42ND ANNUAL MAIZE GENETICS CONFERENCE

PROGRAM
and
ABSTRACTS

16-19 MARCH 2000

COEUR D'ALENE RESORT CONFERENCE CENTER

COEUR D'ALENE, IDAHO

This conference receives financial support from:

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Cold Spring Harbor Laboratory

WE THANK ALL THESE CONTRIBUTORS FOR THEIR GENEROSITY

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"… it was Earl [Patterson] who established the original format for these meetings and successfully propagated the informal atmosphere that is still recognizable in the present-day meetings, in spite of their size…"

GENERAL INFORMATION

Concerning Meals

All meals will be served buffet style in Conference Center Bays across from the Bayview Rooms poster area
Breakfast will be served from 7:00-8:30
Lunch will be served from 12:30-1:30
Dinner will be served from 6:00-7:00 (arrangements will be made to extend the Thursday dinner hour to accommodate slightly late arrivals).

Talks and Posters

Plenary, short talk and concurrent sessions will be held in Conference Center Bays near the front of the Conference Area. Look for signs, as room assignments were not final at press time. Posters will be hung in Conference Center Bays across from the dining area.

Informal Meeting Place

The room used for serving meals will be available for evening socializing. Beer will be dispensed in this room but may be brought across the hall into the poster area during the evening poster sessions. These rooms will close at 2AM but arrangements have been made to accommodate late night discussions in a separate suite (details will be announced at the meeting).

Steering Committee

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

Becky Boston, co-chair
Sue Wessler, co-chair
Ben Bowen
Kelly Dawe
Al Kriz (unable to attend)
Tony Pryor (unable to attend)
Torbert Rocheford
Neelima Sinha
Cliff Weil (also Local Organizer)
Karen Cone, treasurer, ex officio
Mary Polacco, ex officio

Acknowledgements

Many thanks to the members of the Community for participating in this year's move to (mostly) on-line registration and (completely) on-line abstract submission. Your efforts are highly appreciated. The Abstract Book and Conference Web Site are available because of the competent work of Mary Polacco and Steve Schroeder. Registration was outsourced this year through the MU Conference Office and we thank Ms. Lorie Bosquet for her able assistance.

NEXT MAIZE MEETING: **15-18 March 2001**
Grand Geneva Resort, Lake Geneva, Wisconsin
Local Organizer: Marty Sachs, msachs@uiuc.edu

42nd Annual Maize Genetics Conference

March 2000

Thursday, 16 March

6:00-7:00 PM DINNER

7:15-7:30 PM **ANNOUNCEMENTS**

7:30-9:00 PM **PLENARY TALKS** **Chair: Sue Wessler**

7:30 **Jane Langdale, Oxford University**
Cellular differentiation in maize leaves

8:15 **Elliot Meyerowitz, California Institute of Technology**
Cell-cell communication in the *Arabidopsis* shoot apical meristem

9:30PM Posters may be hung Thursday evening and must be removed by noon Sunday

BEER WILL BE AVAILABLE ACROSS FROM THE POSTER AREA UNTIL 2:00AM

FRIDAY, 17 March

7:00-8:30 AM BREAKFAST

8:30-10:10AM **SESSION #1** **Chair: Neelima Sinha**

THE GENE

8:30 Laurel Mezitt, University of California-Davis
Cloning and Characterization of *Sucrose Export Defective1 (Sxd1)*
8:45-8:50 Discussion

8:50 Bradley Till, University of Oregon
CRS1: a nucleus-encoded protein required for the splicing of the maize chloroplast *atpF* group II intron
9:05-9:10 Discussion

9:10 Damon Lisch, UC Berkeley
Mutations that affect paramutation also reverse Mu element methylation
9:25-9:30 Discussion

9:30 Elsbeth Walker, University of Massachusetts, Amherst
Cloning of maize *yellow stripe1 (ys1),* an iron-regulated gene involved in high affinity Fe(III) uptake
9:45-9:50 Discussion

9:50 Donal M. O'Sullivan, IACR-Long Ashton Research Station
Use of a new maize BAC library to study intra-specific variation at the *Rp1* rust resistance superlocus
10:05-10:10 Discussion

10:10-10:40AM BREAK WITH BEVERAGES

10:40-12:20PM **SESSION 2** **Chair: Torbert Rocheford**

THE SEED

10:40 Matthew Evans, University of Wisconsin-Madison
Maternal gametophyte effect genes in maize seed development
10:55-11:00 Discussion

11:00 Philip Becraft, Iowa State University
Positional cues specify and maintain aleurone cell fate in endosperm development
11:15-11:20 Discussion

11:20 Kirsten Nielsen, NC State University
Differential response of pathogenic and non-pathogenic fungi to maize ribosome-inactivating protein
11:35-11:40 Discussion

11:40 Brian Scheffler, USDA-ARS-NPURU, University, Mississippi
Molecular characterization of *In-D: A* semi-dominant mutation of the *intensifier* locus
11:55-12:00 Discussion

12:00 Odd-Arne Olsen, Agricultural University of Norway
Genetic dissection of nuclear endosperm development
12:15-12:20 Discussion

12:30-1:30PM LUNCH

1:30-2:30PM **POSTER SESSION 1** Contributors will be at EVEN-NUMBERED posters

2:30-3:30PM **POSTER SESSION 2** Contributors will be at ODD-NUMBERED posters

BEVERAGES WILL BE AVAILABLE FROM 3:30-4:00

4:00-5:30PM **FORUM:** **Strategies/Plans to Enhance Maize Transformation in the Public Sector**

Chair: **Kelly Dawe**

Speaker: **Steve Moose, University of Illinois**

Panel: **Jeff Bennetzen, Purdue University (Moderator)**
Wayne Parrott, University of Georgia
Michael Spencer, Monsanto
Patrick Schnable, Iowa State University
Vicki Chandler, University of Arizona
Lyuda Sidorenko, Iowa State University
Steve Moose, University of Illinois

6:00-7:00PM DINNER

7:15PM **WORKSHOP: COMPARATIVE GENOMICS** **Chair: Cliff Weil**

Speakers:

Molly Jahn, Cornell University
Gernot Presting, Clemson University
Andy Kleinhofs, Washington State University

9:30-10:00PM **POSTER SESSION #3** Contributors will be at EVEN-NUMBERED posters

10:00-10:30PM **POSTER SESSION #4** Contributors will be at ODD-NUMBERED posters

BEER WILL BE AVAILABLE ACROSS FROM THE POSTER AREA UNTIL 2:00AM

SATURDAY, 18 March

7:00-8:30AM BREAKFAST

8:30-10:10AM **SESSION #3** **Chair: Cliff Weil**

THE PLANT (PART 1)

8:30 Laurie Smith, University of California-San Diego
Molecular analysis of the *Tangled* gene
8:45-8:50 Discussion

8:50 Mark Lubkowitz, University of California-Berkeley
Assembling a genetic network for regional identity along the proximo-distal axis of the leaf
9:05-9:10 Discussion

9:10 Dave Jackson, Cold Spring Harbor Lab
The *fasciated ear2* gene encodes a leucine rich repeat protein that controls inflorescence and floral development in the maize ear
9:25-9:30 Discussion

9:30 Luzie Wingen, Max-Planck-Institute for Breeding Research
A candidate gene for the *Tunicate1* locus
9:45-9:50 Discussion

9:50 Enrico Scarpella, Institute of Molecular Plant Sciences, Leiden University
A role for the rice homeobox gene *Oshox1* in provascular cell fate commitment
10:05-10:10 Discussion

10:10-10:40AM BREAK WITH BEVERAGES

And now for our exploratory foray into the Concurrent Session Experiment

10:40-12:25 CONCURRENT SESSIONS

Session # 4 THE GENOME
Session # 5 THE PLANT (Part 2)
Session # 6 THE GENE

Session # 4 THE GENOME **Chair: Kelly Dawe**

10:40-10:45 Introduction to session

10:45 Mei Guo, Pioneer Hi-Bred Int. Inc.
Insights into molecular basis of heterosis: mRNA profiles of maize hybrids and inbred parents
11:00-11:05 Discussion

11:05 Anjali Dogra, University of Missouri-Columbia
Dosage dependent control of heterosis
11:20-11:25 Discussion

11:25 Edward Braun, Ohio State University
Uncovering complex patterns of evolution for genes encoding *Myb*-domain proteins
11:40-11:45 Discussion

11:45 Evelyn Hiatt, University of Georgia
The *TR-1* knob repeat exhibits extreme levels of neocentromeric activity
12:00-12:05 Discussion

12:05 Nick Lauter, University of Minnesota
Genetic variation for phenotypically invariant traits detected in teosinte: implications for the evolution of novel forms
12:20-12:25 Discussion

Session #5—THE PLANT (Part 2) **Chair: Neelima Sinha**

10:40-10:45 Introduction to session

10:45 Michael Muszynski, Pioneer Hi-Bred Intl. Inc.
Modifying flowering time through modulation of *indeterminate1 (id1)* expression
11:00-11:05 Discussion

11:05 Jennifer Nelson, University of California-Berkeley
Mosaic analysis of a dorsiventral leaf polarity mutant
11:20-11:25 Discussion

