chloroplasts and mitochondria were analyzed by western blot analysis using an anti-LLS1 monoclonal antibody. The LLS1 protein was detected only in fractions enriched for the inner and outer plastid membrane. Thirdly, *in vitro* -labeled LLS1 protein was imported into isolated chloroplasts and found to be specifically localized to the inner chloroplast membrane. Fourthly, transgenic *Arabidopsis* plants expressing an LLS1::GFP fusion protein were examined by confocal microscopy. The LLS1::GFP fusion proteins were localized exclusively to the chloroplast and the predicted LLS1 transit peptide was sufficient to enable this targeting. The LLS1 protein was also detectable by western blot in many plant species including lower plants such as moss and ferns. A bioinformatics survey revealed that *Lls1* homologues exist in algae (*Chlamydomonas*) and cyanobacteria (*Synechocystis*, *Anabaena* and *Trichodesmium*) but not in the anoxygenic bacterium *Chlorobium*. Although the LLS1 protein is always present in photosynthetic tissues the protein is also found in many non-photosynthetic tissues and expression of the *Lls1* gene increases following wounding. Our findings suggest that the *Lls1* gene evolved to help protect oxygenic photosynthesizers from free radical associated damage. This role is of particular importance in the chloroplast compartment where chlorophyll and other pigments can readily become phototoxic.

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Identification of proteins associated with aflatoxin accumulation levels by 2-dimensional gel electrophoresis

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Food and grain contamination by aflatoxin causes worldwide crop loss. Aflatoxin is produced by the fungus *Aspergillus flavus* and is a potent carcinogen. The FDA requires that aflatoxin may not exceed 20 parts per billion for corn going to market. Host plant resistance to *A. flavus* is the best means of solving the problems caused by aflatoxin. One tropically derived maize inbred, Mp313E, has lower aflatoxin accumulation levels when compared to other lines. In contrast,Va35 accumulates high levels of toxin. Our goal is to identify proteins associated with low toxin accumulation in *Zea mays* L. using 2-dimensional isoelectric focusing. In this experiment protein patterns from infected or uninfected ear tissue Mp313E and Va35 were analyzed by means of 2-dimensional gel electrophoresis. All material was self-pollinated 17 days before inoculation with *A. flavus* strain NRRL 3557. Three replicates of inoculated and two replicates of uninoculated material were evaluated for each inbred. Plant material was sampled at 5 days after inoculation. Proteins were prepared according to Hurkman and Tanaka (1986) with modification and analyzed using 11cm, pH 5-8 strips in 12.5% Tris HCl gels stained with Coomassie

Blue. Differences in position and intensity of various protein spots on the gels were determined using PDQuest software. These proteins are candidates for mass spectrometry to determine protein identity.

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Allelic Variation of Gene Expression in Maize Hybrids

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For nearly a century, geneticists and breeders have known that the hybrids between genetically unrelated individuals often exhibit hybrid vigor or heterosis. The genetic factors contributing to hybrid vigor have mainly been described by DNA sequence differences between the parental alleles. However, little is known at the RNA level how the ìhetero-allelesî are expressed and their relevance to hybrid performance. In this study we measured allele-specific transcript accumulation of a random set of 15 genes in an old and new maize hybrid grown together in different environments. We discovered that alleles of 14 genes expressed differentially in at least one hybrid or environment, ranging from expressing both alleles (bi-allelic) to expressing a single allele (mono-allelic). The allelic expression varied during plant development and responded to drought and density stresses. The older hybrid also tended to express one allele whereas the newer hybrid expressed both alleles. The results suggest an unequal contribution of parental alleles in the hybrid and the advantage of bi-allelic expression in hybrids over mono-allelic expression in inbreds, which may contribute to heterosis. The yield gains of the newer hybrids are primarily due to the genetic improvement in tolerance to stresses, which could be the result of breeding selection for alleles that respond to stresses. The large extent of allelic expression variation suggests that a substantial fraction of quantitative trait variation is the result of sequence polymorphisms in the regulatory regions of the loci.

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Cell Death in the lethal leafspot1 mutant of maize and its ortholog in sorghum, dropdead1

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The lethal leafspot1 (lls1) mutant of maize and its orthologous mutant in sorghum, dropdead1 (dd1), are not able to suppress cell death in response to wounding. Although the function of the LLS1 protein is not known, it appears to be localized in chloroplasts. We examined nuclear fragmentation and cytochrome c release from mitochondria, characteristics of programmed cell death (PCD), in wounded leaves of lls1 and wild-type (WT) plants. Fragmentation of DNA was examined 2 and 20 hours following wounding. DNA fragmentation in WT and lls1 plants was evident at 2 hours, however, only lls1 plants displayed DNA fragmentation 20 hours post-wounding. A limited amount of cytochrome c appeared to be released into the cytosol of wounded WT leaves. However, a large amount of cytochrome c was released from the mitochondria into the cytosol, even in unwounded lls1 leaves. This may indicate that these organelles in the lls1 plants are particularly fragile and susceptible to breakage during the mitochondrial isolation procedure. Previously, we have observed that lls1 and dd1 chloroplasts exhibit a decreased number of starch granules. This decreased starch accumulation indicates that lls1 and dd1 plantsí photosynthetic apparatus may be compromised. By using western blotting, we analyzed the levels of eight different light harvesting complex (LHC) proteins in both dd1 and WT leaves and found particular LHC proteins to be increased in the dd1 leaves. We also examined the level of select photosynthetic enzymes (Rubisco and PEP carboxylase) and mitochondrial proteins (alpha and beta subunits of ATP synthase, porin and cytochrome c) in these plants. Rubisco was significantly increased in the dd1 leaves compared to WT, but none of the other proteins showed significant differences. These findings suggest that there is a selective increase in the expression of some proteins involved in photosynthesis, perhaps in response to a decreased overall photosynthetic capability.

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DISSECTION OF PLANT PROMOTER FUNCTION *IN VIVO*

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Combinatorial interactions between MYB and HLH transcription factors are required for the regulation of several important processes in plants. Protein-DNA binding and transient expression experiments established a modular structure for several maize flavonoid biosynthetic gene promoters, in which high- and low-affinity MYB binding sites alternate. While these *cis*-regulatory elements are sufficient to drive expression of naÔve minimal promoters, we have determined that insertions and mutations caused by transposons have effects not predicted from in vitro

protein-DNA binding or transient expression experiments. These results suggest that other *cis*-elements are also important *in vivo*. The behavior of transposon insertions in the promoter of the *anthocyaninless1 (a1)* gene (encoding dihydroflavonol reductase) provides an ideal system to investigate the role of transposons as control elements. To further define which *cis*-elements are involved in the *in vivo* regulation, we analyzed the corresponding promoter sequences of the *a1* and *colorless2* (encoding chalcone synthase) genes in a large number of maize inbred lines, in various *Zea* species, and other grasses. This 'natural variation footprint' method provided information on the degree of variation allowed for experimentally-verified *cis*-regulatory elements, and identified several conserved non-coding sequences (CNS) that may serve as additional binding sites for transcription factors.

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Molecular dissection of the interaction between ZmMRP-1 and the promoter of BETL-1.

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ZmMRP-1 is a transfer cell specific transcriptional activator containing a single MYB-like DNA binding domain. We have previously shown that ZmMRP-1 is necessary and sufficient to trans-activate various transfer cell specific promoters in homologous and heterologous systems. We present here new results on the detailed characterisation of the interaction ZmMRP-1/BETL-1 promoter. We also report on the functional characterisation of ZmMRP-1 through the generation of a collection of 1000 mutated forms of the protein, by microbial transposon insertion, and evaluation of their capacity to transactivate the promoter of BETL-1.

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Expression Analysis of Glu-1Dx5 Transgenic Corn

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Maize endosperm is one of the most significant agronomic tissues because it is the major component of feed made from maize grain. An important trait of endosperm is therefore its content of essential amino acids. Because the majority of endosperm protein is in the form of seed storage proteins, the seed storage proteins have a large impact on the amino acid content of the seed. The main storage proteins in maize endosperm are the zein proteins, which are encoded by large gene families. We set out to manipulate seed protein content by introducing a gene from wheat called Glu-1Dx5 into transgenic corn plants. This gene encodes a seed storage protein called high molecular weight glutenin. We observed differences in nitrogen content between transgenic kernels and non-transgenic kernels from the same ear. To characterize this difference in greater detail, the levels of the Glu-1Dx5 protein and the zeins in transgenic endosperms were quantified by HPLC. A peak of Glu-1Dx5 that reacts with a Glu1Dx5 antibody can be observed in transgenic plants. Through this quantification, we found that the levels of several zeins were altered in the seeds expressing the Glu-1Dx5 transgene. In order to identify differences in transcript levels, a cDNA microarray was used to study the differences in expression patterns of 8064 selected genes between transgenic and non-transgenic endosperms generated on the same ear. Two zein genes with statistically significant differences in transcript levels were identified. Interestingly, the Glu-1Dx5 promoter region contains a G-box like motif that could possibly serve as the binding site for the maize transcription factor Opaque2. We have produced F2 seed of a cross between o2 and Glu1-Dx5 lines. It will be interesting to see the effect of o2 on transgene expression.

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Microarray Analysis of Maize Opaque2 In Developing Endosperms of Eight Inbred Lines

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The Opaque2 (O2) mutation can significantly improve the protein nutritional quality of corn kernels by increasing the lysine content of maize endosperm. Since this discovery, it has been the subject of intense research. Studies conducted over the past 40 years have revealed that the o2 mutation reduces the synthesis of zein storage proteins and increases the level of free amino acids, including lysine. The biochemical and molecular mechanisms leading to these phenomena are only partially understood. We analyzed the grain from eight inbred lines and their backcross o2 conversions and found that the extent of change in amino acid content of an o2 mutant is dependent on its genetic background. In recent years, high throughput microarray studies have been conducted to study the pattern of expression in normal and o2 mutant with a single genetic background. However, background-specific effects of o2 can obscure the main action of o2. In order to eliminate such marginal effects, we sought to identify genome-wide gene expression changes that are consistently maintained in the o2 mutant lines in several genetic backgrounds. Fourteen days after pollination, developing endosperms were harvested from eight pairs of near-isogenic lines. The pooled mRNA was labeled and hybridized to Unigene arrays from ZmDB. A duplicate dye-swap design was used for the microarray experiment and an ANOVA method was used to analyze the data. Our

results showed that more than 100 genes are significantly down-regulated, including expected genes such as zeins, a pyruvate orthophospate dikinase, and ribosome-inactivating protein b32 and a few are up-regulated significantly in o2 mutants. Further analysis will be done on these genes. In the future work, we hope to understand factors influencing amino acid content by studying global expression patterns of o2 at different developmental stages and o2 mutants from specific genetic backgrounds.

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Evaluation of Near-Isogenic Lines for Kernel Oil Concentration QTL in Maize

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Value-added specialized corn hybrids, which may provide efficiencies and economic benefits, have the potential to be grown much more extensively in the Midwest. One such example is high-oil corn (HOC) grown for its livestock feeding value. However, negative associations with grain yield have hindered the success of HOC. We evaluated sets of near-isogenic lines (NILs) for a chromosome region with a major oil concentration quantitative trait locus (QTL) or linked QTL derived from a (Illinois High Oil x B73) B73 BC1S1 population. NILs may facilitate confirmation of QTL, fine mapping, and dissecting the basis of the QTL. Three SSR markers linked to the major oil QTL on chromosome 6 were used in the development of BC1 S5:7 and S7:9 NILs. Testcross progeny were made from BC1S8 NIL sublines to Mo17 for evaluation of NILs in a hybrid context. Grain collected from the lines were evaluated for their kernel oil, protein and starch concentration using near-infrared (NIR) analysis. To better dissect the basis of the QTL, kernel weight, embryo weight and embryo oil concentration, using nuclear magnetic resonance (NMR), of selected sub lines from the S5:7, S7:9, and testcrosses will be reported. Single factor analysis of variance confirmed the kernel oil QTL in the S5:7 NILs evaluated in 2001. Evaluation of a second year of S5:7 NILs and two environments of S7:9 NILs in both per-se and testcross evaluations in 2002 show significant differences in kernel oil concentration between marker class means, confirming presence of QTL. Testcross evaluations for yield detected a negative association with the kernel oil concentration QTL and final grain yield. However, the 2002 growing year was a stressful drought environment and yields were poor in general.