11:25 Debbie Laudencia-Chingcuanco, University of California-Berkeley
Indeterminate floral apex 1 is required for maintenance of meristem identity
11:40-11:45 Discussion

11:45 Sharon Kessler, University of California-Davis
Characterization of *xcl,* a mutation affecting planes of cell division
12:00-12:05 Discussion

12:05 Matt Sauer, University of Pennsylvania
EPC, a gene controlling juvenile to adult phase change in maize
12:20-12:25 Discussion

Session #6—THE GENE **Chair: Becky Boston**

10:40-10:45 Introduction to session

10:45 Jay Hollick, University of California, Berkeley
Diverse roles of *required to maintain repression (rmr)* factors in gene silencing
11:00-11:05 Discussion

11:05 Subbaiah Chalivendra, University of Illinois
Altered patterns of sucrose synthase phosphorylation and localization precede root tip death in anoxic maize seedlings
11:20-11:25 Discussion

11:25 Brian Dilkes, University of Arizona
Cell cycle regulatory components in the endosperm endoreduplication cycle
11:40-11:45 Discussion

11:45 Binzhang Shen, Rutgers University
Ac tagging and characterization of a terpenoid cyclase gene induced by herbivore damage
12:00-12:05 Discussion

12:05 Suzy Cocciolone, Iowa State University
Regulation of the maize *a1* promoter in transgenic plants
12:20-12:25 Discussion

12:30-1:30PM LUNCH

1:30-2:30PM **POSTER SESSION #5** Contributors will be at ODD-NUMBERED posters

2:30-3:30PM **POSTER SESSION #6** Contributors will be at EVEN-NUMBERED posters

BEVERAGES WILL BE AVAILABLE FROM 3:30-4:00

4:00 **WORKSHOP: Genomics Resources** **Chair: Sue Wessler**

Speakers: **Vicki Chandler, University of Arizona**
Functional genomics of chromatin genes

Virginia Walbot, Stanford University
Maize gene discovery project

Jeff Bennetzen, Purdue University
Genomic sequence comparisons between maize BACs and orthologous regions of barley, rice, sorghum and wheat

Ed Coe, USDA-ARS, Columbia, Missouri
Comprehensive genetic, physical, and database resources for maize

Kelly Dawe, University of Georgia
Functional genomics of maize centromeres

Rob Marteinssen, Cold Spring Harbor Laboratory
Center for maize targeted mutagenesis

Lisa Harper, University of California-Berkeley
An integrated map of cytological, genetic and physical information of maize

Ron Phillips, University of Minnesota
Radiation hybrid and cloning system for the genetic and physical mapping of the corn genome

Jo Messing, Rutgers University
The international rice genome sequencing project

Don McCarty, University of Florida
Applied genomics: strategies for efficient molecular analysis of complex genetic systems

6:00-7:00PM DINNER

7:30-9:00PM **PLENARY TALKS** **Chair: Ben Bowen**

7:30 **Graham Moore, John Innes Centre,**
Wheat, a model or commodity--chromosome pairing and polyploidy

8:15 **John Doebley, University of Wisconsin,**
Genetic evidence and the evolution of maize

9:30-10:00PM **POSTER SESSION #7** Contributors will be at ODD-NUMBERED posters

10:00-10:30PM **POSTER SESSION #8** Contributors will be at EVEN-NUMBERED posters

BEER WILL BE AVAILABLE ACROSS FROM THE POSTER AREA UNTIL 2:00AM

Sunday, 19 March

7:00-8:30AM BREAKFAST

8:30-10:10AM **SESSION #7** **Chair: Becky Boston**

THE GENOME

8:30 Mark Settles, University of Florida
Genomic approaches to seed development
8:45-8:50 Discussion

8:50 Jeffrey Wong, University of Illinois
Molecular marker mapping of chromosomal regions associated with carotenoids and tocopherols in maize
9:05-9:10 Discussion

9:10 Jeffry Thornsberry, North Carolina State University
Association tests of candidate genes regulating plant height and flowering time
9:25-9:30 Discussion

9:30 Tim Helentjaris, Pioneer Hi-Bred Int., Inc
Insights from applying expression profiling to female development under stress
9:45-9:50 Discussion

9:50 Peter Carlton, UC Berkeley
Centromeres, telomeres, and meiotic chromosome pairing
10:05-10:10 Discussion

10:10-10:40AM BREAK WITH BEVERAGES

10:40AM MEETING ADJOURNS

LIST OF POSTERS

I Biochemical Genetics

1	Miguel Cervantes-Cervantes	Preliminary characterization of the geranylgeranylpyrophosphate synthase (GGPPS) gene family of maize.
2	Prem Chourey	Metabolic analyses of a double mutant of sucrose synthase (SuSy) genes in developing endosperm.
3	Prem S. Chourey	Analysis of a double mutant of sucrose synthase (SuSy) genes that shows evidence of a third SuSy gene.
4	Joanna Cross	ADP-Glucose Pyrophosphorylase Activity from Maize-Potato Hybrids
5	Jorg Degenhardt	Biochemical and molecular characterization of the terpene synthase gene family in *Zea mays*
6	Karsten Frenzel	Characterisation of zmKCS, a fl-Ketoacyl-CoA-Synthase from Maize possibly involved in Wax Biosynthesis
7	Diane Janick-Buckner	Characterization of the camouflage 1 mutant of maize
8	Kirsten Nielsen	Differential response of pathogenic and non-pathogenic fungi to maize ribosome-inactivating protein
10	Bruce R Thomas	Regulation of sugar production in cereal seedlings
11	Elsbeth Walker	Cloning of maize yellow stripe1 (ys1), an iron-regulated gene involved in high affinity Fe(III) uptake.
12	Jonathan Walton	Functional Genomics of Hemicellulose Biosynthesis
13	Xuelu Wang	QTL mapping of elongation factor 1-alpha (eEF1A) content and characterization of eEF1A genes in maize endosperm
14	Chunyuan Wu	Biochemical and reverse genetic analysis of the maize starch debranching enzyme ZPU1
15	Eleanore Wurtzel	A heterologous system to identify strategic genes for metabolic engineering of the maize carotenoid biosynthetic pathway.
16	Galina Zayakina	Highly polymorphic zeins of maize represent a useful source of genetic markers.

II Cytogenetics

17	Amie Franklin	Analysis of desynaptic 2 supports an involvement of the Rad51 recombination protein in homologous chromosome synapsis
18	Inna Golubovskaya	The pam1 gene: Bouquet Formation and Homologous Synapsis
19	Lisa Harper	Towards an Integrated Map of Cytological and Genetic Information
20	Eetienne Kaszas	Phosphorylation of histone H3 is correlated with changes in sister chromatid cohesion during meiosis in maize
21	Yong Li	Development and characterization of maize-Tripsacum F1 hybrid population segregating for apomixis
22	Joshua Marshall	FRETM as an optical technique to determine molecular interactions in maize kinetochore/centromere on a sub-optical scale
23	Graham Moore	Wheat, a model or commodity-chromosome pairing and polyploidy
24	Ron Okagaki	Towards an oat-maize radiation hybrid panel
25	Brent Page	Evidence For and Evolutionary Relationship Between Chromosome 4 and the B Chromosome, Based on Related Centromere Repeats
26	Monther Sadder	Comparison of the genetic map to the physical map of molecular markers related to QTLs for resistance against southwestern corn borer (Diatraea grandiosella D.) on pachytene chromosomes using in situ hybridization in maize (Zea mays L.)
27	Stephen Sowinski	Effect of Abnormal Chromosome 10 on the Frequency of Recombination in maize
28	Nathan Springer	Epigenetic inheritance of an aneuploid induced phenotype
29	M. Isabel Vales	Maize-chromosome 9 rearrangements in progenies of oat-maize chromosome 9 radiation hybrids
30	David F. Weber	Use of the r-X1 Deficiency System to Recover Trisomics for Chromosome 8 in Maize
31	Pascale Williams	CRP1: A Translational Activator in Maize Chloroplasts
32	Hong-Guo Yu	Maize single-kinetochore chromosomes can align at the equator by tension-sensitive interactions with opposite spindle poles
33	Yin-Zhou Zheng	Analysis of a small cluster of B specific repeat sequences in the long arm of the B chromosome

III Cytoplasmic Inheritance

34	Christine Chase	Transposon tagging of nuclear genes that regulate mitochondrial gene expression
35	Chester Dewald	Comparison of Seven Inbred Maize Lines with Their BC-3 Derivatives in Tripsacum Cytoplasm
36	Susan Gabay-Laughnan	Genetic characterization of CMS-S restorer-of-fertility alleles in Mexican races of maize and teosinte

IV Developmental Genetics

37	Debbie L. Alexander	Phenotypic analysis of corkscrew; a recessive mutation affecting shoot development
38	Robert Baker	Characterization of nl*1179, a mutant affected in leaf development
39	David Barnes	ramosa2 affects a determinacy switch point in the developing maize inflorescence
40	Peter Bommert	Analysis of embryo-specific mutants in Zea mays reveals that radial organization of the maize proembryo preceeds the establishment of the shoot apical meristem

41	David Braun	Genes controlling later events in leaf development: liguleless1 and tie-dyed1
42	Gladys Cassab	Three maize root-specific genes are not correctly expressed in regenerated caps in the absence of the quiescent center
43	Andrew Doust	Inflorescence development in Setaria
44	Matthew Evans	Maternal Gametophyte Effect Genes in Maize Seed Development
45	Marcelo Friedlender	Activation of maize defense markers by Les9
46	Giulini A. Gavazzi G.	Characterization of maize mutants affecting embryogenesis
47	Angela Hay	Characterization of the dominant leaf mutation Wab (Wavy Auricles in Blades).
48	Momoyo Ito	Epidermal cell differentiation and radial pattern formation in grass embryogenesis.
49	Lynne Jesaitis	laminate coleoptile is required for coleoptile identity and normal leaf pattern
50	Nick Kaplinsky	rgo1 and ids1 interact to control spikelet meristem identity and new spikelet identity mutants.
51	Michael Kolomiets	Characterization of clear spot-1 (csp1), a disease lesion mimic mutant of maize.
52	Yew Lee	To understand how plants sensesce
53	Jun Lim	Radial patterning during regeneration of the root apical meristem in maize
54	Mark Lubkowitz	Discerning the function of liguleless3 in leaf development: a search for downstream targets
55	Paula McSteen	barren inflorescence2 (bif2), barren stalk1 (ba1), Barren inflorescence1 (Bif1) and Suppressor of sessile spikelet1 (Sos1): multiple pathways for axillary meristem development in the maize inflorescence
56	Jennifer Moon	Cloning and Characterization of Maize Lazy-1
57	M.G. Neuffer	Diurnal Response of Selected Chlorophyll Mutants Under Different Combinations of Light and Temperature
58	Asuka Nishimura	Analyses of genes involved in the lateral organ formation from the shoot apical meristem
59	Odd-Arne Olsen	Genetic dissection of nuclear endosperm development
60	Woong June Park	Tissue-specific Expression of AUX1 in Maize Roots
61	Scott Poethig	The phase-specific identity of a leaf is specified after leaf initiation
62	Dorien Postma-Haarsma	CHARACTERIZATION OF KNOX CLASS HOMEOBOX GENES FROM RICE
63	Peter Rogowsky	Esr genes show different levels of expression in the same region of maize endosperm
64	Mark Running	Mutations in thick tassel dwarf 1 affect meristem function
65	Matt Sauer	EPC, a gene controlling juvenile to adult phase change in maize
66	Ruairidh Sawers	Bundle Sheath Defective2 (BSD2); a novel protein required for the accumulation of RuBisCO
67	Michael Scanlon	Clonal analysis of NS1, cloning of ns2?: progress toward elucidating the function of the narrow sheath duplicate genes during maize leaf development.
68	Enrico Scarpella	A role for the rice homeobox gene Oshox1 in provascular cell fate commitment
69	Susanne Hansen	Expression analysis of ZmMADS1 and ZmMADS3 in different tissue cultures of maize
70	Anne W. Sylvester	Observing the predictability of random events during leaf development: Analysis of *rli1-warty*and other cell pattern mutants.
71	George Theodoris	Characterization of genes involved in organ development in maize
72	Bruce R Thomas	Maize beta-glucanases - multiple genes and multiple roles in plant development
73	Leszek Vincent	Developing systematic descriptors and containment hierarchies for maize

74	Erik Vollbrecht	Characterization of ramosa1, a gene regulating indeterminacy in the maize inflorescence
75	Luzie U. Wingen	A candidate gene for the Tunicate1 locus
76	Yuan Zhang	Glucocorticoid Inducible cr4 Transcription in Transgenic Maize

V Genome Structure/Synteny

77	Chris Carson	Mutant Mapping in the Missouri Maize Project
78	Doug Davis	Progress On Maize Whole-Genome Radiation Hybrids
79	Georgia Davis	A High-Resolution Genetic Map of the B73 x Mo17 Population.
80	Michael Freeling	Announcing: The "Grass Hybrids" Public Database and Website
81	Huihua Fu	Genomic organization of the highly recombinogenic bz region of maize
82	Shailesh Lal	Gene discovery using the maize genome database ZmDB
83	Bruce May	Maize Targeted Mutagenesis: A Knockout Resource for the Maize Community
84	Wade Odland	Current and future uses of oat-maize addition and radiation hybrid lines
85	Leonore Reiser	The Arabidopsis Information Resource (TAIR)
86	David Remington	EVALUATING DISEQUILIBRIUM AMONG POLYMORPHISMS WITHIN AND BETWEEN CANDIDATE GENES IN MAIZE
87	Hector Sanchez-Villeda	MaizeDB - Gateway to All Public Maize Genome Data.
88	Steve Schroeder	Data Management in the Missouri Maize Project
89	David Selinger	Comparison of nucleotide substitutions and multi-base insertions in *b* alleles from *Zea mays* ssp and *Zea luxurians* suggests a relatively recent origin for most insertions.
90	Natalya Sharopova	Microsatellites in maize - development and mapping.
91	Xianghe Yan	Use of the transposon Ac as a gene-searching engine in the maize genome.

VI Molecular Genetics

92	Greenland Andy	Nuclear expression of T-urf13 in the tapetum mimics male sterility in CMS-T maize.
93	Donald Auger	Nuclear dosage effects on mitochondrial gene expression
94	Don Baldwin	Transcript profiling of the maize defense response to a fungal pathogen and its toxin, a histone deacetylase inhibitor
95	Deverie K. Bongard	SNP discovery using the maize EST database.
96	Brent Buckner	Sequence analysis of a recessive allele of the y1 gene of maize
97	Anne Bunner	Characterization of two Novel Arginine/Serince-Rich Splicing Factors that are Differentially Spliced in Maize
98	Todd Christensen	Identification and Characterization of Seven Rop GTPases in Maize
99	Maureen Clancy	Maize shrunken1 first intron-mediated enhancement of gene expression
100	Kathryn Clayton	Construction of a consensus SSR map for maize using a high-throughput marker screening system
101	Cintia M. Coelho	Genetic control of endosperm endoreduplication and modes of maternal control
102	Jennifer Cooper	Chromosome Arm Aneuploidy Causes Dosage Effects on *sucrose synthase1* and *shrunken1* RNA levels in maize plants
103	Chuck Dietrich	Characterization of the maize gl8 gene family and its role in the fatty acid elongase complex.
104	Brian Dilkes	Cell cycle regulatory components in the endosperm endoreduplication cycle.
105	Anjali Dogra	Dosage dependent control of heterosis

106	Jane Dorweiler	*Mediator of Paramutation2* is a dominant inhibitor of the establishment of paramutation
107	Cynthia Ernst	Utility of marker assisted selection for introgression of commercially important genes into elite germplasm
108	Wolfgang Goettel	Heritable allelic interaction between *P-pr* and *P-rr*
109	John Gray	A CHLOROPLAST PROTECTIVE FUNCTION FOR *lls1 (lethal leaf-spot 1)* IN PLANTS ?
110	Baozhu Guo	Identification of a gene at the syntenic sh2-a1 region in maize acting as a QTL affecting silk maysin synthesis
111	Mei Guo	Insights into Molecular Basis of Heterosis: mRNA Profiles of Maize Hybrids and Inbred Parents
112	Jose Gutierrez-Marcos	Imprinted genes in maize endosperm
113	Linda Harris	Maize/Gibberella ear rot- maize genes induced in the plant/pathogen interaction
114	Linda Harris	Maize genomics at ECORC
115	Tim Helentjaris	Insights from applying expression profiling to female development under stress.
116	Zihua Hu	Genome-scale RNA profiling of parentally imprinted genes in maize endosperm
117	Jinsheng Lai	Stable expression of the high methionine storage protein gene in transgenic progenies of various maize inbred lines
118	Carolyn Lawrence	Rooting the Kinesin Tree: A Phylogenomic Analysis
119	Jin Li	Site-selected Mutagenesis of the rad51b Gene in Maize
120	Dennis J. McCormac	Translation of the chloroplast *atpB/E* mRNA requires a nuclear gene in maize.
121	Venugopal Mikkilineni	Genomic Organization of the Fatty Acid Desaturase-2 (FAD-2) EST's in Maize.
122	Snezana Mladenovic Drinic	Chromatin polymorphism dependent gene expression in maize
123	Rita-Ann Monde	Genetic analysis of thylakoid protein targeting
124	Daniel Moran	Expression of a wheat high molecular weight glutenin in transgenic maize: A comparison of seed-specific promoters.
125	Rebecca J. Mroczek	ANALYSIS OF THE ORGANIZATION OF THE ABNORMAL-10 CHROMOSOME OF MAIZE
126	Jorge Nieto-Sotelo	Characterization of five maize *hsp101-m-::Mu* lines obtained by reverse genetics
127	E. Owusuwaa Owusu	The Maize Tousled-Like Kinase Gene Family
128	UTA PASZKOWSKI	dinf1 and nope1, two mycorrhiza-specific mutants in maize
129	Varaporn Sangtong	Expression and inheritance of a wheat endosperm storage protein in maize
130	Yutaka Sato	Knock-out the knox genes
131	David Selinger	Characterization of a tissue-specific gene silencing phenomenon involving *B-Bolivia* and CaMV 35S/B chimeric transgenes.
132	Binzhang Shen	Ac tagging and characterization of a terpenoid cyclase gene induced by herbivore damage
133	Lyudmila Sidorenko	Novel type of P1-rr suppresison is caused by transgene carrying full length P1-rr promoter
134	David Skibbe	Characterization of the Zea mays Aldehyde Dehydrogenase Gene Family
135	Karolin Stahl	Expression of the DIMBOA biosynthesis genes
136	Maike Stam	The involvement of long distance communication in a natural case of gene silencing in plants, paramutation at the b locus in maize
137	Ann Stapleton	Wax helps: the glossy1 mutant is more sensitive to ultraviolet radiation by some physiological measures
138	Shannon Stenehjem	Identification of genes transcribed from a QTL
139	Masaharu Suzuki	Conservation of maize VP1 function in the dicot, Arabidopsis.
140	Richard Thompson	rgf, a mutation reducing grain filling in maize through effects on basal