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A DNA methyltransferase mutation increases expression of the epigenetically-regulated maize gene, Pl-Blotched

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Changes in DNA methylation and chromatin structure have been associated with altered gene expression in a variety of different organisms, including maize. In maize, an example of such an epigenetic effect has been observed at Pl-Blotched, an allele of the anthocyanin regulatory gene purple plant1 (pl1). Pl-Blotched leads to a variegated pattern of anthocyanin pigmentation compared to the uniformly pigmented phenotype of plants with the Pl-Rhoades allele. The phenotypic difference of Pl-Blotched is accompanied by a different pattern of DNA methylation and by a more condensed chromatin state. To help us understand the relationship of DNA methylation to chromatin structure and expression of Pl-Blotched, we introduced a mutant allele of the DNA methyltransferase Zmt2. This DNA methyltransferase gene is one of two duplicates in the maize genome that encode enzymes with methylation specificity for CNG sequences. In Pl-Blotched plants with the Zmt2 mutant, anthocyanin pigmentation was higher than in plants with the wild-type DNA methyltransferase gene. The increase in pigmentation coincided with a modest decrease in cytosine methylation at diagnostic restriction sites in the gene, opening of chromatin structure in the 3' region of the gene, and an increase in pl1 mRNA accumulation. These results indicate that disrupting DNA methylation leads to a more open chromatin state and higher levels of gene expression. These observations point to the importance of DNA methylation of the Pl-Blotched gene in maintaining the chromatin structure of this epiallele.

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Genomics analysis of lipoxygenase gene family

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Lipoxygenases (LOX) are dioxygenases that catalyze the hydroperoxidation of polyunsaturated fatty acids such as linolenic and linoleic acids. Several lines of correlative evidence strongly implicate lipoxygenases and their metabolic products in the resistance mechanisms to diverse plant pathogens and in accumulation of mycotoxins such as aflatoxin. As the first step in our attempt to clearly define LOX functions, we have undertaken a genomic scale analysis and identification of the entire LOX multigene family of maize, rice and Arabidopsis using wealth

of Pioneer and DuPont EST collection as well as public EST and genomic databases. Using bioinformatics analysis of ESTs representative of more then 130 different cDNA libraries, full insert sequencing and 5íRACE cloning technique we have identified that maize genome contains at least 11 different LOX genes. In comparison, rice contains at least 14 different genes and Arabidopsis genome encodes only 6 LOX genes. This number has been independently confirmed by using gene-specific probes in Southern blotting analysis and by identification of chromosome location of each individual gene by using gene-specific PCR primers and genomic DNA from oat-maize addition lines. Homologs of two of these LOX genes have never been reported in any other plant species and can be grouped into two novel classes of plant LOX genes. RNA profiling and Northern blotting results suggested that many of the LOX genes are up-or down-regulated by pathogens, their elicitors or defense-associated signaling molecules such as JA, ethylene and SA suggesting their involvement in pathogen-induced defense responses. By using reverse genetics strategy, we have identified maize mutants in which function of 8 LOX genes is interrupted by insertions of Mutator transposable elements in their coding sequences. These mutants will undergo thorough testing for disease resistance and mycotoxin accumulation levels to unambiguously define LOX function in defense responses and interaction between pathogens and the plant host.

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A maize *Y1* homolog encodes a functional phytoene synthase.

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Plant-derived carotenoids are important in human health and include a class of over 600 structures. In plants, carotenoids function in light harvesting, protect against photo-oxidative damage and act as precursors to abscisic acid (ABA). The carotenoid biosynthetic pathway takes place on plastid membranes by nuclear-encoded enzymes that require appropriate plastid-targeting domains. The marked difference in plastid membrane architecture between endosperm and photosynthetic tissue suggests possible differences for pathway assembly depending on tissue/plastid type. As part of an ongoing effort to investigate regulation of the pathway, we are characterizing gene families and enzymes for the entire pathway, including isoprenoid precursors. In this study, we investigated the first step of the carotenoid biosynthetic pathway, synthesis of phytoene from geranylgeranyl pyrophosphate (GGPP), a key compound which lies at a metabolic crossroad. This step is catalyzed by phytoene synthase (PSY) and is rate-controlling for carotenoid accumulation. We identified a maize cDNA (*PSY2*) showing homology to maize *Y1*, the previously identified structural gene for PSY. When the *PSY2* cDNA was used as a hybridization probe of maize genomic DNA, a different hybridization pattern was produced as compared when *Y1* was used as a probe, suggesting that *PSY2* represented a second gene. To test whether this second gene encoded a functional phytoene synthase or was a pseudogene, we used a heterologous complementation system to confirm function of PSY2. A gene cluster encoding the carotenoid biosynthetic pathway enzymes, with the exception of PSY, was introduced into *E. coli*. Only when a cDNA encoding a functional PSY was introduced, would zeaxanthin accumulate. When the *Y1* homolog cDNA (*PSY2*) was introduced, zeaxanthin accumulated. These results indicate that there is a second phytoene synthase gene in maize which encodes a functional phytoene synthase. We are currently investigating the roles of both phytoene synthase enzymes and their corresponding genes in conditioning carotenoid accumulation in maize. This research was funded in part by the National Institutes of Health.

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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 nondisjuction 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.

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Color complementation in *E. coli* for functional testing of a cDNA required for maize carotenoid biosynthesis

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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. The biosynthetic pathway takes place on plastid membranes by nuclear-encoded enzymes that require appropriate plastid-targeting domains. The marked difference in plastid membrane architecture between endosperm and photosynthetic tissue suggests possible differences for pathway assembly depending on tissue/plastid type. As part of an ongoing effort to investigate regulation of the pathway, we are characterizing gene families and enzymes for the entire pathway including isoprenoid precursors. From limited sequence in GenBank, we identified a cDNA predicted to encode a betacarotene hydroxylase (HYD), an enzyme that catalyzes the addition of hydroxyl groups at position three on the cyclic rings of betacarotene to form the xanthophyll, zeaxanthin. This maize (*Zea mays*) cDNA was sequenced and found to encode a 359 amino acid polypeptide with 56% identity to a homolog from the dicot, *Arabidopsis thaliana*. Since sequence homology implies, but does not always indicate enzyme specificity and activity, we used a heterologous bacterial system to demonstrate function of the putative maize betacarotene hydroxylase. The cDNA was cloned in-frame into the pET23c vector and then tested by co-transformation with a second plasmid conferring accumulation in *Escherichia coli* of the HYD substrate, betacarotene. Presence of the maize HYD cDNA was associated with altered colony pigmentation as compared to the control strain accumulating betacarotene but lacking the maize cDNA. HPLC analysis confirmed accumulation of the HYD enzymatic product, zeaxanthin and the intermediate, betacryptoxanthin, thereby proving the cDNA encoded a functional betacarotene hydroxylase. This undergraduate research project was funded in part by the National Institutes of Health.

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Isolation and characterization of a maize cDNA encoding a telomere repeat DNA oligonucleotide-binding protein

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To determine the molecular basis of meiotic telomere behavior, we have set out to identify genes that encode telomeric complex proteins using a conserved myb-like telomere-binding domain of human TRF1 to query EST databases. From these searches and by cDNA library screens, we have discovered and sequenced five full-length maize cDNAs that represent a small gene family with a novel domain organization that is apparently unique to plants. Here we describe the preliminary characterization of one member (called MHCP5) of this family. The ORF of MHCP5 was expressed in E. coli, and the recombinant protein was found to bind double-stranded telomere repeat oligonucleotides in vitro. Gel mobility shift assays with 21 different mutant variant oligos established that MHCP5 binds most strongly to the wild-type telomere repeat. Southern blots revealed that the MHCP5 cDNA hybridizes to several restriction fragments at moderate stringency, consistent with the idea that MHCP5 belongs to a small gene family. RNA gel-blot analysis with a 3í UTR probe indicated that this gene is expressed at low levels in a few plant tissues. A pair of primers for MHCP5 produced two PCR products with DNA from either B73 or Mo17 inbred lines. One PCR product was from MHCP5 and the other from a putative duplicate gene termed MHCBOP5. All four PCR products were cloned, sequenced, and then genetically mapped with recombinant inbred lines using the 96 well PCR-based IBM DNA Mapping Kit. MHCP5 and MHCBOP5 were mapped in duplicated regions on two different chromosomes. Possible functions of MHCP5 and this novel gene family will be discussed.

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Characterization of an oligopeptide transporter

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Peptide transporters are a well-studied physiological phenomenon in prokaryotes and in fungi. They function in transporting small peptides across the plasma membrane in an energy-dependant manner. In lower eukaryotes internalized peptides serve primarily as a source of amino acids for protein synthesis or as a nitrogen source. Peptide transporters have been classified into different groups based on the energetics and the length of the

substrate. The PTR (Peptide TRansporter) family uses the proton motive force to translocate peptides two to three amino acids in length. The OPT family (OligoPeptideTransporters) also uses the proton motive force for translocation but the substrates are 3-6 amino acids in length. OPT and PTR transporters have been studied in Arabidopsis but none have been characterized in grasses. We have identified several putative OPT type transporter in Zea mays using orthologous sequences from yeast and Arabidopsis. We are in the process of characterizing one of these genes named ZmOPT3. We are using heterologous expression of ZmOPT3 in yeast to determine if ZmOPT3 does indeed encode an oligopeptide transporter. Furthermore, we are attempting to discern if phytosulfokine, a pentapeptide plant hormone, is a substrate for ZmOPT3.

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Isolation of Genes Controlling Agronomic Traits in Maize

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GENOPLANTE, the French Genomic initiative, has initiated in 1999 a Maize Programme devoted to the identification of genes playing a key role in the control of drought or cold tolerance, earliness, grain filling and maturation, and whole plant digestibility. Among the overall objectives, one project focused on two main goals: (1) production of 100,000 ESTs (Expressed Sequence Tags) and (2) identification of sequences differentially expressed under specific conditions. This work has been performed in collaboration (and under contract) with the Celera AgGen Company (Davis, California). Plant tissues were sampled from various sources of material differing in genotype, organ, stage of development, conditions of plant growth (temperature, water availabilityÖ). Libraries were constructed from 76 mRNA samples. Three kinds of libraries were produced: (1) standard libraries without any normalisation or subtraction procedure; (2) normalised libraries, with SSH protocol procedure (the mRNAs were subtracted against themselves), and (3) subtraction libraries (tissue 1 ñ tissue2 Ö). Sequences were produced for 15 standard libraries, 12 normalised libraries and 30 subtraction libraries. The EST sequencing, completed by May 2001, produced 107,559 sequenced > 99bp, merging into 24,775 contigs. The combined assembly of these sequences with publicly available maize ESTs (70,807 in May 2001) produced 31,664 contigs. A modified cDNA AFLP display developed by Celera AgGen and called AFLP-Transcript Imaging (TI) was used to obtain the transcript profile of 40 mRNA samples (partially redundant with the 76 mentionned above). In each sample, about 20,000 tags (Genetags) are characterised for their size and expression level. The comparison among the various samples allows the identification of differentially expressed genes (TI bands). The sequence determination of these bands is made possible by the Genetag database that contains about 150,000 cDNA AFLP tags (Genetags). These were produced through the sequencing of all bands produced with the same TI protocol on reference samples. Identification is based on comparing the size of the TI bands scored on capillary electrophoresis runs and the length of the Genetag sequences for corresponding primer pairs. Sequences corresponding to EST sequencing and the Genetag sequences, in total about 250,000, will be publicly released by July 1st, 2003. This represents so far the largest release of maize sequences in the public domain. Comparisons were made between samples relevant for earliness, kernel filling, drought and cold tolerances. 4,562 non redundant differentially expressed bands (TI bands) were observed and a sequence identification is proposed for 3,352 of them using the Genetag database. Verification of these differentially expressed bands, currently under process, is necessary as ambiguity in sequence identification may remain when several Genetags sequences overlap the confidence interval of the size of the TI bands. This verification process made by RT-PCR using the same samples as used above (for differential expression discovery) allows in a single experiment the confirmation of sequence identity and confirms the differential expression between the two conditions. The presentation will illustrate the diversity/redundancy and the specificity of sequences obtained within and across different libraries in relation with (1) the nature and the complexity of dissected organs, (2) the library construction methodologies. The presentation of the contig assembly merging EST from this project, public EST and Genetag sequences will document the complementarities of EST Sequencing versus Transcript Imaging approaches. In silico northern analysis of these sequences will also be presented and finally the use of some sequences in a functional genomic programme will be exemplified.

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Ectopic knotted1 expression does not perturb gibberellin biosynthesis in maize.