		endosperm and pedicel development
141	Mark van Haaren	High resolution AFLPÆ genetic maps of Maize
142	Rik van Wijk	Linkage Map Integration: An integrated genetic map of Zea mays L.
143	Hong Yao	Characterization of the 140-kb Multigenic a1-sh2 Interval
144	Suling Zhao	Phosphate Transporters in Maize

VII Quantitative Traits

145	Edward Bruggeman	Relationships between yield, stability, and density tolerance
146	Shaun Bushman	Genetics of Chlorogenic Acid and Maysin Synthesis in Maize Silks
147	Ana Butron	Effect of p1 locus on synthesis of silk maysin, apimaysin, 3'-methoximaysin and chlorogenic acid in maize
148	Nick Lauter	Genetic variation for phenotypically invariant traits detected in teosinte: implications for the evolution of novel forms
149	Cesar Lopez	Heterotic Patterns Among Elite Flint Maize Populations from Argentina
150	Larissa Wilson	Associating Phenotypic Traits With Sequence Variation in Maize id1

VIII Transposable Elements

151	Ryuji Ishikawa	New members of RiceMutaor elements by deletion and non-homologous recombination with ectopic DNA segments
152	Ning Jiang	Tourist traps in the maize genome
153	Richard Langham	*MuDR-like Sequences are Widespread in the Grasses*
154	Zenaida V. Magbanua	ASSESSING THE UTILITY OF MITES AS MOLECULAR MARKERS
155	Adriano Marocco	Study of the chilling-induced chlorosis by using the virescent mutants of maize.
156	Robert Meeley	An Overview And Some Observations From Work On Mutator-Based Reverse Genetics
157	Christine Schaefer	Development of an En/Spm transposon system for barley
158	Richard Slotkin	Transposition Frequency of *Rescue Mu*
159	Xianghe Yan	Jittery, a low-copy, Mu-related transposon apparently mobilized by BSMV infection
160	Xiaoyu Zhang	mPIF Elements: Possible Non-autonomous Members of the PIF Transposable Elements
161	Jianbo Zhang	Non-linear Ac/Ds transposition and maize genome reorganization

Memorial in Honor of
Earl B. Patterson

Earl B. Patterson passed away on Saturday May 1, 1999. He was 75 years old. He is survived by his children, Mark and Anne. His wife Betty passed away August 1, 1999.

His name is synonymous with the Maize Genetics Cooperation Stock Center whose current thriving status is attributable, in large measure, to his unstinting effort in its behalf. His deep imprint also remains with the annual Maize Genetics Conference, which he organized and presided over through the 60s, 70s and early 80s.

Earl Patterson was born on a farm in southeastern Nebraska near the town of Reynolds, on July 21, 1923, the youngest of nine unusually gifted children in a closely-knit family of four girls and five boys. Earl attended the University of Nebraska where, in 1947, after serving three years in the U.S. armed services during WWII, he received his B.S. degree in technical science, graduating first in his class. Dr. Frank Keim, long-time head of the Department of Agronomy at the University of Nebraska, and a genetics teacher who was familiar with Earl's excellent qualifications and interest in the subject, encouraged him to pursue advanced studies with Dr. E. G. Anderson, himself of Nebraska origin, at the California Institute of Technology in Pasadena. Upon Dr. Keim's recommendation, Earl's application was accepted and his graduate years were spent in the Biology Division at Cal Tech with Dr. Anderson as his mentor. He received his Ph.D. degree in genetics at that institution in 1952, and stayed at Cal Tech for another year as a postdoctoral fellow.

In 1953 Earl accepted a position in the Departments of Botany and Agronomy at the University of Illinois in Urbana. Here he was responsible for the Maize Genetics Cooperation Stock Center which had just been moved from Cornell University to Urbana. Two years later, in 1955, he became project leader of that program in the Department of Agronomy. Earlier maintenance of the maize genetic stocks at Cornell led to selection of strains that were adapted to the short growing season at Ithaca but only poorly suited to culture in the Corn Belt and most other corn growing regions. As a result, Earl Patterson's first task in his new position at Illinois was to commence the conversion of these many genetic stocks to inbred and hybrid backgrounds that were better adapted to most corn growing regions. Earl maintained the stock center through these formative years until 1966 when he relinquished his stock center responsibilities to concentrate on research. Earl’s research focused on the isolation and characterization of male sterility mutants in maize. He found numerous new nuclear male-sterile mutations. When Southern Corn Leaf Blight, a disease specific to T-type male-sterile cytoplasm, struck the hybrid corn industry, seed companies reverted to manual detasselling. Earl developed a method to use his nuclear male-sterile traits to replace cms-T to avoid detasselling. Combining his male-sterile traits with various chromosomal aberration stocks, he developed a new method for producing hybrid corn seed. This work resulted in the issuing of two patents.

In 1977, Earl stepped up to fill the gap left by the retirement from teaching of the head instructor of the introductory genetics course. He was lead instructor for this course, in collaboration with faculty from the Animal Sciences Department, until 1987. The average enrollment was 80-90 students per semester.

When the Director position at the Maize Genetics Cooperation Stock Center again became vacant in 1986, Larry Schrader, then Head of the Agronomy Department at Illinois, persuaded Earl to resume management of the Stock Center. It was to the great benefit of all maize researchers that Earl returned to that position at a time when future support and direction of the center were uncertain. He continued that effort until his retirement in 1993.

Earl always gave "distribution" of seed stocks very special attention. On each request for seed, he brought to bear his encyclopedic knowledge of maize genetics lore. A request for seeds often resulted in the shipment of more packets than requested because of Earl's uncanny ability to anticipate needs and problems associated with growing and handling the items requested. All manner of useful suggestions were likely to be found in the letters that accompanied the packets of seeds requested. There is no doubt that a collection of letters that Earl has sent in response to seed requests over the years would be a valuable resource for maize geneticists.

While the Maize Genetics Cooperation Stock Center is today well supported and a thriving organization, it was not always so. In its earlier years at Illinois, funds for its operation were uncertain and often meager. With an improved internal status for the Stock Center in recent years has come increased support from the Agricultural Research Service of the United States Department of Agriculture, and in 1992 this agency assumed responsibility for operations and funding of the program. To Earl, whose labors, and sometime frustrations, have been so closely associated with the development of the Stock Center, the strong position that it has recently achieved was a source of great satisfaction and pride.

In 1958, Earl Patterson along with John Laughnan, Ed Coe, and Gerry Neuffer, talked about the possibility of an annual informal get-together of maize geneticists and their graduate students. The first meeting was in January 1959, and took place at Allerton Park, a part of a farm facility owned by the University of Illinois and located just outside of Monticello, Illinois. There were about twelve participants at that first meeting, so few that it could be held in the quite small Oak Room in Allerton Park House. These maize meetings as they came to be called were delightfully informal and grew in numbers of participants over the years. They were presided over by Earl. He made all the arrangements for use of the facility and dates of the meetings each year. He sent out notices of meetings to potential participants and arranged for ground transportation to Allerton House. There was no prearranged program of speakers; participants would arrive on Friday evening and at that time or early the next morning Earl would talk with people interested in sharing their research experiences and in that way developed a program for the get-together. At first, there was no need for a microphone, even for the most soft-spoken individuals, but as the meetings grew in size it necessarily moved to amplification. Earl introduced the speakers, adjusted the microphone, operated the overhead, arranged for the right kind of soft chalk and erased the blackboard, all with a special finesse that earned for him the position of permanent chair of all sessions. In addition to all these things Earl presided over the gene mapping sessions usually held on Saturday evenings. As the meetings grew in size, it was recognized that some modest level of organization was needed. Earl's suggestion of establishing a steering committee for the annual meetings was approved by the maize group. Today this committee continues to serve an important function in the Maize Genetics community.

After 25 years, the maize meetings grew to such a size that Allerton House could no longer accommodate them and so, regretfully, the maize genetics community was obliged to move the meetings from this treasured site. This past March the 41st annual meeting of maize geneticists, now called the Maize Genetics Conference, was held at the Grand Geneva Convention Center in Lake Geneva, WI, with over 400 teachers and researchers in attendance. Younger members of the maize genetics group are probably not acquainted with Earl Patterson nor aware of the reverence in which the Allerton meetings are still held by their predecessors. However, they should know that it was Earl who established the original format for these meetings and successfully propagated the informal atmosphere that is still recognizable in the present-day meetings, in spite of their size.

(Reprinted from the University of Illinois memorial)

7:30 pm Thursday Plenary Talk

T1 Cellular differentiation in maize leaves

Langdale, Jane A.(1); Alexander, Debbie(1); Cribb, Lizzie(1); Fitter, David(1); Martin, David(1); Rossini, Laura(1); Sawers, Ruairidh (1); Tsiantis, Miltos(1) (1) University of Oxford, South Parks Rd, Oxford, UK

The differentiation of distinct cell-types in a plant can be considered to occur in two partially overlapping stages - first cells become specified and then they differentiate. Work in our group is aimed at characterizing genes that control these two processes. To date, we have isolated three genes, one of which [rough sheath2 (rs2)] acts in the leaf to negatively regulate homeobox genes that normally act in the meristem. In so doing, the rs2 gene specifies leaf cell fate. The rs2 gene encodes a myb-like transcription factor and is orthologous to the Antirrhinum PHANTASTICA gene. Work is currently aimed at understanding how the rs2 gene interacts with other genes to maintain leaf development programs. A candidate gene is corkscrew (cks). Recessive cks mutants define a distinct locus from rs2 but exhibit phenotypes very similar to rs2 mutants. A second gene, Golden2 (G2) appears to regulate the differentiation of bundle sheath cells in the C4 maize leaf. If G2 is inactive, bundle sheath cell chloroplasts fail to develop and photosynthetic enzymes do not accumulate. The G2 gene also appears to play a role in the differentiation of photosynthetic cell-types in C3 leaves. Sequence data revealed the existence of a bipartite nuclear localization signal in the first exon of the gene and we have shown that G2-reporter gene fusions are targeted to the nucleus in onion epidermal cells. Further sequence analysis indicated the presence of a novel domain within the deduced protein sequence that shares some features with TEA DNA binding domains. As such, we proposed that G2 acts as a novel transcriptional regulator of cellular differentiation in maize. Subsequent analysis has shown that families of G2-like genes exist in maize, rice and Arabidopsis. Using forward and reverse genetics, we are currently analyzing G2 function in all three species with a view to understanding how the gene fulfils its role in cellular differentiation.

8:15 pm Thurday Plenary Talk

T2 Cell-cell communication in the Arabidopsis shoot apical meristem

Meyerowitz, Elliott, California Institute of Technology

No abstract.

8:30 am Friday

T3 Cloning and Characterization of Sucrose Export Defective1 (Sxd1)

Mezitt, Laurel A.(1); Sinha, Neelima(1); Lucas, William J.(1) (1) University of California-Davis, Davis, California 95616, USA

In sxd1 mutants of maize, the export of sugars from source leaves is dramatically reduced, and minor veins in the leaf blade exhibit distortion and plasmolysis of phloem parenchyma cells. In the affected vascular bundles, plasmodesmata (PD) in cell walls between bundle sheath (BS) cells and phloem parenchyma (PP) cells are occluded by the deposition of a layer of cell wall along the BS-PP border (1). The specific occlusion of this subset of PD seems to be under developmental control, as immature portions of leaves show normal minor vein structure and open PD between all cell types. AIMS (2) was used to identify a portion of the sxd1 locus, and part of the gene was isolated from a subgenomic library made from sxd1 plants. The full cDNA sequence was obtained through 5' and 3' RACE experiments using WT RNA as template. The sxd1 mutation involves a deletion of the 5' end of the coding sequence, effectively knocking out expression of the gene. In WT plants, in situ hybridization experiments show expression of Sxd1 in bundle sheath cells, and Northern blots indicate up-regulation of expression as the leaf blade matures. The Sxd1 gene encodes a novel 53kD protein with no known functional domains. Single-copy orthologs to Sxd1 have been identified in the Arabidopsis and Synechocystis genomes. Further characterization of the function of SXD1 in both maize and Arabidopsis will allow us to begin to dissect a pathway that results in PD occlusion. This characterization could ultimately lead to our ability to manipulate the pathway, and engineer controlled symplasmic isolation of specific cell types or tissues. 1. Russin et al (1996). Plant Cell 8, 645-658. 2. Frey et al (1998). Plant Journal 13, 717-721.

8:50 am Friday

T4 CRS1: A nucleus-encoded protein required for the splicing of the maize chloroplast *atpF* group II intron.

Till, Bradley(1); Jenkins, Bethany(1); Schmitz-Linneweber, Christian(1); Kulhanek, Doris(1); Carrier, Roz(1); Klaus, Sebastian(1); Barkan, Alice(1) (1) University of Oregon, Eugene, OR 97403, USA

The splicing of introns from plastid RNAs is an essential step in the formation of a functional chloroplast and is subject to developmental regulation. To study factors involved in the regulation of plastid RNA splicing, we identified *Mu*-induced mutations that affect this process. Two such genes have been identified, *crs1* and *crs2*, each involved in the splicing of different subsets of plastid RNAs (Jenkins, *et. al.*, (1997) Plant Cell **9,** 283-296). Here we describe the cloning and characterization of *crs1*. Mutations in *crs1* specifically affect the splicing of the chloroplast *atpF* group II intron. We identified two alleles of *crs1*. The *crs1-1* allele was identified in a forward genetic screen and is caused by a *MuDR* insertion upstream of the putative start codon. A second allele, *crs1-2*, was identified through a screen of a reverse genetics resource we have developed that is tailored to studies of chloroplast biogenesis. This resource was assembled from approximately 1000 *Mu*-induced mutants with chlorophyll-deficient leaves. A PCR screen of pooled DNAs from this collection with a *crs1* primer in conjunction with a *Mu* TIR primer yielded *crs1-2*. The *crs1-2* mutation is caused by a *Mu1-2* insertion in the crs1 gene 100 base pairs downstream of

the putative start codon. The predicted *crs1* gene product contains a putative chloroplast transit peptide and is related to a family of *Arabidopsis* proteins of unknown function. Interestingly, the *crs1* mRNA is alternatively spliced. To date, CRS1 is the only identified nucleus-encoded protein that activates the splicing of a specific group II intron. Biochemical characterization of the gene product(s) is in progress.

9:10 am Friday

T5 Mutations that Affect Paramutation also Reverse Mu element Methylation

Lisch, Damon(1); Hollick, Jay (1); Carey, Charles(2); Chandler, Vicki(2) (1) UC Berkeley, Berkeley CA 94720; (2) University of Arizona, Tucson AZ 85721

Transposon silencing and paramutation are two of the more mysterious phenomena in maize. Each involves heritable changes in gene activity in the absence of changes in DNA sequence. Very little is known about the means by which these changes are affected. Here we present evidence that several mutations that affect paramutation in maize also affect *Mu* element silencing. The *mop1* mutation prevents paramutation at three different loci in maize. It can also reliably prevent and even reverse the methylation of *MuDR* and *Mu1* elements induced by the activity of a dominant suppressor of Mutator activity, *MuK*. These changes in methylation status are correlated with changes in *MuDR* expression. Interestingly, the *mop1* mutation can reverse the methylation of non-autonomous *Mu1* elements even in the absence of the regulatory *MuDR* element. Thus, the hypomethylation of *Mu* elements in a *mop1*/*mop1* background is not mediated by expression of *MuDR* transposase. The *mop1* mutation does not affect the methylation of some other transposable elements, including ones just upstream of a paramutable *b* gene, suggesting that *mop1* acts on a very particular subset of metastable sequences in the maize genome. A series of additional mutations in genes that are require to maintain the repressed state of paramutant alleles of *pl* were also examined for their effect on *Mu* element methylation. In each case, although the families examined did not appear to have intact *MuDR* elements, hypomethylation of *Mu1* termini cosegregated with the mutant phenotype. The observation that both *Mu* silencing and paramutation are affected by the same suite of mutations suggests that both phenomena utilize a similar epigenetic regulatory system.

9:30 am Friday

T6 Cloning of maize yellow stripe1 (ys1), an iron-regulated gene involved in high affinity Fe(III) uptake.