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The maize knotted1 homeodomain transcription factor functions to maintain the indeterminate properties of meristem cells. In maize, ectopic expression of kn1 and other class 1 knox genes through dominant mutations

results in displacement of the ligule into the blade, ectopic ligule formation and the production of tissue outgrowths or ìknotsî. Ectopic expression of class 1 knox genes in dicots results in altered leaf shape, as well as formation of ectopic meristems. Recent evidence from dicots indicates that knox misexpression phenotypes maybe mediated by plant hormones. Ectopic knox expression can result in increased cytokinin (CK) accumulation and reduced gibberellic acid (GA) levels. In tobacco, the KNOX protein NTH15 directly inhibits transcription of the GA 20-oxidase biosynthetic gene Ntc12. In order to elucidate targets of maize knotted1, the lipid-transfer protein2 promoter (Ltp2) from barley was used to drive ectopic expression of kn1 in transgenic maize plants. Ltp2:Kn1 transgenic plants display phenotypes resembling class 1 knox dominant mutations (e.g. Knotted1-O, Rough Sheath1-O and Gnarley1-R). Transgenic plants have displaced blade-sheath boundaries, ectopic ligules in the blade and tissue outgrowths localized along the mid-rib. Leaf tissue from transgenic plants shows abundant accumulation of kn1 mRNA. We tested if genes known to modulate GA biosynthesis in maize, including GA 20-oxidase, were regulated by kn1. None of the genes tested showed differential expression between the transgenic and non-transgenic sibs. Endogenous GA levels were measured in 1-week and 2-week seedlings. No differences in endogenous GA levels were detected in 2-week seedlings and a modest (1.9-fold) reduction in GA1 was detected in 1-week seedlings. The 1-week seedling experiment was not replicated; therefore the slight reduction in GA1 levels cannot be distinguished from experimental error. These preliminary data suggest that the ectopic kn1 phenotype in maize is not mediated through altered GA levels.

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Chromosomal Distribution of Maize Repetitive Sequences

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The maize genome is composed largely (85%) of repetitive sequences, most of which are retrotransposons. Many of these elements are present in high copy numbers and widely dispersed across the genome. A series of oat-maize chromosome addition (OMA) lines, each with an individual maize chromosome added to a hexaploid oat genome, provides the opportunity to assess the relative copy number of specific repetitive elements of each maize chromosome. Microarray technology measures the relative abundance difference of a sequence between two labeled DNAs. Labeling two OMA lines and hybridizing them to an array with immobilized maize repetitive sequences can directly compare the amount of an element present between the two maize chromosomes. The probes in this research were designed to detect whole elements or unique motifs within specific repetitive elements. Preliminary trials have shown that copy number differences can be detected between some of the chromosomes. Trials have been done comparing chromosome 4 with 6 and 3 with 9. Maize chromosomes 3 and 9 have distinctive compositions with respect to certain repetitive sequences. Probes for Zeon1 and Opie2a show the greatest differences between these two chromosomes. The repetitive sequences of chromosomes 4 and 6 are similar in copy number (within 8%). Such results will improve the clarity of the repetitive sequence organization within maize. This research is based upon work supported by the National Science Foundation under Grant No. 0110134.

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The Effects of Host Genetic Background on Aboveground Microsymbionts: Endophytes on Smut-resistant and Smut-susceptible Corn

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In agricultural systems, selection for resistance is usually to a particular pathogen, normally the one that is most economically detrimental at the time. However, the ecological and evolutionary effects of this change in plant environment for other members of the microbial community is not well understood. We are using the Zea mays-Ustilago maydis (corn-corn smut) system to address this issue. Recombinant inbred lines of corn resistant or susceptible to corn smut have been developed and genetic differences associated with resistance or susceptibility have been documented (R. Phillips, see Baumgarten et al. poster). Here, we present preliminary data on endophyte diversity from a field survey conducted in 2002. Endophytes were cultured from surface-sterilized corn tissue samples on media selective for fungi. Endophytes were categorized morphologically, based on colony characteristics. Over 120 unique colony types were identified and over 80 unique colony types were present in the most fully sampled line. We found that over 300 samples were needed to characterize the endophyte diversity of each line. A trend toward lower endophyte diversity in smut-susceptible lines is noted, suggesting that susceptibility to one microbial symbiont, smut, may not increase susceptibility to other symbionts. Next, we plan to use molecular approaches for endophyte identification in order to more fully characterize the endophyte diversity on these corn lines.

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Rmr7 is necessary for silencing at paramutable maize loci.

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The required to maintain repression 7 (rmr7) locus encodes a trans-acting factor necessary for maintaining the silencing that occurs at the maize purple plant 1(pl1) locus as a result of paramutation. The pl1 locus encodes an R2R3 Myb-type transcription factor required for anthocyanin production in specific plant tissues. The Pl1-Rhoades (Pl) allele confers high levels of anthocyanin production, but it occasionally changes to a low expressing state designated Pl1í-mahogany (Plí). In plants heterozygous for Pl and Plí, the Pl allele is changed to the Plí state, and is therefore an example of paramutation. In an EMS mutagenesis screen for factors affecting pl1 paramutation, we isolated two mutant alleles of rmr7 (rmr7-1 and rmr7-2). Genetic analyses show that both mutations represent single locus recessive alleles of a novel genetic locus, located on chromosome 2S. The rmr7 mutant plants can be identified in a Plí background because anthocyanin pigmentation is increased to levels seen in Pl plants. Molecular data reflects this increase, as rmr7-1 plants have significantly higher levels of pl1 mRNA than do rmr7-1/+ plants. Furthermore, tests with other recessive pl1 alleles show that the increased anthocyanin production in rmr7 mutants occurs only in Plí plants, confirming that rmr7 affects Plí silencing specifically and not anthocyanin biosynthesis in general. The rmr7 locus is not required for normal plant development, as rmr7 mutants are morphologically similar to their wild-type siblings, even after five generations of inbreeding. In addition to its role in maintaining Plí silencing, genetic tests indicate that rmr7 function is required to establish paramutation at the booster1 (b1) locus, which encodes another transcription factor involved in anthocyanin pigmentation. The role of rmr7 in epigenetic repression at both pl1 and b1provides evidence of a mechanistic link between paramutation at these loci. Furthermore, the developmental stability of rmr7 mutants suggests a specific rather than global role in gene silencing.

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EFFECTS OF SELECTION ON SEQUENCE DIVERSITY AND LINKAGE DISEQUILIBRIUM AROUND THE MAIZE Y1 LOCUS

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The extent of linkage disequilibrium (LD) in the maize population has important consequences for the usefulness of genetic association mapping. Recent reports in maize have described a rapid decline in LD, ranging from 100-200 bp to approximately 1500-2000bp [Tenaillon et al., 2001; Remington et al., 2001; Thornsberry et al., 2001]. To further evaluate the patterns of LD and sequence diversity in maize, the sequences within and surrounding the Y1 yellow endosperm locus were analyzed. The Y1 gene determines the endosperm color of maize kernels and is thought to have undergone repeated selection for the yellow endosperm phenotype due to its higher nutritional content. Taking advantage of the availability of a 1Mb BAC contig with this locus at a central position [Morgante, 2001], various portions of the Y1 gene and its surrounding genomic regions were amplified and sequenced from a set of 75 white and yellow corn inbreds. All polymorphic sites were identified. Significantly higher nucleotide diversity was found in the white endosperm inbreds at the Y1 locus, while the yellow endosperm inbreds shared a common haplotype throughout the 6kb region, thereby showing significant LD. The low copy regions surrounding Y1 evinced similar patterns of diversity up to 500kb+ away from the Y1 locus on one side only. LD appears to extend much farther than was observed previously, presumably due to the region being subject to selective forces.

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Methylation analysis of tissue culture-induced P white cob maize mutants

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Plant tissue culture is a mutagenic process producing a high frequency of genetic changes among regenerated plants and their progenies. Studies linking mutant phenotypes with molecular changes indicate that transposons, base changes, and small insertions/deletions contribute to tissue culture-induced mutation. The objective of this study was to characterize a potential case of tissue culture-induced epigenetic silencing of P-wr. Five independent p-ww alleles were identified based on segregation for a white cob phenotype among regenerant-derived progeny, and confirmed by allelism tests. The explant source, LH51, contains a P-wr allele. DNA gel-blot analysis indicated that no major rearrangement of the repeated locus had occurred, but revealed hypermethylation in all five new p-ww alleles. DNA gel-blot analysis and PCR have been used to determine the pattern of hypermethylation across the mutant alleles. Results of the methylation analysis will be reported and interpreted in

the context of previous studies on the P locus.

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Transgenic Luciferase Constructs That Respond to Abiotic Stress in Maize

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We have transformed maize with two stress-responsive reporter constructs. The first construct contains the maize rab17 promoter linked to firefly luciferase structural gene. The second contains the maize Adh1-S promoter and luciferase. Both constructs were introduced in separate events into maize HiII embryogenic callus at the Iowa State Plant Transformation Facility by particle bombardment. Selected plants containing either construct were regenerated. Events containing rab17-LUC are being assayed for luciferase expression after applying osmotic stresses (limited irrigation and irrigation with sodium chloride or polyethylene glycol solutions) and abscisic acid. Response of the transgene with each treatment is measured by CCD imaging. Various tissues at several developmental stages can be screened non-destructively with this method. Likewise, the Adh1-LUC plants are assayed for luciferase expression after exposure to hypoxic conditions and cold temperatures. We now have seed from both types. Those showing patterns of luciferase expression similar to that expected for the native rab17 or Adh1 genes are being advanced to generate maize lines with reporters that specifically respond to various environmental stresses. These should be useful tools for studies in plant environmental stress physiology and genetics.

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Gene Expression in the Maize Endosperm Revealed by Genome-wide mRNA Profiling

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We have taken a genomic approach to examine global gene expression in the maize endosperm in relation to dosage and parental effects. Endosperm of eight hybrids generated by reciprocal crosses and their seven inbred parents were sampled at three developmental stages; 10, 14 and 21 days after pollination. These samples were subjected to GeneCalling, an open ended mRNA profiling technology, that simultaneously analyzes thousands of genes. Results indicated that the overall level of gene expression in the maize endosperm was dosage dependent; that is, the gene expression was proportional to the parental genome contribution of 2n maternal : 1n paternal. However, approximately 8% of the genes deviated from such allelic dosage expression and exhibited differential expression in hybrids of reciprocal crosses, resembling either maternally or paternally expressed genes. There were more genes with maternal-like expression than with paternal-like expression. Allele-specific expression analysis of four selected genes using the WAVE denaturing HPLC (dHPLC) system revealed several mechanisms responsible for the deviation from the allelic additive expression in the hybrid endosperm: heterochronic allelic variation, allelic variation in the level of expression and genomic imprinting. A novel imprinted gene, the maize homologue of Non Apical Meristem (NAM) was identified.

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Expression Profiling Across Diverse Maize Germplasm

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The quality and nutritional value of maize kernels varies greatly in diverse germplasm. Many genes are involved in the biosynthesis of starch, protein, and oil in the kernel. Upwards of 100 genes are involved in the regulation and accumulation of zein protein storage bodies alone. To identify additional candidate genes involved in energy biosynthesis in the maize kernel we completed expression profiles from microarrays produced by the Maize Gene Discovery group that are specific to the genes expressed during maize endosperm development (series 605). Expression profiles as well as numerous other phenotypes were collected for analysis in our standard 102 inbred maize lines that represent much of the diversity of modern maize inbreds. Ears were successfully harvested 14 days after pollination from 45 and 89 diverse inbred lines and mutant stocks throughout the 2000 and 2001 summers, respectively, in North Carolina. Total RNA was isolated, labeled and hybridized to arrays in a loop design. Through a range of analysis approaches, we show the variation in expression across diverse germplasm, we examine the heritability of the expression profiles, we relate these expression profiles to kernel quality, and we relate variation in expression to genetic relatedness based on SSR data. This study demonstrates that expression profiling is a powerful way to characterize germplasm diversity.

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Functional analysis of a sorghum myb orthogolous transcription factor promoter in transgenic maize

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The central flavonoid pathway is highly conserved in the plant kingdom. However, functions of the end products and expression mechanisms of the pathways have diverged during the course of evolution. The comparison of phlobaphenes (flavan 4-ols) accumulation in maize and sorghum tissues exhibit conservation as well as divergence. The genes p1 in maize and y1 in sorghum regulate the accumulation of phlobaphenes. The p1 and y1 genes are MYB transcription factors and are highly similar in their coding region. However the 5í and 3í regions as well as genomic organization of these genes show divergence. In this study we tested the hypothesis that the difference in the expression pattern of the phlobaphenes accumulation in two related grass species is due to the divergence of their 5í regulatory sequences. Maize line was transformed with the Y1::GUS construct and expression of the promoter analyzed. Expression results demonstrating the similarity and differences for tissue preferred function of the orthologous gene promoter in maize will be presented.

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A novel screen for single-locus kernel composition mutants.

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Visible mutant analysis of maize kernel composition has identified ~25 genes involved in starch biosynthesis and seed storage protein accumulation. The mutant alleles of these genes have been used to partially decipher these pathways and develop specialized corn varieties with dramatically increased value. All of these genes are sensitive to genetic background modifiers, and only a fraction of the biochemically predicted genes that should influence starch and protein composition have identified mutant phenotypes. These observations suggest that there are kernel composition loci that have phenotypes undetectable to the human eye. We have used single-kernel near infrared (NIR) spectroscopy to screen for mutants that affect kernel composition but do not affect the visible appearance of the kernel. Initially, kernels from visible mutants with composition defects were characterized by NIR to develop a strategy to identify novel mutants with visibly normal kernels. In a screen of M2 families from the UniformMu population, we identified putative mutants based solely on NIR spectra. A subset of the mutants were identified as putatively allelic through tight linkage to the bz1 locus. These mutant isolates share a novel polymorphism in the sh1 locus suggesting that they have a subtle defect in sucrose synthase. These results suggest a strategy for identifying weak alleles of known visible mutant loci and strong alleles of unknown kernel composition modifiers.

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Assessment of transgenic maize events produced by particle bombardment or Agrobacterium-mediated transformation

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We have previously published a protocol to produce transgenic maize using Agrobacterium-mediated transformation. In this study, we compared transgenic events produced by particle bombardment or Agrobacterium-mediate transformation with regard to transgene copy number and RNA expression level using real-time PCR or real time RT-PCR technology. Our results using this new analysis method correlated well with those obtained using traditional Southern and Northern analyses. Results showed that the majority of Agrobacterium-derived transgenic lines contained 1 to 3 copies of the transgene, whereas most of the bombardment-derived lines contained 10 to 277 copies of the transgene. The average amount of transgene transcript in Agrobacterium-derived lines was four times more than that in bombardment-derived lines. Transgene expression in the R2 generation was greater than the R1 progenitor for 77% of the Agrobacterium-derived lines. In contrast, 89% of the bombardment-derived lines had lower transgene expression in the R2 than in the R1 generation. We conclude that transgene expression is more stable in Agrobacterium-derived transformants than in bombardment-derived transformants. Interestingly, we observed that more than 70% of Agrobacterium-derived transgenic lines contained various lengths of the bacterial plasmid backbone DNA sequence, indicating that Agrobacterium transformation was not as precise as previously perceived using the current binary vector system. In conclusion, although the transformation efficiency of Agrobacterium-mediated transformation using a standard binary vector is one-half of particle bombardment in our laboratory, the resulting transformants have fewer transgene copies, and higher and more stable gene expression than their bombardment-derived counterparts.

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Determination of the physiologically significant substrate of RF2A in fertility restoration

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Cytoplasmic male sterility is a maternally inherited inability to produce viable pollen. Texas (T)-cytoplasmic male sterility can be overcome by dominant alleles of two genes, *rf1* and *rf2a*. The *rf2a* gene encodes a mitochondrial aldehyde dehydrogenase (ALDH) whose enzyme activity is required for fertility restoration in T-cytoplasm. Since RF2A can oxidize many aldehydes, the physiologically significant substrate is not known. Three additional ALDH genes (*rf2b*, *rf2c*, and *rf2d*) have been identified in maize and are being used to reduce the number of candidate substrates. A two-pronged approach is being used. First, *in vitro* kinetic enzyme analyses for over 25 commercially available aldehydes have been performed on RF2A, RF2B, RF2C, and RF2D. Second, complementation constructs containing the *rf2a*, *rf2b*, *rf2c*, or *rf2d* coding region, driven by the *rf2a* promoter and targeted to mitochondria with the RF2A mitochondrial targeting sequence, were transformed into maize. If the transformed complementation construct is able to oxidize the physiologically significant substrate in a (T) *rf2a/rf2a* background, then viable pollen will be produced. Fertility observations have revealed that both the *Rf2a* (control) and *Rf2c* constructs, but not the *Rf2b* and *Rf2d* constructs, complement the male sterile phenotype. Since *Rf2c* can complement the male sterile phenotype, the RF2A and RF2C proteins can both oxidize the physiologically significant substrate. Comparison of the kinetic data between RF2A and RF2C has identified four aldehydes that have common kinetic characteristics for both proteins. To determine which of these substrates is physiologically significant, microarray analyses will be performed on tapetal cells of RF2A and RF2C complementing plants to identify pathways that are coordinately regulated between these two constructs and that are therefore likely to be involved in the generation of these aldehydes.

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Microarray Analysis of Trichostatin A and 5-aza-2-deoxycytidine Treatments in Maize Tissue Culture

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Microarrays allow for the simultaneous screening of multiple genes for changes in transcription in response to experimental conditions. The goal of this experiment was to detect genes whose expression is sensitive to alterations in DNA methylation or histone acetylation. Maize suspension cultures were treated with either the DNA methyltransferase inhibitor, 5-aza-2-deoxycytidine, or the histone deacetylase inhibitor, trichostatin A. RNA was isolated and used to interrogate maize 1-1-03 Unigene arrays using untreated cultures as a control. Data were analyzed to determine which genes were altered in one or both treatments. The results of this experiment will be presented.

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Molecular and genetic characterization of a 'gain of function' Mu insertion P1-wr allele

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The maize p1 locus encodes a MYB-homologous regulator of red phlobaphene pigment biosynthesis in pericarp, cob glumes, and other plant tissues. The P1-wr allele of the p1 gene specifies the accumulation of pigment in the cob, but not the pericarp (Styles and Ceska, Maydica 34: 227-237, 1989). In order to gain a greater understanding of the tissue specificity associated with this allele, induced mutations were identified using the Trait Utility System for Corn (TUSC) developed by Pioneer Hi-Bred International, Inc. (Meeley and Briggs, MNL 69: 67-82). Using several P1-wr specific primers, we have detected 13 unique Mu insertion alleles. One of the insertion alleles shows ëgain of functioní in pericarp. We tested if the gain of function is the result of Mu suppression, as has been observed in hcf106 mutation (Barkan and Martienssen, Proc. Natl. Acad. Sci. USA 88: 3502-3506, 1991). Genetic and molecular analysis of this ectopic P1-wr-mum1 expression and pericarp suppression in the w-t allele will be presented

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Proteins associated with *Spodoptera frugiperda* resistance in Zea mays L.

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Spodoptera frugiperda, fall armyworm (FAW), can cause significant economic loss in maize. Commercial non-transgenic maize lines are susceptible to feeding damage. We have identified several quantitative trait loci (QTL) conferring resistance to whorl-stage feeding damage in maize. Tropical maize provides the source for the resistance genes. One QTL corresponds to the *Glossy15* gene, a regulatory gene that causes abbreviated expression of the juvenile phase in maize leaves. The remainder of the QTL are unknown. An abbreviated juvenile phase has been linked to resistance. Protein patterns from juvenile and adult leaf tissue of corn (*Zea mays*

L.) inbreds that are resistant or susceptible to fall armyworm were analyzed through 2-dimensional electrophoresis. Tissue was collected from F3 families of a cross between the resistant parent (Mp705) and the susceptible parent (Oh28). Bulks were made from the 13 most resistant and 13 most susceptible F3 families. Adult and juvenile tissues were analyzed separately. Both presence/absence and protein intensity differences were identified. Differently expressed proteins were excised from the two-dimensional gels and analyzed through mass spectrometry to determine protein identity. The goal of this comparative protein research is to identify putative *S. frugiperda* resistance genes in maize corresponding to our QTL. These resistance 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.

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The molecular characterization of the sequences required for b1 paramutation and high expression

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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 maize pigmentation pathway, the high expression of the B-I allele is downregulated to the lower expression level of the Bí allele, in B'/B-I heterozygous plants. New B' alleles behave like Bí, they paramutate B-I alleles with a 100% efficiency. The sequences required for Bí paramutation and B-I expression locate in a 6 kb region ~100 kbp upstream of the b1 coding region, suggesting the involvement of long range communication between sequences far upstream and the promoter proximal region (Stam et al., 2002, Genes & Dev. 16:1906; Stam et al., 2002, Genetics 162:197). Within the 6 kb region, Bí and B-I have 7 tandemly repeated copies of a 853 bp sequence which are involved in paramutation. There are no sequence differences between Bí and B-I in this region, establishing they are epialleles. The Bí and B-I repeats are currently being compared at the molecular level making use of trans-acting mutations affecting paramutation. Analyses of the repeat DNA methylation levels using methylation sensitive restriction enzymes revealed a complex pattern of correlations. Some sites are methylated only in Bí and other sites only in B-I. The methylation of the former sites is partially relieved in a mop1-1 mutant background, but not in a Mop2-1 background. We speculate that the differential methylated regions may have different functions. Some sites might be involved in paramutation, others in enhancing expression. Nuclease accessibility assays revealed that the B-I repeats have a more open chromatin structure than the Bí repeats. New Bí alleles had an intermediate chromatin state relative to B-I and Bí. The question which chromatin structure changes are correlated with paramutation is currently being studied in more detail.

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Identification and Expression of Flavonoid 3'-Hydroxylase in Maize and Sorghum

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Flavonoids are secondary metabolites that function in plant growth and development through pollen tube germination, responses to stress, pigmentation, protection from UV light, and as antimicrobial phytoalexins such as anthocyanidins. Here we attempt to further elucidate the role of flavonoid-3í-hydroxylase, a cytochrome P450, and its possible function in the flavan-4-ol pathway which leads to the synthesis of phlobaphene pigments. Previous research has shown that the pr gene may encode for the F3íH activity required for the conversion of dihydrokaempherol to dihydroquercetin in the 3-hydroxyanthocyanidin pathway (Larson et al., 1986, Biochem. Genet. 24:615-624), however, the f3íh gene has not been cloned. Our preliminary attempts indicate that f3íh may also play a role in the flavonoid 3-deoxyanthocyanidin biosynthetic pathway. Comparisons of available genomic and proposed amino acid sequences were used as a starting point in isolating f3íh cDNA and genomic clones. Results from expression studies of f3íh will be presented.

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MAR-induced variegation

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Chromatin loop organization is based on interactions between DNA matrix attachment regions (MARs) and the nuclear protein matrix. In this study, we recreated an in vivo pre-existing chromatin loop based on MARs. We used the 5í and the 3í MARs of maize Adh-1 gene to bracket a Gus expression cassette. Unexpected quantitative results, obtained in various tissues of fully regenerated plants, led us to check the transgene expression pattern. Surprisingly, maize Adh-1 MARs induced a systematic variegated expression pattern in maize roots. To our knowledge this constitute the first report of intraspecific MAR induced variegation. Furthermore, we found that

tissue and developmental specificity were superimposed to variegation. Our results reveal new aspects of MAR influence gene regulation.

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LOCALIZATION OF A BACTERIAL PROTEIN IN STARCH GRANULES OF TRANSGENIC MAIZE KERNELS

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The B subunit of Escherichia coli heat labile enterotoxin (LT-B) is a potent oral immunogen, with potential for use as a vaccine and an adjuvant to co-administered vaccine. LT-B has been used as a model antigen to demonstrate the feasibility of producing effective oral vaccines in transgenic plants. We expressed a synthetic gene encoding the LT-B subunit in transgenic maize kernels. Using immunogold labeling /electronmicroscopy and cell fractionation/western analysis, we demonstrate that the LT-B protein, when produced with either its native bacterial signal peptide (BSP) or the signal peptide from the maize 27 kDa gamma-zein, accumulates in starch granules of transgenic maize kernels. These observations suggest that the BSP in is not required for localization of LT-B to starch granules, and that starch localization is a feature of the mature protein. Analysis of ground kernel endosperm by ELISA and Western analysis also indicates the presence of LT-B in the soluble (extra-granular) fraction. The functional properties of starch encapsulated LT-B are yet to be determined. The unexpected localization of LT-B in starch granules suggests the existence of a novel amyloplast targeting mechanism. This targeting property is a potentially valuable cell biology tool for further elucidation of protein translocation into the amyloplast. Furthermore, LT-B could be used to direct other proteins to the amylopast, conferring stability to the conjugated proteins, a feature that is desirable in edible vaccines.

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Targeting Induced Limited Lesions IN Genomes (TILLING) for Maize: Reverse Genetic Analysis of Point Mutations

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TILLING is a reverse genetic method that combines chemical mutagenesis, typically EMS, and PCR-based screening in gene regions of interest. The approach complements other reverse genetic methods and provides a range of allele types, including missense and knock-out mutations, potentially useful in gene function and interaction studies. The Arabidopsis TILLING Project (ATP) in Seattle has developed into a, low-cost, high-throughput operation for plant functional genomics and we are establishing a maize TILLING venue modeled after it. ATP has developed, interactive, web-based bioinformatics tools that allow users to identify gene regions most suitable for TILLING, design optimal PCR primers for mutational screening, and to map, analyze and evaluate the mutations that are discovered. Preliminary data indicate that standard EMS pollen mutagenesis protocols provide sufficient mutation density to carry out efficient TILLING in maize. Mutagenizing both the W22 and B73 inbreds, we have set a target of developing ~10,000 mutant lines for each inbred. M1 leaf tissue is used for DNA analysis and M2 seed will then be distributed to users. Gene segments will be screened, mutations identified and sequenced and seed stocks for identified mutants made available through the Maize Genetics Stock Center. TILLING technology has potential benefits for agriculture, where methods are needed for crop improvements that avoid the expensive regulatory process required for transgenics and that are widely acceptable to consumers. We provide an overview of the methodology and bioinformatics tools, and we present preliminary data and our progress to date.