Panaviene, Zivile(1); Curie, Catherine(2); Loulergue, Clarisse(2); Dellaporta, Stephen L. (3); Briat, Jean-Francois(2); Walker, Elsbeth L.(1) (1) University of Massachusetts, Amherst, Amherst, MA 01003, USA; (2) Université Montpellier 2 et École Nationale Supérieure d'Agronomie, Place Viala, F-34060 MONTPELLIER cedex 1 (France); (3) Yale University, New Haven, CT 06520

Crop plants frequently do not take up adequate amounts of iron from the soil, leaving them starved for iron and leading to chlorosis, poor yeild, and decreased nutritional quality. Extremely limited soil bioavailability of iron has led plants to evolve uptake strategies that can be broadly defined as chelation, extrusion and re-uptake of iron-binding molecules (Mori, S., 1999, Curr Opin Plant Biol 2, 250-253); and reduction, plasma membrane localized ferric reductases coupled with iron transporters (Eide, D.et al., 1996, Proc. Natl. Acad. Sci. USA 93, 5624-5628; Yi, Y. & Guerinot, M. L., 1996, The Plant Journal 10, 835-844; Robinson, N. J.,et al., 1999, Nature 397, 694-697). The world's major grain crops acquire iron by extrusion and re-uptake of phytosiderophores, a process fundamentally different from that used by other plant groups (Briat, J.-F. & Lobreaux, S., 1997,Trends Pl. Sci. 2, 187-193). Several

lines of evidence have suggested that the maize yellow stripe1 (ys1) mutant is deficient in Fe(III)-phytosiderophore uptake (von Wiren, N.,et al., 1994, Pl. Physiol. 106, 71-77; Hopkins, B. G., et al., 1992, J. Pl. Nutr. 15, 1599-1612; Jolley, V. D. & Brown, J. C., 1991, J. Pl. Nutr. 14, 45-58.). As we will report, cloning of ys1 by transposon tagging with Ac reveals a new class of membrane protein that mediates iron uptake. Expression of YS1 in a yeast iron uptake mutant restores growth specifically on Fe(III)-phytosiderophore media. Under iron deficient conditions, ys1 mRNA levels increase in both roots and shoots. Cloning of ys1 is an important step in understanding iron uptake in grasses, and may have implications for mechanisms controlling iron homeostasis in all plants.

9:50 am Friday

T7 Use of a new maize BAC library to study intra-specific variation at the Rp1 rust resistance superlocus

O'Sullivan, Donal M. (1); Ripoll, Pierre-Jean(1); Daviere, Jean-Michel(1); Edwards, Keith J.(1) (1) IACR-Long Ashton Research Station, Long Ashton, Bristol BS41 9AF, United Kingdom

We report the construction and characterisation of a three genome equivalent BAC library from the European flint inbred line F2. The library contains 84,652 HindIII clones and 2,206 BamHI clones with a combined average insert size of approx. 90kb. Plastid sequences detect 5.1% of clones, while knob and ribosmal RNA sequences represent 0.2 and 0.1% of the library respectively. The library has been screened for a number of single copy sequences by either PCR on DNA pools or by hybridisation to robotically gridded colony filters, with on average 2.5 positive clones per single copy probe. The Rp1 locus is a genetically complex disease resistance locus. The Rp1-D allele has been cloned [Collins et al. (1999) Plant Cell 11(7):1365-76] and identified as a member of a genetically clustered family of NBS-LRR genes. We wanted to determine the physical order and spacing of these Rp1-D homologues, and to study variation in the physical organisation among highly diverged genotypes using available large insert libraries. To this end, we have assembled Rp1 contigs of YAC clones from our LH82 (dent) library, and BAC clones from the F2 (flint) library. Locus-specific markers derived from sequence analysis of the LH82 clones have been used to align the F2 and LH82 contigs, giving us a preliminary view of the comparative structure of the locus in lines representative of both flint and dent heterotic groups.

10:40 am Friday

T8 Maternal Gametophyte Effect Genes in Maize Seed Development

Evans, Matthew M. S.(1); Kermicle, Jerry L.(1) (1) University of Wisconsin-Madison, Madison, WI 53706, USA

Seed development begins after double fertilization of the embryo sac by the two sperm cells of the pollen grain. One sperm cell fertilizes the egg to produce the embryo, and the other fertilizes the central cell to produce the endosperm. Proper development of the seed depends on two classes of maternal effect genes as well as the expression of genes from the zygotic and endosperm genomes. Mutations in genes with maternal sporophyte effects and with maternal gametophyte effects have been identified. We have isolated several mutants with maternal gametophyte effects. In some mutants of this class these effects are the consequence of abnormal embryo sac morphology. Here we concentrate on mutants, maternal effect lethal1 (mel1), mel2, and mel3, whose primary defect is the production of defective seed from mutant gametophytes. All three also show reduced pollen transmission suggesting a requirement in the male

gametophyte. Interestingly, the defective kernel phenotype of mel1 is only conditioned in seeds that inherit mel1 maternally and are homozygous for the recessive allele (endogenous to the W22 inbred line) of a second gene, snm1, demonstrating redundancy between maternal effect and zygotically acting genes. In contrast, mel2 and mel3 do not require snm1 or any other zygotically acting mutations to condition their phenotypes. mel2 maps to the long arm of chromosome 5 near dappled aleurone1 but does not condition a dappled phenotype. snm1 maps to the short arm of chromosome 2.

11:00 am Friday

T9 **Positional cues specify and maintain aleurone cell fate in endosperm development.**
Becraft, Philip W.(1); Asuncion-Crabb, Yvonne(1) (1) Iowa State University, Ames, IA 50011, USA

A genetic analysis of aleurone development in maize endosperm was conducted. A cell lineage analysis showed that, contrary to what has been reported, the aleurone does not comprise a separate lineage from starchy endosperm. Thus positional cues specify aleurone fate. The dek1 mutant was utilized to examine questions of aleurone cell fate determination. Mutants in dek1 block aleurone formation at an early stage and cause peripheral endosperm cells to develop as starchy endosperm. Revertant sectors of a transposon-induced dek1 mutant showed that peripheral endosperm cells remain competent to differentiate as aleurone cells until late in development. Ds-induced chromosome breakage was used to generate sectors of Dek1+ loss. Events occurring up through late development caused aleurone cells to switch fate to starchy endosperm indicating that cell fate is not fixed. Thus, positional cues are required to specify and maintain aleurone fate, and Dek1 function is required to respond to these cues. An analysis of additional mutants that disrupt aleurone differentiation suggests a hierarchy of genes functions first to specify aleurone cell fate, and then to control aleurone differentiation. These mutants disrupt aleurone differentiation in reproducible patterns suggesting a relationship to endosperm pattern formation.

11:20 am Friday

T10 **Differential response of pathogenic and non-pathogenic fungi to maize ribosome-inactivating protein**
Nielsen, Kirsten(1); Payne, Gary A.(1); Boston, Rebecca S.(1) (1) NC State University, Raleigh, NC 27695-7612, USA

The maize endosperm ribosome-inactivating protein, RIP1, is a potent translational inhibitor with a putative role in plant defense. RIP1 is produced as a zymogen (proRIP1) during endosperm development and then proteolytically cleaved during seed germination to produce the active RIP1 enzyme. This enzyme readily inactivates ribosomes

from non-plant eukaryotes but has little activity against ribosomes from plant species. RIP1 was tested for antifungal activity with a microculture assay in which fungal conidia treated with RIP1 or control proteins were monitored over time. We have previously shown that growth of the maize pathogen *Aspergillus flavus* was inhibited by RIP1. However, the organism could overcome this inhibition by producing a new hyphal tip that led to a branched phenotype. When conidia from a closely related non-pathogenic species, *Aspergillus nidulans*, were treated with RIP1, we observed a striking decrease in hyphal proliferation, followed by autolysis. To determine whether or not the antifungal effect of RIP1 was due to its enzymatic activity, we constructed an expression plasmid with a point mutation that encodes a Ser-Thr change near an active site residue. The resulting protein had no ribosome inactivating activity *in vitro*. In addition, it had no effect on either the growth or development of *A. nidulans*. These results indicate that the antifungal activity of RIP1 requires the enzymatic ribosome-inactivating activity. However, these results also lead to the question - How does RIP1 enter the fungal cell to inactivate the ribosomes? The size limit for peptide transport in fungi is thought to be less than 1 kD. At 25 kD, maize RIP1 would appear to be too large to cross the fungal cell membrane. We are investigating the means by which RIP1 exerts its antifungal effect by using immunolocalization to detect RIP1 during its interaction with fungi and by attempting to define the signal transduction pathway that leads to autolysis.

11:40 am Friday

T11 Molecular characterization of In-D: A semi-dominant mutation of the intensifier locus

Scheffler, Brian E.(1); Rojek, Regina(2); Pusch, Inka(2); Stübe, Sabine(2); Watson, Susan B.(1); Wienand, Udo(2) (1) USDA-ARS-NPURU, PO Box 8048, University MS, 38677, USA; (2) Institut fur Allgemeine Botanik, Universitat Hamburg, Ohnhorststr. 18, 22609 Hamburg, Germany The intensifier1 (in1) locus represents a unique regulatory gene of flavonoid biosynthesis. Although it has protein homology to the r1/b1 family of transcription activators, it appears to repress production of chalcone synthase (CHS) encoded by the whp1 locus. Initial work on the in1 wild-type allele, isolated from color converted W22 (In-W22) (Burr et. al., 1996, The Plant Cell 8:1249-1259), demonstrated that the vast majority of the transcripts from this allele were misspliced. We have extended this work to characterize the different missplicing patterns of In-W22 and In-D (a semi-dominant mutation of in1 that inhibits overall production of anthocyanins in the aleurone tissue). Northern and Western analysis demonstrate that In-D is expressed at significantly higher levels when compared to In1-W22, and that total CHS production is inhibited or delayed. Genomic analysis of In-D indicates it consists of two complete, but structurally different, copies of the "wild-type" allele. Comparison of In-W22 to In-D uncovered a CACTA transposable element in In1 that is common to the anthocyanin loci C2, Whp1, and C1. Complete sequence analysis clearly demonstrates that the In-W22 allele is not the true wild-type allele. In fact, In-W22 is a low expressing allele due to a large insertion near the TATA box. Analysis of the 5' regions of all in1 copies revealed they all are closely linked to the KAPP (kinase associated protein phosphatase 1) gene (1749- 2368 bp from the start of transcription), indicating that the duplication that resulted in the formation of the In-D allele included extended to this region.

The intensifier1 (in1) locus represents a unique regulatory gene of flavonoid biosynthesis. Although it has protein homology to the r1/b1 family of transcription activators, it appears to repress production of chalcone synthase (CHS) encoded by the whp1 locus. Initial work on the in1 wild-type allele, isolated from color converted W22 (In-W22) (Burr et. al., 1996, The Plant Cell 8:1249-1259), demonstrated that the vast majority of the transcripts from this allele were misspliced. We have extended this work to characterize the different missplicing patterns of In-W22 and In-D (a semi-dominant mutation of in1 that inhibits overall production of anthocyanins in the aleurone tissue). Northern and Western analysis demonstrate that In-D is expressed at significantly higher levels when compared to In1-W22, and that total CHS production is inhibited or delayed. Genomic analysis of In-D indicates it consists of two complete, but structurally different, copies of the "wild-type" allele. Comparison of In-W22 to In-D uncovered a CACTA transposable element in In1 that is common to the anthocyanin loci C2, Whp1, and C1. Complete sequence analysis clearly demonstrates that the In-W22 allele is not the true wild-type allele. In fact, In-W22 is a low expressing allele due to a large insertion near the TATA box. Analysis of the 5' regions of all in1 copies revealed they all are closely linked to the KAPP (kinase associated protein phosphatase 1) gene (1749- 2368 bp from the start of transcription), indicating that the duplication that resulted in the formation of the In-D allele included extended to this region.

12:00 noon Friday

T12 Genetic dissection of nuclear endosperm development

Olsen, Odd-Arne (1); Lid, Stein Erik (1); Meeley, Bob (2); Chamberlin, Mark (2); Brown, Roy C. (3); Lemmon, Betty E. (3); Olsen , Karin S.(1); Nichols, Scott E. (2) (1) Agricultural University of Norway, Ås, Norway; (2) Pioneer Hi-bred International, Johnston, Iowa , USA; (3) University of Louisiana at Lafayette, Lafayette, Louisiana, USA; (4) (6) ; (7) ; (8) Following fertilization, the nuclear type endosperm develops into a multinucleate syncytium lining the central cell. Cellularization results in an endosperm consisting of four major cell types, starchy endosperm, aleurone, transfer cells and cells of the embryo surrounding region (ESR). Recent studies in cereals and Arabidopsis have revealed a comparatively simple, conserved developmental pathway for endosperm cellularization. Cellularization is initiated by organization of the syncytium into nuclear-cytoplasmic domains (NCDs) defined by nuclear-based radial microtubule systems. Anticlinal walls deposition at boundaries of polarizing NCDs establish a honeycomb arrangement of open ended compartments or alveoli. Alveolar walls continue to grow centripetally in association with adventitious phragmoplasts formed at interfaces of microtubule systems emanating from adjacent NCDs. Periclinal cell division in alveoli results in a peripheral layer of cells and displacement of the alveoli inward. Repeated cycles of anticlinal wall growth and periclinal cell division cellularize the endosperm. Cell differentiation in endosperm appears to occur via positional information laid down in the central cell or during endosperm early development, marking off the periphery of the endosperm as different from the interior. Cell types with peripheral positions include transfer cells and aleurone cells, whereas the interior is occupied by starchy endosperm cells. In contrast, differentiation of the ESR region seems to depend on an existing proximal distal axis in the central cell. In order to analyze endosperm development genetically, we have carried out a systematic microscopy-based screen of the Pioneer TUSC collection for mutants perturbed in endosperm development. Mutant phenotypes include complete lack of aleurone cells, abnormal pattern of cell divisions in the aleurone layer, aleurone cells with abnormal contents, multiple layers of aleurone cells, and endosperm containing aleurone, but no starchy endosperm cells. Efforts are currently underway to clone selected mutant genes.

8:30 am Saturday

T13 Molecular Analysis of the Tangled Gene

Smith, Laurie G.(1); Gerttula, Suzanne (1); Levy, Joshua (2) (1) University of California-San Diego, La Jolla, CA; (2) University of North Carolina-Chapel Hill, Chapel Hill, NC

Analysis of the tangled (tan) mutant phenotype demonstrated that this gene is required for the spatial regulation of cell division during the development of leaves and other organs. In tan mutant leaves, cells frequently divide normally in the transverse orientation, but rarely divide in the longitudinal orientation. The majority of cells divide abnormally such that the new cell wall is crooked or curved (1). Analysis of the cytoskeleton in dividing cells of tan mutant leaves has shown that abnormally oriented cell divisions are due mainly to frequent failure of the phragmoplast (the cytokinetic apparatus of plant cells) to be guided to the site previously occupied by a preprophase band of microtubules and actin filaments (2). Making use of a Mu1-tagged allele, we have cloned the Tan gene. Consistent with our analysis of the tan phenotype, Tan gene expression is tightly correlated with cell division in the shoot. The Tan gene encodes a predicted protein of approximately 41kD that is highly basic (pI 10). This protein is related to the basic region of the APC (adenomatous polyposis coli) protein, which has been identified in mammals and several other metazoans. The basic region of APC has been shown to mediate its interaction with microtubules, suggesting that the TAN protein may also be a microtubule-binding protein. Consistent with this conclusion, monoclonal antibodies raised to the TAN protein produced in E. coli stain all the microtubule structures in dividing plant cells: preprophase bands, spindles, and phragmoplasts. These antibodies are not specific to TAN, however; they apparently recognize other, TAN-related proteins as well. Biochemical studies and intracellular localization of epitope-tagged TAN fusion proteins will be used to further pursue the localization and function of TAN protein in dividing cells. (1) Smith, LG, Hake, S, and Sylvester, AW (1996). The tangled1 mutation alters cell division orientations throughout maize leaf development. Development 122:481-489. (2) Cleary, AL, and Smith, LG (1998). The Tangled gene is required for spatial control of cytoskeletal arrays associated with cell division. Plant Cell 10:1875-1888.

8:50 am Saturday

T14 Assembling a genetic network for regional identity along the proximo-distal axis of the leaf

Lubkowitz, Mark A(1); Theodoris, George(1); Bauer, Petra(2); Freeling, Mike(1) (1) University of California-Berkeley, Berkeley, CA 94720; (2) Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK),Corrensstr. 3D-06466 Gatersleben

Development occurs through the coordinated temporal and spatial regulation of various genes and genetic programs. In recent years our laboratory has focused on leaf development and has described several genes that are involved in this process. Work from our laboratory and others has demonstrated that the knotted1-like homeobox (KNOX) gene family plays an important role in leaf development. Mutants that ectopically express KNOX genes in the developing leaf cause proximal cell identities such as sheath cells to occur in more distal regions such as the blade. This phenotype manifests itself as a displaced ligule-auricle boundary with sheath extending into the blade region. A molecular analysis of this phenomenon revealed possible interactions

between the KNOX gene product of liguless3 and other KNOX gene products. We further analyzed the leaf developmental module by biochemically characterizing the interaction between the negative regulator roughshteath2 and the KNOX gene roughsheath1 and by using a modified yeast screen to look for downstream targets of liguleless3. This upstream and downstream approach has now added "players" to the network of function that identifies regional identity along the proximo-distal axis of the leaf.

9:10 am Saturday

T15 The fasciated ear2 gene encodes a leucine rich repeat protein that controls inflorescence and floral development in the maize ear.

Jackson, Dave (1); Taguchi Shiobara, Fumio(1); Hake, Sarah (2); Yuan, Zhuang (1) (1) Cold Spring Harbor Lab, Cold Spring Harbor, NY 11724; (2) USDA UC Berkeley Plant Gene Expression Center, Albany, CA 94710

Shoot development depends on the coordinated activities of groups of stem cells called meristems that are responsible for organ initiation and positioning. Within the meristem, a finely controlled balance between cell proliferation and incorporation of cells into new primordia ensures that the meristem is maintained as an organized structure. During ear development in maize the inflorescence apical meristem, which consists of a few hundred cells, supplies many tens of thousands of cells to make spikelet primordia. In certain cases, which can be environmental or genetic in nature, the fine balance in the meristem is upset and it over proliferates, in a process known as fasciation. In maize there are several loci that mutate to give a fasciated ear phenotype, and we have cloned one of them, fasciated ear2. FAE2 encodes a leucine rich repeat protein which by analogy to similar proteins in other plants likely acts as a receptor to control meristem organization. FAE2 is expressed in inflorescence and floral meristems, and closer examination of fae2 flowers revealed that their development and positioning is also abnormal. fae2 shows a genetic interaction with ramosa3, a mutation that also causes fasciation in some genetic backgrounds, therefore it is likely that fae2 and ra3 interact directly or closely in a pathway to control ear meristem development. Control of ear meristem size may be one mechanism by which the number of rows of spikelets is controlled, and it is possible that subtle changes in this class of genes was involved during the domestication of crop plants and the selection for example for larger fruits or increased numbers of seeds.