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The origins of sweet corn

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The first mention of US sweet corn, referred to as ìshriveled cornî in the literature is from Thomas Jefferson in 1810. By 1909, Correns had characterized the sugary1 locus, and much later is was discovered that this locus produces a starch debranching enzyme of the isoamylase type (James et al, 1995), responsible for building starch molecules in concert with the starch branching enzymes. A mutation at this locus is responsible for producing kernels that contain a high concentration of phytoglycogen and taste sweet. The phenotype appears as a wrinkled, translucent kernel that we recognize as normal commercial sweet corn. The data presented here clarify the origin of sweet corn, for which there were two competing hypotheses. One theory argues a single origin from a Peruvian race (Chullpi) (Manglesdorf, 1974), whereas others propose independent origins from recurring mutations (Erwin, 1951; Tracy, 2001). Morphological and molecular analyses have confirmed 4 and possibly 5 unique, independent mutations responsible for the sugary phenotype in 64 sweet corn accessions from North America and South

America. Three of the mutations are single amino acid changes at residues that are highly conserved across plants, Archaebacteria and Eubacteria. The fourth mutation is the result of a transposable element in exon 1, which disrupts translation. The fifth putative mutation, present in accessions from Peru, is most likely a modification of the 5í end of the gene, but further investigation is required. There is strong geographic patterning to these mutations. The abundance of single amino acid changes responsible for several of the sweet phenotype mutations is quite surprising and may reflect constraints on the su1 locus for germination.

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Genotype-specific trans-acting Factors Influence Meiotic Recombination in the 140-kb a1-sh2 Interval and Elsewhere in the Genome

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Rates of meiotic recombination can be affected by *trans*-acting factors: genotype-specific modifiers not residing in the interval of interest. Such *trans* factors were previously shown to affect rates of recombination in an identical 140-kb *a1-sh2* interval of chromosome 3 that was introgressed into the A632, W64A and Oh43 inbred backgrounds. Rates of recombination were increased in the A632 and W64A inbred backgrounds two-fold and high-resolution recombination break-point mapping showed that *trans*-modifiers in these backgrounds increased rates of recombination at existing hotspots. To test the hypothesis that patterns of breakpoint distribution differ within a hotspot, fine-structure mapping of the distribution of breakpoints within the *yz1* gene was conducted. These analyses revealed different patterns of breakpoint resolution among the inbred backgrounds. These findings demonstrate that *trans*-factors can affect both the rates and distributions of meiotic recombination in the *a1 sh2* interval. To test the hypothesis that *trans*-acting factors in these inbred backgrounds can also affect rates of recombination in other regions of the genome, the genetic distances associated with a genomic interval that is not located on chromosome 3 were determined in each of the three inbred backgrounds. This genomic interval has a significantly greater genetic distance in the A632 background as compared to the other inbred backgrounds.

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Molecular analysis of the maize P1-rw allele

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The maize p1 gene encodes a Myb-like transcription factor which regulates the synthesis of a phlobaphene-like red pigment in kernel pericarp, cob and other floral organs. Some p1 alleles elicit differential pigmentation of pericarp and cob: for example, P1-rr, P1-wr, and P1-rw specify red pericarp/red cob, white pericarp/red cod, and red pericarp/white cob, respectively. These alleles provide excellent resources to study the mechanism of organ-specific gene expression. The P1-rr and P1-wr alleles have been previously characterized; we are now investigating the gene structure and molecular expression pattern of the P1-rw allele. Genomic Southern analysis indicates that, similar to P1-rr, P1-rw contains a single copy gene flanked by long direct repeats. RT-PCR results showed that the P1-rw 5°Ø UTR and 5°Ø coding sequences are identical to P1-rr and P1-wr. However, the P1-rw 3°Ø coding sequences and 3°Ø UTR are identical to that of the p2 gene, a tightly linked, paralogous gene. To reconcile these results, we isolated and sequenced two overlapping P1-rw genomic clones. Interestingly, although P1-rw is structurally similar to that of P1-rr (both contain a coding sequence flanked by two long direct repeats), P1-rw differs from P1-rr in two major characteristics: First, the P1-rw coding sequence is chimeric, and consists of a p1-like 5°Ø UTR, followed by p2-like exons and introns, followed by a truncated P1-wr-like exon 3. This chimeric structure appears to have been generated by recombination between the p1 and p2 genes. Second, P1-rw has a major polymorphism in the 1.2 kb distal enhancer region which is located 5 kbp upstream of the p1 transcription start site. Our current hypothesis is that polymorphisms in the coding sequences and/or the distal enhancer region are responsible for the distinct gene expression pattern of P1-rw. Nuclear run-on assay is underway to test whether P1-rw expression is regulated at the transcriptional or post-transcriptional level, or both.

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Nutritionally Improved Transgenic Sorghum

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Sorghum is the dietary staple food to over half a billion people in developing countries. However, sorghum grain is low in protein quality due to its low contents of essential amino acids, such as lysine. The reliance on sorghum results therefore in malnutrition, especially of children, in many countries in Africa and Asia. Advancements in

sorghum tissue culture and transformation research in Pioneer have led to the development of the first efficient method to genetically transform sorghum by Agrobacterium. With the objective to use this novel technology to improve the nutritional quality of its grain, we introduced into sorghum a high-lysine gene (HT12) that we previously found to be efficacious when transgenically expressed in corn. Sorghum was transformed using a ìsuper-binaryî Agrobacterium vector containing two unlinked T-DNA cassettes. One of these cassettes contained the HT12 gene and the other contained a herbicide-resistance gene as a selectable marker. In progeny of the primary transformants, this potentially permits the segregation of the marker gene from the trait gene. The elimination of the marker gene is important, because sorghum can cross-breed with wild relatives and herbicide-resistant plants can not be released into the environment. Of four independent transgenic events obtained, three expressed high levels of the HT12 protein in the grain. Several plants from these events were selfed and the progeny was analyzed for segregation of the herbicide marker and the HT12 gene. Preliminary data demonstrate the successful elimination of the herbicide-resistance gene from one event. Heterozygous seed from this event showed a 40-60% increase in lysine. A transgenic, lysine-enriched sorghum variety has the potential to directly benefit large populations in developing countries. Assistance in the development and in the release of such a variety would therefore demonstrate to a wide public the possibility of biotechnological solutions to important agricultural and nutritional challenges.

Quantitative Traits

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Identification of QTLs controlling Ustilago maydis resistance in two populations of recombinant inbred lines

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We report the identification of QTLs contributing to Ustilago maydis (corn smut) resistance in two populations of recombinant inbred lines of maize. The two RI populations were generated by crossing a susceptible inbred (CMV3) to two inbred lines (A188 and W23). During the summers of 2000-2002, the frequency and severity of U. maydis infection on these RI lines was evaluated in St. Paul, MN and Waseca, MN. We found that resistance to U. maydis infection was highly heritable (h2 = 0.65-0.84) in both populations of RI lines and several lines segregated for U. maydis resistance in specific tissues. 100 previously mapped SSR markers were surveyed across RI lines from both populations and examined for association with U. maydis infection. Several QTLs for U. maydis resistance were discovered, but significant associations were found in all replicate blocks between markers mapped to chromosome 2, 4, and 6. Some QTLs were observed only in specific environments, indicating that genetic by environment interactions may be influencing the U. maydis resistance of several RI lines. Similar to other studies mapping QTLs for pathogen resistance, QTLs for U. maydis resistance mapped to chromosome regions containing resistance gene homologs, defense-related genes, and QTLs for pathogen resistance.

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Association mapping of starch candidate genes with kernel composition and starch viscosity traits

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Starch production in the maize kernel is a complex trait controlled by multiple genes. Depending on the specific needs of production, maize breeders select for kernel composition traits, such as the amount of starch produced in the kernel. An equally important aspect of maize kernel quality and of great interest for food processing applications is the pasting and viscosity profile of the starch produced. This study used 97 diverse maize lines in an association mapping approach to locate DNA polymorphisms within six candidate genes that had an effect on kernel composition traits (starch, oil, protein) or starch pasting/viscosity traits. Starch candidate genes Ae1, Bt2, Sh1, Sh2, Su1, and Wx1 were amplified, sequenced, and tested for associations using the software package, TASSEL. Principle component factor analysis was used to reduce multiple testing and to examine underlying factors contributing to the multidimensional nature of kernel physicochemical properties. Overall gene analyses indicated significant results for kernel composition traits from Sh1, Sh2, and Bt2. Significant associations for starch pasting/viscosity traits were seen in Ae1 and Sh2. In order to assess the effects of Sh2 on starch traits, haplotype analysis of a 500 bp region was performed. Although a significant effect on amylose was seen in the winter field season ($P < 0.04$), it was not significant in the summer field season ($P = 0.20$). However, Sh2 showed a significant genotype x environment effect on kernel composition, therefore we propose the hypothesis that with

higher ambient temperatures, the heat labile nature of ADP-glucose pyrophosphorylase (Green and Hannah, 1998), in which Sh2 encodes a subunit, varies between Sh2 haplotypes and has an epistatic effect on amylose production.

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A large region upstream to the teosinte branched 1 (tb1) locus was selected during maize evolution

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Quantitative trait locus (QTL) mapping studies have suggested that a major locus, teosinte branched 1 (tb1), is responsible for many of the morphological differences between maize and its wild ancestor (teosinte). The gene for tb1 was cloned by transposon tagging and is predicted to encode a transcriptional regulator. Levels of tb1 RNA are two fold higher in maize as compared to teosinte, and sequence evolution studies have demonstrated that the tb1 promoter region, but not the coding region, shows a significant signature of selection. These results suggest that differences in tb1 regulation were the target of selection during maize domestication from teosinte. Nonetheless, the molecular changes that were selected at tb1 are not known. To better understand the nature of selection at tb1, we have generated 220 kb of contiguous genomic sequence around the tb1 locus, and assessed sequence diversity at sites 5' to the tb1 coding sequence. Sequence analysis has revealed that a large 162 kb region between tb1 and the next 5' gene contains only transposable elements and non-genic unique sequences. Nucleotide polymorphism analysis at sites throughout this intergenic region has revealed low sequence diversity (a sign of selection) among maize lines from the tb1 promoter region extending to at least 58 kb upstream. In contrast, at 93 kb upstream to tb1, as well as at the next gene 5' to tb1, sequence diversity in maize is nearly that observed at neutral sites in the maize genome. Sequence diversity throughout the region in teosinte is high. These results suggest that a large region of 58 to 93 kb was selected during maize domestication and that this region is likely to harbor sequences responsible for the derived maize morphology. The large size of the selected region may reflect the fact that multiple polymorphisms in the region are required for maize morphology. Alternatively, low recombination rates upstream to tb1 may have contributed to maintenance of an extensive region of low sequence diversity following selection at a given site during domestication. The pattern and extent of linkage disequilibrium in the region will also be discussed.

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Genetic Control of the Number of Ears per Plant and Related Morphological Traits in the Golden Glow Maize Population

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Prolificacy is a natural characteristic in maize. Humans, however, have tended to select for the concentration of resources in a single large ear borne at the apex of the primary lateral branches. Since 1971, bi-parental mass selection for increased number of ears per plant has been carried out in the open-pollinated maize population Golden Glow at the University of Wisconsin - Madison. To better characterize the changes in morphology accompanying selection for prolificacy in Golden Glow, a mapping population has been developed from the cross of inbred A679 with a highly-prolific plant from cycle 23 of Golden Glow. The objective of this study was to determine the genetic basis of prolificacy and related traits and to determine whether the genetic control of prolificacy was related to genetic events associated with the domestication of maize. Significant phenotypic variation was observed for most traits analyzed in this experiment. Twenty-four QTL were found for the nine traits described here. In a number of cases, regions of the genome controlled more than one morphological trait at a time. The number of significant associations between molecular markers and a particular trait, as well as the total percentage of phenotypic variance accounted for by each QTL, varied significantly from trait to trait. The largest QTL found in this study explained 36.5% of the phenotypic variance for the frequency of tassels at the end of tillers. Eleven of the 24 associations described here involved linkage group 1. A particularly interesting region was located on bin 1.09 of the mapping population. This region shared many similarities with the pattern of development suggested for the teosinte branched1 mutant. This indicates that the genetic control of prolificacy and associated traits in Golden Glow resembles the process of maize domestication at least in regard to its branching morphology.

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Selection for Methionine and Tryptophan Content in Maize

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Some essential amino acids in maize are nutritionally limiting for animal diets. In plant breeding, selection to

improve a population while conserving genetic variation within the population is referred to as recurrent selection. The potential of this method to improve maize populations for their composition in two amino acids such as Methionine (Met) and Tryptophan (Trp) was tested. The objectives of this study are (1) to determine the effect of selection on the amino acid composition in maize, (2) to conduct divergent selection for both Trp and Met, and (3) to determine the effect of selection on the nutritional value of the populations. Initially two random-mated maize populations, BS11 and BS31, were chosen for this study on the basis of their protein content and variability. The two populations were treated independently and selections were categorized for high Met, low Met, high Trp, and low Trp within each population in Cycle 0 in 2001. The first cycle of half-sib family selection for Met and Trp was completed in the summer of 2002. For both populations in Cycle 1, the values for the high Met and Trp selections were significantly higher than the values for the low Met and Trp selections. These data suggest that a divergent selection program is a useful method for producing populations with improved Met and Trp content. These populations will be a valuable resource for researchers interested in the genetics and biochemistry of kernel amino acid content.

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Candidate Gene Association of Kernel Composition in Diverse Maize Inbreds

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Kernel quality in maize is an important quantitative trait that has been studied extensively and many QTL have been identified for starch, protein, and oil content. Candidate gene association mapping, also known as linkage disequilibrium mapping, can be used to dissect QTL on a finer scale than traditional QTL mapping. Previously, association studies have identified that starch content and quality associate with polymorphisms in genes late in the starch pathway.

Our lab is also interested in earlier events that lead up to kernel development and starch biosynthesis. *Lachrima1* (*lac1*) is a defective kernel mutant (*dek34*) with an unknown function, *brittle1* (*bt1*) is an amyloplast adenylate translocator, and *dull endosperm1* (*du1*) is a soluble starch synthase. Regions of these genes were sequenced in 32 inbred lines chosen for diversity in kernel composition and analyzed for association with kernel composition traits, i.e. kernel protein, oil, starch, and amylose.

Several polymorphisms in each gene have been identified that significantly associate with kernel composition. Efforts are currently underway to sample these polymorphisms in a larger set of 102 inbred lines in the hope of identifying the causative site. We have also initiated a study of the invertase gene family, which may be involved in assimilate translocation as their gene products cleave sucrose into glucose and fructose.

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The genetic and molecular basis of cell wall digestibility in silage maize

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Maize feeding value is mainly related to the digestibility of the vegetative parts, and especially of cell wall components. Lignin is the cell wall biopolymer hindering forage digestibility the most. Several lignification parameters influence silage maize digestibility: lignin content and composition, and chemical cross-linking with other cell wall components. In order to understand relationships between cell wall lignification and digestibility, we have performed QTL analyses of cell wall lignification traits, and investigated allelic polymorphisms of a candidate gene. The analysis of genetic variation in cell wall traits was performed on 25 lines covering a wide range of origins and digestibility. Traits related to cell wall digestibility were measured. QTL for some of these traits were identified in a Recombinant Inbred Line population derived from F288 x F271, and compared with QTL for digestibility. QTL for *p*-coumaric acid and ferulic acid were different. Digestibility QTL co-localized with QTL of lignin and phenolic acid content. Analysis of allelic variation of the gene coding for caffeic acid *O*-methyltransferase (*COMT*) was performed. This gene was selected because mutations in this gene result in the *brown midrib3* phenotype, which is associated with changes in cell wall composition and a major improvement in digestibility. Seven haplotypes of this gene were characterized. The high degree of polymorphism was partly due to several recombination events. It supports the idea of screening for favorable alleles to achieve higher digestibility. Screening of allelic variation of *COMT* should be performed in a larger germplasm collection. This will provide a database for the definition of relationships between allele polymorphism and digestibility, which is helpful for the identification of favorable alleles. They can later be introgressed in breeding material. The various parts of the genome where QTL were detected will lead to the identification of other candidate genes suitable for allelic variation study.

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Whole-Genome Mapping of Maize UV Responses

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Understanding of the mechanisms plants use for adaption to ultraviolet radiation stress is key in predicting plant responses to our changing environment. Study of UV perception has been complicated by uncertainty about whether a particular response is due to damage/inhibition or due to signal transduction. Many different UV responses have been measured (growth, shape change, photosynthetic parameters, etc.) but these studies generally suffer from difficulty in determining the underlying mechanism. We are analyzing UV responses at multiple levels, from gene expression to morphological change. We have carried out the first round of genome wide-scans to identify and map the most important UV response control loci in the IBM mapping population. We have begun comparisons of the QTL pattern of UV responses; there are some QTL which are shared among a subset of responses and others which are response-specific. Statistical analysis of the number and location of the QTL will allow us to determine if there is a ìmaster switchî that controls all UV responses, or if there is a set of separate switches for each different kind of response. Mapping of control loci, and examination of map patterns, will allow integration of a great deal of data that would otherwise exist in isolation.

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Identification of QTL Responsible for Root Architecture

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Drought resistance is the single most limiting factor to crop productivity worldwide. Many factors are associated with drought response including anthesis-silking interval, leaf surface area and root architecture. Our objective is to identify the QTL for root architecture in Zea mays and investigate their role in drought response. Two experiments were conducted. Ten lines with different drought tolerance levels were evaluated in two reps in a randomized complete block design in the greenhouse. After two weeks of growth taproot length, overall root branching, number of primary branches, root mass, shoot mass and number of leaves were measured. A subset of 94 mapping lines from the IBM population were planted in five reps and the same traits measured. Genotypic data for 251 markers , evenly distributed throughout the genome, were used for QTL analysis. The genetic map was constructed with Mapmaker Exp version 3.0 for Unix. QTL analysis was performed using QTL Cartographer Version 1.16. QTL positions were compared to QTL identified in prior drought tolerance experiments.

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Identification and characterization of Al tolerance genes in the Intermated B73 x Mo17 (IBM) population by quantitative trait locus mapping

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Aluminum (Al) is the third most abundant element in the earthís crust and toxic to plant roots if solubilized at acidic pH. Aluminum toxicity, which results from inhibition of root growth and nutrient/water acquisition, is a serious limitation to crop production, as up to one-half of the worldís potentially arable land is acidic. Aluminum toxicity is one of the primary soil constraints for Latin America and Sub-Saharan Africa, where maize is the primary staple crop. Increasing plant aluminum tolerance has been an objective of plant breeders worldwide for many years, yet no tolerance genes have yet been cloned. The physiological mechanisms that underlie aluminum tolerance are somewhat better understood. The best-characterized mechanism involves an aluminum-activated release of low molecular weight di- and tri-carboxylic organic acids (e.g. malate, oxalate, citrate) that can bind soluble Al in the rhizosphere and prevent its uptake into the root. Other mechanisms (e.g. alteration in rhizosphere pH, release of phenolics, ameliorating Al-induced free radicals) quite possibly also can be contributing to Al tolerance in some plant species, but their presence have yet to be confirmed. We initiated a quantitative trait loci analysis of aluminum tolerance in maize, using the IBM population as our subject, with the intention of identifying the genes responsible for tolerance to aluminum stress and the underlying physiological mechanisms. The IBM population is especially attractive, as B73 and Mo17 appear to utilize different mechanisms to create similar levels of tolerance. Transgressive segregation has generated varieties with tolerance similar to that seen in elite aluminum tolerant materials developed by EMBRAPA Maize and Sorghum, far more tolerant than either parent. Using a preliminary dataset, we identified five QTL regions important for aluminum tolerance and explain 48% of the variance observed; Mo17 contributes the superior allele for four of the five loci. Interestingly, these four loci appear to act epistatically with one another suggesting they function in a common pathway. Comparative mapping of QTLs between our study and one from EMBRAPA Maize and Sorghum will also be discussed.

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Searching for Virus Resistance Genes-is mdm1 a Universal Virus Resistance Gene?

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Previously, a gene controlling resistance to Maize dwarf mosaic virus strain A (MDMV) was identified in the inbred line Pa405. The gene was closely linked to the RFLP marker umc85 on the short arm of chromosome 6. This gene also conferred resistance to two other viruses in the family Potyviridae: Sugarcane mosaic virus (SCMV/MDM-B) and Wheat streak mosaic virus (WSMV). To determine whether resistance to these viruses was linked to the gene on chromosome 6S in a diverse array of maize germplasm, we identified 42 MDMV-resistant inbred lines, then crossed these lines to three MDMV susceptible inbreds and developed F2 populations. The F2 populations were screened with MDMV and leaf tissue from susceptible and resistant pools was screened with RFLP and SSR markers. In 40 of the 42 inbreds, chromosome 6S markers associated with the resistant parent were found in the resistant, but not the susceptible pooled tissue. In addition, markers associated with SCMV/MDM-B and WSMV resistance on chromosome 3 and WSMV resistance on chromosome10 were found only in the pooled resistant tissue for 12 and 7 inbreds, respectively. These data suggest that mdm1 is associated with MDMV resistance in most germplasm, but that other loci may also affect resistance. In addition to resistance genes, rating date and environmental factors influenced the development of symptoms and phenotypic classifications. Consequently it was not possible to determine the type of gene action.

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Molecular evolution of genes encoding AGPase in endosperm of Zea mays

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Sh2 and Bt2 genes encode for subunits of a major enzyme in endosperm starch biosynthesis pathway in Zea mays, ADP Glucose Pyrophosphorylase (AGPase). We study the molecular evolution of both genes in wild and cultivated forms, including different subspecies of teosinte, landraces and inbred modern lines. For Sh2, natural selection, acting from well before domestication, has strongly reduced nucleotide diversity among both wild and cultivated forms. Similar haplotypes are found among maize and teosinte. For Bt2, the severe reduction of nucleotide diversity during maize domestication and the phylogenetic reconstruction suggest that Bt2 is involved in the domestication syndrome. Although these two genes encode for interacting subunits of the same enzyme and are both under strong selective pressure, they show independent evolutionary histories, leading to reduced genetic diversity and no evidence of coevolution at the molecular level.

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Marker Assisted Selection for Resistance to Aspergillus flavus and Aflatoxin production in Maize.

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Genetic studies were performed on Aspergillus ear rot and aflatoxin production in (Tex6xB73)xFRB73 BC1S1 and Tex6xFRB73 F2:3 mapping populations derived from maize inbred Tex6, associated with relatively low levels of aflatoxin production. Two major QTL regions for low aflatoxin were identified on chromosomes 5 and 10 in Tex6, explaining 41% of total phenotypic variation. Two largest QTLs for resistance were identified in Mp313E chromosome 4L in Mississippi (Davis et al., 1999). Our objective is to pyramid the two different chromosomal regions of resistance genes in Tex6 and 4L chromosome regions in Mp313E into a commercially used susceptible inbred line FR1064 background through marker assisted selection. Different stages of back crossing onto FRB73 with the various combinations of significant genomic regions for resistance from Tex6, and FR1064 with the significant fraction of resistance from Mp313E were genotyped. The homozygous families for the significant chromosomal regions associated with the resistance in Tex6 and Mp313E, were identified. The initial preliminary analyses of some homozygous lines for Mp313E 4L segment in FR1064 showed that the level of aflatoxin is in the range of 150-200ppb as opposed to FR1064, in which the range is 700-800ppb. Selected homozygous lines for 4L from Mp313E and chromosome regions 5 and 10 from Tex6 were crossed; (Mp313E x FR1064)FR1064 BC2S1 with (Tex6 x FRB73)FRB73 BC2S1 to produce F1s. Segregating progenies for these regions in F2 were genotyped in summer, 2002. Plants were selected for either homozygous or heterozygous for the significant regions from both resistant parents and then selfed. Six families were identified and individual plants were genotyped in the winter green house. A few plants were identified that were homozygous for all chromosome segments, while few more plants were identified with different combinations of major regions. These advanced lines will be selfed, genotyped further to pyramid homozygous resistance genes from different

genetic sources in multiple lineages. Lines will be evaluated for resistance recovery and yield performance in the subsequent years.

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Xenia effect on phosphorus concentration in outcrossed maize seed

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Xenia effects have been reported for seed weight and oil concentration in maize seed. However, the effect of foreign pollen on seed mineral nutrient concentration have not been studied. The objective of this research was to determine if foreign pollen alters seed phosphorus (P) concentration. Fifteen maize inbreds were crossed onto two hybrids, B73xMo17 and Oh43xW64A, over three years. Seed weight and P concentration were measured and used to calculate seed P uptake. A xenia effect was found for P uptake with some inbred crosses resulting in increased P uptake and some in decreased P uptake compared to the selfed hybrid control. A significant xenia effect was also found for seed size. Although a difference in P uptake was found, the difference is small enough that open pollinated maize populations can be used for studies measuring P content of seed.

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The impact of farmer management practices on maize landrace genetic diversity

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We report on the impact of traditional farmer management of maize populations in structuring molecular and phenotypic diversity and on the population dynamics of maize landraces. These populations, from a sample of local landraces cultivated by farmers in six villages in the Central Valleys of Oaxaca, Mexico, show small among-population differentiation (Fst=0.011). Most surprisingly, there is no isolation by distance and small among-village differentiation (Fst=0.003). One would expect populations to fit Hardy-Weinberg equilibrium, but significant homozygote excess (Fis=0.13) was found. This homozygote excess shows remarkable interpopulation and interloci differences. We show that this pattern is related to the variation for flowering traits of a given population. A short anthesis-silking interval and high level of heterogeneity in flowering precocity will favor assortative mating. This leads to a loci dependent population substructure giving an unusual case of Wahlund effect and inbreeding while high levels of seed exchange among farmers prevent population differentiation at both village and regional levels. In spite of considerable gene-flow, high levels of population differentiation for quantitative traits were observed (i.e., an among-village Qst value of 0.535 for kernel weight). We show that phenotypic diversification is the result of farmer divergent selection in spite of predominant, but not strict, conservative selection. Furthermore, we characterized nonproportional changes in the G matrix structure both within and among villages that are consequences of farmer selection. As a consequence of these differences in the G matrix structure, the response to multivariate selection will be different from one population to another. Large changes in the G matrix structure could indicate that farmers select for genes of major and pleiotropic effect. Farmersí decision and selection strategies have a great impact on phenotypic diversification in maize landraces.

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Allele Frequency Changes of Oil Candidate Genes in the High Oil Lines ASK Cycle0 ñ Cycle 28: Selection or Drift?

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In this study, we attempt to validate candidate loci for high oil content in maize kernels by following the allele frequency at these loci in a high oil long term selection population, ASK (Alexho Single Kernel Synthetic). This population was bred for high oil over a total of 28 cycles of selection (Misevic, D & Alexander, DE. 1989. Crop Sci. 29:320-324). The hypothesis is that certain allele(s) of a gene involved in the high oil trait will be selected for, and therefore will either increase in frequency or become fixed after several rounds of selection. Candidate loci identified in a microarray study (J.-M. Lee, unpublished data; see also J.-M. Lee et al., 2002 Funct. Integr. Genomics 2 :13-27) were selected for analysis. For each of the candidate genes, SNP haplotypes were identified and their frequencies in the ASKc0, c5, c20 and c28 populations were determined. The same analysis was also performed for a set of control loci. Pronounced changes in haplotype frequency at most of the loci examined were observed, including some of the control loci. To help distinguish between selection and random drift, computer simulation of the selection process has been conducted. The simulation allowed estimation of the likelihood that the observed changes in allele frequency could be explained by the effects of genetic drift due to the structure and

operation of the breeding program. Thus, allele frequency changes that has a low estimated likelihood under the drift model were identified as selected alleles for further investigation In addition to the candidate loci, we have also examined the genetic diversity at 100 SSR loci for 43 individuals from selection cycles 0,5,10,20, 25 and 28. These results allowed us to evaluate oil QTLs and candidate loci identified by gene expression analysis, and to identify new oil-associated loci in the ASK population. Examples of the allele frequency study with SNP, SSR markers and computer modeling on Chromosome 9 will be presented.

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A test for a heritable epigenetic component of heterosis

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Our lab is interested in developing a molecular explanation of heterosis. Toward this end, we conducted a test of whether there is a *heritable* epigenetic component to hybrid vigor/inbreeding depression. Classical studies of the effect of homozygosis of the r locus on its expression served as the impetus for these studies. Styles (1967) showed that maintaining R mottling alleles in the homozygous state would produce an accumulating decrease, over several generations, of their paternally imprinted expression. This effect is reversed by R allele heterozygosity. If this behavior were characteristic of many regulatory genes, then such a phenomenon could contribute to the basis of heterosis/inbreeding depression. To examine this question, inbreds of Mo17 and B73 and the two reciprocally produced hybrids were crossed by stock 6 to generate four classes of maternal haploids. This protocol produces the hybrid condition for one generation and then eliminates it for all genes in the subsequent generation to assay for any heritable effect. The haploid kernels were selected using anthocyanin markers and embryo morphology. The mature haploid plants were measured for numerous quantitative traits including plant height, culm circumference, leaf length, leaf width, number of tassel branches, number of nodes, position of the ear, ear length and number of tillers among others. If inbreeding depression results from an accumulating heritable effect that is reversed by the hybrid state, one would expect the haploids derived from the hybrids to perform better than those derived from the inbred lines. Previous work by Kato (2002) on doubled haploids indicates that inducing the haploid state itself does not invoke any obvious change in the quantitative characteristics of an inbred line. The haploids derived from the hybrids showed greater variance as expected from the fact that each individual has a unique genotype that is different from the inbred lines but overall they did not exhibit greater performance than the mean of the inbreds. These data do not support the idea that inbreeding depression has a heritable accumulating component.

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Molecular Dissection of a Stalk Quality Quantitative Trait Locus on Maize Chromosome 3

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Maize stalk quality is a quantitative trait associated with yield stability. This study was conducted to validate a QTL effect on maize chromosome 3 and characterize candidate genes using structural and functional genomics analyses. The QTL was validated using a marker-assisted backcrossing strategy. Marker-assisted introgression of the donor segment created a population of NIL contrasts. Phenotypic assessments measuring agronomic traits and disease inoculations in 2000 and 2001 across 5 locations were completed. The allele effect in the BC2 NIL contrasts across 2000 and 2001 were a 15, 15, and 12% reduction in stalk rot, stalk lodging, and crown rot means, respectively. Allele effects in the BC3 NIL contrasts in 2001 were a 6, 6, and 4% reduction in stalk rot, stalk lodging, and crown rot means, respectively. In 2001, BC2 and BC3 NIL contrasts indicated a 5 and 4% increase in rind strength, respectively. Candidate genes were characterized using expression profiling of two NIL-derived testcross entries grown under greenhouse conditions in 2002. The results of total lignin content and the ratio of lignin monomers assays of whorl tissues, sampled at 4 vegetative stages, indicated heritable differences under greenhouse conditions. Messenger RNA was extracted from whorl tissues per entry for each stage. Expression profiles were obtained using an Affymetrix RiceChip array. The results indicated 982 oligos were differentially regulated. Comparative genomic analyses of mapped maize loci linked to the QTL region aligned to the rice genome identified a syntenic region on rice chromosome 1. Structural queries within this region have aligned 12 of the 982 RiceChip oligos with gene predictions at this syntenic interval. Efforts to validate these candidate genes are in progress.

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Diverse Maize Germplasm Phenotypes: Population Structure and Heterosis

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Germplasm diversity is the raw material needed for maize improvement and dissection of complex genetic traits. Although there is a tremendous amount of variability for many traits in diverse inbred lines, most maize research is done on a small number of inbred lines, e.g. B73 and Mo17. We have now extensively characterized 300 lines, a germplasm set that captures about 85% of all the SNPs in maize, and their B73 hybrids for up to 50 phenotypes. Phenotypes included tassel measurements, leaf measurements, plant and ear height, flowering dates, node counts, ear measurements, and kernel characteristics.

Unlike most germplasm surveys, we have accurate estimates of relatedness and population structure based on 100 SSR loci. In this study, we estimate that heritabilities ranged from 0.15 to 0.65 across all traits. We also discuss which traits are highly correlated with population structure. These correlations with population structure have important implications for association studies and for allele mining projects. We will also present preliminary data on how heterotic effects correlate with genetic diversity for a range of non-yield traits. Characterization of this germplasm at phenotypic and molecular levels will aid in the dissection of complex traits and the planning of projects to exploit the rich diversity of maize.

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Determination of QTL associated with root lodging in maize

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Root lodging continues to be a serious agronomic issue in maize. In an effort to determine QTL associated with root lodging, an F2 mapping population of 47 individuals was created from two siblings derived from two lines exhibiting extreme phenotypes averaging 0.7 and 7.7 percent root lodging. These F2s, the F1 cross of the siblings, and the originating lines were crossed with a tester historically experiencing no root lodging. Percent root lodging was evaluated for 20 reps of each testcross in 5 environments and averaged over two years. Sibling parents of the F2 population were screened with 174 SSR markers for polymorphisms, and testcross progeny were analyzed for QTL using the resulting 74 SSRs. Three areas of significant loci were found on chromosomes 3, 9, and 10 with single marker analysis, which correlate to QTL regions found in previous studies of specific root system morphological traits. Interval mapping showed two of these QTL, near marker bnlg1160 on chromosome 3 and bnlg1185 on chromosome 10, explained 21.7 and 24.3 percent of the phenotypic variation respectively. The LOD score of bnlg1185 was above the calculated threshold of 2.6; bnlg1160 was slightly lower with a LOD score of 2.5. Both markers were also found to have significant allele effects, potentially allowing prediction of root lodging in certain genotypes.

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Association Mapping of Phenotypic Variation in Maize Flowering Time

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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. Previous association mapping experiments had concentrated upon SNP discovery in a set of 100 inbred lines and subsequent statistical tests to identify polymorphisms that associated with phenotypic variation observed in the field. We will discuss current efforts at confirming associations by scoring specific polymorphisms in an expanded set of 200 additional inbred lines. We will also discuss our efforts to streamline the process of SNP discovery for use in association mapping. This approach is based upon our knowledge of linkage disequilibrium and genotypic diversity, which allows us to perform initial SNP discovery in a much smaller core set of inbred lines. Understanding the effects of such nucleotide polymorphisms and their presence in the germplasm will improve selective breeding techniques for these quantitative traits.

Transposable Elements

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DNA Repair Genes and Ac/Ds Transposition

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Host cell genes are likely to be involved in repairing the DNA damage created when transposons excise. To investigate this further, we have been exploiting a system engineered to allow Ac/Ds transposition in yeast. Data from yeast suggest that the repair of Ac excision sites occurs through a single-strand annealing mechanism and that it involves, among other host factors, the Mre11/Rad50 and Ku complexes. Data are presented describing unexpected results from yeast Mre11 separation-of-function mutations that begin to address the DNA processing roles of this protein. We have also begun to examine the roles in transposition for these complexes in plants. For example, Ku80-deficient mutants of Arabidopsis have a radiation hypersensitive phenotype and fail to produce transposon footprints following Ac excision, when compared with wild-type sibs. Current data on mre11, rad50, ku70, and ku80 mutants of Arabidopsis and mre11 and rad50 mutants of maize are presented.

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***Ac* insertional mutagenesis of the *vp7/ps1* locus of maize**

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We are currently developing a series of near isogenic maize inbred lines homozygous for a single active *Ac* transposon. The *Ac* elements are precisely positioned in each line and mapped using publicly available recombinant inbred populations (IBM94 and BNL96).
http://bti.cornell.edu/Brutnell_lab2/Projects/Tagging/BMGG_pro_tagging.html

Ultimately, approximately 200 lines will be generated with *Ac* insertions placed at roughly 20 cM intervals throughout the genome. To demonstrate the utility of this system we have performed a targeted mutagenesis of the *vp7/ps1* locus. We selected 400 transposition events from an *Ac* located approximately 4 cM from the *ps1* locus and self-pollinated the plants. Phenotypic screens of 386 ears revealed 7 putative *ps1*-insertion alleles. Using an inverse-PCR protocol, we cloned *ps1* sequences flanking one of the *Ac* insertions. To clone the entire *ps1* gene, we developed a novel PCR-based protocol termed *'Ac* casting'. This method exploits the tendency of *Ac* to insert locally in somatic tissues of the plant to create a library of insertion alleles. These somatic insertions can be recovered through two rounds of PCR using gene-specific and *Ac*-specific primer sets. In theory, this technique could be utilized to clone genomic sequences flanking any *Ac* insertion in the maize genome. Finally, sequence analysis and HPLC data will be presented that strongly suggests that the *ps1/vp7* locus encodes for lycopene b-cyclase. This enzyme is necessary for the production of b-carotene derivatives including ABA and a-carotene derivatives including lutein.

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Assembly and Analysis of *RescueMu* Transposon-tagged Maize Genome Survey Sequences

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The **M**aize **G**ene **D**iscovery **P**roject (MGDP) is using a genetically engineered *Mu* transposon (*RescueMu*) for insertional mutagenesis and gene tagging in maize. Plants are grown in grids of up to 48 rows x 48 columns with an individual *E. coli* library prepared of *RescueMu* insertions from each row and column. Maize genomic inserts captured by *RescueMu* cloning are PCR-amplified and sequenced from the transposon ends. So far, MGDP has deposited over 100,000 genome survey sequences (GSSs) derived from Grids G, H, I, K, and M; grids P, S, Q and AA are in progress. The *RescueMu* GSS assembly is more challenging than the usual genomic fragment assembly. First, *RescueMu* transposons are recovered for use as sequencing templates after digestion of DNA with *Bam*H1 + *Bgl*II (http://zmdb.iastate.edu/zmdb/library-plate/GridGprep.html). The resulting right and left sequences flanking *RescueMu* are trimmed when either restriction site(s) or a ligation site is encountered. Consequently, a *RescueMu* clone can produce up to four genomic DNA sequence fragments. Second, somatic *RescueMu* insertions should be sequenced only once, while heritable insertions are likely to be recovered several times. We developed a

*RescueMu*GSSAssembler program to remove the redundancy in the *RescueMu* sequences and to derive a GSS-based maize gene index. Presently, over **8,400** GSS clusters, each representing a unique maize genomic region, have been assembled from somatic plus heritable sequences. About **56%** of those regions match either plant ESTs or proteins, **indicating *RescueMu* inserts into genic regions.** Likely germinal insertions show an even higher bias for genes. Based on DNA hybridization blot analysis and *RescueMu* sequences, heritable transpositions range from **15% to ~100% per grid**. To compare the *RescueMu* tagging approach with other gene-enrichment methods, we assembled >55,000 GSSs from methylation filtration methods. Our preliminary results show only **7%** of the *RescueMu* insertions overlapping with the regions sequenced after methylation filtration. Research is supported by the NSF.

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Genome-wide analysis of mariner-like transposable elements in rice reveals complex relationships with Stowaway MITEs.

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Stowaway is a superfamily of miniature inverted repeat transposable elements (MITEs) that is widespread and abundant in plant genomes. Like other MITEs, however, its origin and mode of amplification are poorly understood. Several lines of evidence point to plant mariner-like elements (MLEs) as the autonomous partners of the nonautonomous Stowaway MITEs. To better understand this relationship, we have taken advantage of the nearly complete genome sequences of two rice subspecies to generate the first inventory of virtually all MLEs and Stowaway families co-existing in a single plant species. Thirty four different MLEs were found to group into three major clades and 26 families. More than 22,000 Stowaway MITEs were identified and classified into 36 families. Based on detailed sequence comparisons, MLEs were confirmed to be the best candidate autonomous elements for Stowaway MITEs. Surprisingly, however, sequence similarity between MLE and Stowaway families was restricted to the terminal inverted repeats (TIRs) and, in a few cases, to adjacent subterminal sequences. These data suggest a model whereby most of the Stowaway MITEs in rice were co-mobilized by MLE transposases encoded by distantly related elements.

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Discovery of Helitron Type Transposable Elements in Maize Genome

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Despite the diversity of transposable elements, virtually all make a duplication of host sequences upon entry. Also the 3í terminus is either a copy of a poly (A) tail or a duplication of their 5í end. An exception to these rules is a recently described family of transposable elements called Helitrons. Sophisticated computer searches revealed the presence of elements in some eukaryotic genomes that do not bear terminal repeats, do not duplicate host sequences upon insertion and apparently transpose through replication and strand replacement. Until recently, the lack of large invariant sequence motifs precluded their detection. Despite their abundance, there is no genetic evidence of an autonomous Helitron. We recently described a maize mutant sh2-7527 that contains an insertion within the Sh2 gene. The insertion bears striking similarity to Helitrons. Like Helitrons, the insertion lacks terminal repeats, does not duplicate host sequences and was inserted between the host dinucleotide AT. Also like Helitrons, the element contains the sequences TC and CTRR at its 5í and 3í termini, respectively. This mutant was isolated from a conventional breeding program in the 1970s, strongly suggesting that Helitron-type transposable elements are present and active in the present day maize genome. A search of maize sequences deposited in the public databases identified three other Helitrons that bear sequence similarity to the sh2-7527 element only at both the termini. These elements ranging from approximately 9kb to 35kb in length contain randomly distributed copies of retroelements or pseudogenes. Based on the comparison of the insertion sites with maize ESTs and PCR amplified genomic sequences, these elements have apparently inserted within a host AT dinucleotide sequence. The possible impact of these elements on genome evolution is discussed.

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Horizontal transfer of a Mu-like element (MULE).

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Although many instances of horizontal transfer have been inferred from analysis of a wide variety of animal transposons, to our knowledge there have been no documented instances of similar transfers of plant transposable elements. This is surprising given the fact that hybridization is known to have occurred throughout plant

evolution. Here we present a case for the horizontal transfer of a MULE element between two subtribes of maize: *Panicoideae* and *Oryzoideae*. The two species examined (*Oryza sativa* and *Setaria italica*) are separated by more than 50 million years, and yet they carry MULE elements that are roughly 90% identical at the nucleotide level over more than 3.8 kilobases. This sequence similarity, which is as high as that of the well conserved *Adh1* gene, includes both coding and non-coding nucleotides, suggesting that it is not the result of conservation for protein function. These data is consistent with a horizontal transfer between an ancestor of *S. italica* and an ancestor of *O. sativa.* Neither element contains an intact *mudrA* transposase gene, suggesting that subsequent to transfer both elements became inactive. Although well distributed and present in multiple copies in several species of *Setaria*, we only detect a single hybridizing fragment in rice, suggesting that the transfer was from *Setaria* to rice. These observations match previous results that indicate that the distribution of many subfamilies of MULE elements are remarkably patchy, suggesting that horizontal transfer of MULE elements may have been a common feature of the evolution of these elements

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STRUCTURES, AGES AND CHROMOSOMAL DISTRIBUTIONS OF LTR RETROELEMENTS IN THE RICE GENOME

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LTR retrotransposons constitute a large part of the repetitive DNA fraction in the nuclear genomes of higher plants. LTR retrotransposons and other transposable elements play major roles in the structural and functional evolution of higher plant genomes. Moreover, LTR retrotransposons are 'relatively neutral' sequence components within the genome. Hence, study of the relationship between recombination, age and distribution of retroelements in a relatively complete genome can provide insights into genome-wide mechanisms of sequence evolution. We analyzed structures, ages and distribution of over 1000 LTR retroelements belonging to 11 families in the genome of rice variety Nipponbare. Our preliminary analyses indicate that, though the majority of the intact LTR retroelements are younger than 5 mys, over 75% of the elements have undergone sequence deletion and/or recombination. Illegitimate recombination and unequal recombination are primarily responsible for LTR retrotransposon removal. In general, the retroelements investigated are distributed over all the rice chromosomes and show the typical pericentromeric clustering distribution, but the retroelements belonging to some families seem to be dispersed over all the chromosomes. Our data suggest that the degree and nature of deletion of LTR retroelements is related to their ages and chromosomal distribution in the rice genome.

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Nested Deletions: A new tool for plant genomics research

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Plant genomes are laden with local sequence duplications and clusters of homologous genes. To simplify the analysis of these duplications and gene clusters, this project will develop a new genetic technology to generate large deletions quickly and efficiently. This approach is based on the finding that transposable elements can participate in alternative transposition pathways that generate novel recombination products, including large deletions and duplications. The system described here utilizes a transgene construct containing maize Ac/Ds transposon ends in tandem orientation within a I/dSpm transposon (Ned1; Nested deletions 1). The Ned1 construct will be transformed into maize; subsequent crosses will introduce the En/Spm transposase to mobilize Ned1 to various genomic locations, and the Ac transposase to activate the deletion process. The action of Ac transposase on the Ac termini within Ned1 generates an unlimited set of nested deletions with one end anchored at the transgene locus. The Ned1 construct contains marker genes for detection of both Ned1 transpositions and Ac-induced deletions, as well as sequences for easy cloning of deletion endpoints via plasmid rescue. If this demonstration project is successful, this approach could, in the future, be extended to the production of a set of maize lines containing Ned1 elements at dispersed sites throughout the genome. Researchers could then use these lines to isolate deletions and other rearrangements in specific regions of the genome for a variety of research applications, including 1) to map molecular and genetic markers to defined intervals; 2) to assign functions to individual gene copies within complex loci; 3) to test the effects of gene copy number on expression and silencing. For further information, contact Tom Peterson, 2206 Molecular Biology, Iowa State University, Ames, IA 50011. thomasp@iastate.edu. Supported by National Science Foundation-Plant Genome Program Award 0110170.

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Mechanisms of Mu inactivation in the UniformMu population

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Efficient genetic control of transposon activity is essential for functional genomics applications of the Robertsonís Mutator system. The autonomous MuDR element is required for transposition activity of more numerous non-autonomous Mu transposons in the maize genome. In the UniformMu transposon tagging population, somatic transposition activity is monitored using the spotted aleurone phenotype of the bz1-mum9 mutation. In advanced generations of UniformMu, selection of stable Mu-off derivative lines is facilitated by the frequent segregation of Mu-inactive kernels on selfed ears. Because MuDR elements present in the population are made heterozygous through continual backcrossing to W22, we hypothesized that loss of MuDR by segregation is a major mechanism of Mu-inactivation in F2 families. However, epigenetic mechanisms of Mu-inactivation are also known. To address this question we performed a genetic analysis of Mu-activity in 25 UniformMu BC4 families. Spotted and non-spotted (stable bronze) kernels were counted on each F2 ear and the goodness of fit to Mendelian ratios evaluated by chi square. Individuals were grown from spotted and non-spotted classes and analyzed by Southern blotting using MuDR and Mu1 probes. The Mu1 probed DNAís were digested with HinfI in order to correlate methylation of Mu1 TIRís with spotting activity. Families that segregate 3:1 and 15:1 spotted:non-spotted kernels showed a good correlation with segregation of MuDR elements. Other families that fit 1:3, 1:1 or intermediate ratios did not show a correlation with MuDR detected by Southern suggesting an epigenetic or dominant mechanism of Mu inactivation. We conclude that both MuDR segregation and epigenetic mechanisms contribute to Mu-inactivation in UniformMu lines.

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Identification of the Transposase Controlling the Insertional Activity of *MuDR/Mu* Elements

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Transposition activity of *MuDR/Mu* elements is directed by the autonomous element *MuDR*. This element contains two genes *mudrA* and *mudrB*. The *mudrA* encodes several forms of MURA transposase resulting from alternative splicing. The gene has two transcription start sites at positions +169 and +252 and four exons. 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 truncated MURA736 when the last intron is retained. Transgenic maize containing 35S:MURA823 (Raizada and Walbot, 2000) or MURA823 and MURA736 transcribed from the native 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 expressing either the native cDNA (frameshift required) or a frameshift corrected form. MURA854 protein is produced in vivo from both constructs and programs somatic excision of *Mu* elements. MURA854 lines were crossed with [1] *mudrB* lines to look for germinal insertion and [2] RescueMu lines to monitor for somatic insertion. We have now identified first *Mu* insertion events suggesting that MURA854 is the functional transposase of *MuDR/Mu* family. In addition, we provide evidence that MURB might be essential for *Mu* insertion.

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***Mu killer (Muk)* epigenetically silences the *Mutator* family of transposons**

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Mu killer (Muk) is a single dominant locus that epigenetically silences the *Mutator* family of transposable elements in maize. *Muk* completely silences both simple minimal *Mutator* lines as well as highly active multiple-*MuDR* transposon tagging lines. Silencing by *Muk* is not dependent on either the position of the *MuDR* element to be silenced, or on the gender of parent from which *Muk* was inherited. The silencing of *MuDR* by *Muk* occurs gradually through plant development and is complete by the development of floral structures. Once silenced by *Muk, MuDR* elements remain inactive for multiple generations even after *Muk* is segregated away, suggesting that *Muk* is involved in the initiation but not the maintenance of the silenced state. Active *MuDR* elements express two transcripts, the putative transposase, *mudrA*, and the helper gene, *mudrB*. When active *MuDR* elements are crossed to *Muk*, transcript levels of *mudrA* decrease substantially. In contrast, *mudrB* transcript remains in the

total RNA fraction but fails to accumulate in the poly-A RNA fraction. In progeny plants that carry silenced *MuDR* but that lack *Muk*, neither *mudrA* nor *mudrB* transcript is present in either the total or poly-A fraction. From our studies with *Muk*, we hope to understand how the maize genome has evolved to epigenetically regulate transposable elements.

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Distribution and Evolution of the PIF/IS5 Transposon Superfamily and Its Association with Tourist-like MITEs

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Two recently discovered active DNA transposons, the maize P Instability Factor (PIF) and the rice Pong element, define a new eukaryotic transposon superfamily named ìPIF/IS5î. In order to examine the distribution and evolution of this superfamily, a systematic survey combining database searches and a degenerate PCR assay was performed. Hundreds of related TPase sequences were identified (or isolated) from a variety of plant, animal and fungal species, indicating that PIF/IS5 is one of the most widespread and abundant superfamilies in eukaryotes. Subsequent phylogenetic analyses distinguished three major groups (plant PIF-like, plant Pong-like and an animal group), each represented by multiple distinct lineages. Several lineages of Pong-like elements recently amplified to a surprisingly high copy number (~1,000) in B. oleracea after its divergence from A. thaliana. PIF and Pong are responsible for the amplification of two Tourist-like miniature inverted-repeat transposable element (MITE) families (mPIF in maize and mPing in rice, respectively). Relationships between PIF/Pong-like elements and Tourist-like MITEs were further examined in rice, where it was found that most rice Tourist-like MITEs are related to either PIF- or Pong-like. Furthermore, clear-cut associations between PIF- or Pong-like elements and Tourist-like MITEs were readily identified. Taken together, these results suggest that members of the PIF/IS5 superfamily are responsible for the origin and amplification of Tourist-like MITEs. `

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