9:30 am Saturday

T16 A candidate gene for the Tunicate1 locus

Wingen, Luzie U. (1); Werth, Susanne (1); Muenster, Thomas(1); Deleu, Wim(1); Faigl, Wolfram(1); Saedler, Heinz(1); Theissen, Guenter(1) (1) Max-Planck-Institute for Breeding Research,Carl-von-Linne-Weg 10, 50829 Koeln, Germany

Tunicate1 (Tu1) is a co-dominant, gain-of-function mutation on the long arm of maize chromosome 4 that causes a foliaceous elongation of the glumes and other changes in male and female inflorescences, resulting in the famous phenotype of pod corn. We have isolated a MADS-box gene termed zmm19, encoding a putative transcription

factor, which maps very closely to the Tu1 locus. Members of the respective gene family often work as organ identity genes. Expression studies in a population segregating for mutant and wildtype plants revealed that in all wildtype plants, zmm19 is expressed weakly in vegetative leaf blades and strongly in leaf sheaths and husk leaves, but not in inflorescences. In all Tu1 mutant plants, expression of this gene in leaves is the same as in wildtype plants, but in addition, there is a strong ectopic expression in male and a very strong expression in female inflorescences, i.e. exactly in the structures which show morphological changes in the mutant. Analysis of the promotor region of the zmm19 gene in Tu1 mutant and wildtype plants showed that in all mutant plants there is a deviant allele of zmm19 which contains a fragment of a Mu-like transposable element upstream of the coding region. This special allele of zmm19 has not been found so far in a great diversity of phenotypically wildtype maize races. Expression studies employing a single nucleotide polymorphism in zmm19 mRNAs revealed that only the mutant allele is ectopically expressed in Tu1 mutant plants. The by far most plausible explanation for all these findings is that the zmm19 gene represents the Tu1 locus. We thus assume that the pod corn phenotype is caused by the ectopic expression of a leaf developmental program in the inflorescences of maize. Work to corroborate this hypothesis is in progress.

9:50 am Saturday

T17 A role for the rice homeobox gene Oshox1 in provascular cell fate commitment

Scarpella, Enrico(1); Rueb, Saskia(1); Hoge, J. Harry C.(1); Meijer, Annemarie H.(1) (1) Insitute of Molecular Plant Sciences, Leiden University, Leiden, The Netherlands

The vascular tissues of plants form a network of interconnected cell files throughout the plant body. The transition from a genetically totipotent meristematic precursor to different stages of a committed procambial cell and its subsequent differentiation into a mature vascular element, involve developmental events whose molecular nature is still mostly unknown. The rice protein Oshox1 is a member of the homeodomain leucine zipper family of transcription factors. Here, we show that the strikingly precise onset of the Oshox1 gene expression marks critical, early stages of provascular ontogenesis in which the developmental fate of procambial cells is specified but not yet stably determined. This observation suggests that the Oshox1 gene may be involved in the control of the proper conditions required to accomplish the developmentally necessary restriction of genomic potential of procambial cells. In support of this hypothesis, ectopic expression of Oshox1 in provascular cells that normally do not yet express this gene results in an anticipation of procambial cell fate commitment, eventually culminating in premature vascular differentiation. Oshox1 represents the first example of a transcription factor whose function can be linked to specification events mediating provascular cell fate commitment.

Concurrent Sessions 5,6,7

Session # 5 THE GENOME

10:45 am Saturday

T18 Insights into Molecular Basis of Heterosis: mRNA Profiles of Maize Hybrids and Inbred Parents

Guo, Mei(1); Rupe, Mary(1); Smith, Howie(1); Yang, Sean(1); Bowen, Ben(2); Crasta, Oswald (3) (1) Pioneer Hi-Bred Int. Inc., Johnston, IA 50131, USA; (2) Lynx Therapeutics Inc., Hayward, CA 94545, USA; (3) CuraGen Corporation, New Haven, CT 06511, USA

Heterosis is a term used to describe the increased vigor of hybrid progeny in comparison to their inbred parents. Although heterosis has been widely used in plant breeding for many decades, the molecular mechanisms underlying the phenomenon remain unknown. We have chosen to study heterosis as a phenotype, and are using CuraGen RNA profiling technology to examine differences in RNA expression between hybrids and their inbred parents. Preliminary data indicate less than 5% of the profiled CuraGen cDNA fragments differ between a hybrid and its

inbred parents. At the mRNA level, little heterosis was found in the hybrids. Instead, transcript level of the majority of the genes in the F1 fell within the range of the two parents. Interestingly, in all hybrids examined we observed a tendency towards the male parent in the level of gene expression. Such a “male parental preference” was found negatively correlated with hybrid yield. Preliminary data suggest that silencing or partial silencing of the female parental allele in the F1 may have contributed to this unbalanced expression pattern.

11:05 am Saturday

T19 Dosage dependent control of heterosis

Dogra, Anjali(1); Coe, Edward H.(1); Birchler, James A.(1) (1) University of Missouri-Columbia, Columbia, Missouri 65211 USA

Heterosis is the phenomenon in which the performance of a hybrid obtained by crossing two different lines is superior to the better parent. Heterosis has long been used commercially, but the genetic basis is still poorly understood. Two theories have been the most prevalent to explain heterosis. According to the dominance theory, hybrid vigor is the result of a complementary effect of dominant over recessive alleles. This theory does not readily explain how a hybrid outperforms the better parent. The overdominance theory holds that heterozygosity per se is advantageous, but there are very few examples of such behavior exhibited by single genes. We sought a novel experimental approach to evaluate the genetic basis of heterosis. We compared diploid and triploid inbreds and hybrids of B73 (B) and Mo17 (M). Triploid "inbreds" (BBB, MMM) and hybrids (BMM, MBB) were made using the trifluralin tassel treatment, which induces diploid sperm. Various morphological characters such as plant height, leaf number, stalk circumference etc. were measured to assay hybrid vigor. We did not use yield as a criterion because triploids are mostly sterile due to irregular distribution of chromosomes during meiosis. We observed that the diploid and triploid inbreds were significantly different from each other. This is not due to the effect of triploidy because triploid hybrids are comparable or better than the diploid hybrids. The two types of triploid hybrids exhibited different levels of heterosis, suggesting an influence of allele dosage. Dosage dependent factors appear to control quantitative traits which may result from the fact that many regulatory genes in diploid eukaryotes exhibit additive behavior. We suggest that heterozygosity at regulatory loci increases target gene expression of metabolic or developmental pathways that contribute to hybrid vigor. Homozygosity may have the opposite effect. According to the dominance theory, the performance of diploid and triploid hybrids should be comparable, but for characters which show heterosis in triploids the magnitude of heterosis is much better than in diploids except for leaf length. Our data do not agree fully with the overdominance theory also, because it proposes that the two kinds of triploid hybrids would be similar in performance. Therefore we propose a role of heterozygosity at dosage dependent regulatory loci to control heterosis.

11:25 am Saturday

T20 Uncovering complex patterns of evolution for genes encoding Myb-domain proteins.

Braun, Edward L.(1); Dias, Anusha(1); Grotewold, Erich(1) (1) Department of Plant Biology, Ohio State University, Columbus, Ohio 43210, USA

Myb proteins, which are characterized by the presence of one to four conserved ca. 50 amino acid motifs (myb motifs), have been identified in diverse eukaryotic groups. However, the gene family encoding one specific group of Myb-domain transcription factors, the R2R3 Myb proteins, has undergone a remarkable expansion within the land plants. In sharp contrast to the diverse *R2R3 myb* gene family, a small number of genes encoding structurally distinct Myb-domain proteins can also be identified in the plants. These structurally distinct proteins include

Myb homologs characterized by one, two, and three myb motifs. We will present the results of analyses which establish the evolutionary roots of Myb-domain proteins containing single and multiple myb motifs as well as the results of surveys of maize cDNAs encoding Myb-domain proteins. Patterns of diversification for these gene families within the land plants and within the monocots, based in part upon sampling from maize, will be presented.

11:45 am Saturday

T21 The TR-1 knob repeat exhibits extreme levels of neocentromeric activity

Hiatt, Evelyn N.(1); Kentner, Edward K.(1); Dawe, R. Kelly(2) (1) Dept. of Genetics, University of Georgia, Athens, GA 30602; (2) Depts. of Genetics and Botany, University of Georgia, Athens, GA 30602

Knobs are large heterochromatic regions found in specific locations on maize chromosomes. In the presence of Abnormal chromosome 10 (Ab10), knobbed chromosomes exhibit neocentromeric activity during meiosis and genes linked to knobs show meiotic drive. Ab10 differs from normal chromosome 10 (N10) by an additional piece of chromatin added to the long arm of N10. The additional chromatin includes a differential region (with three chromomeres), a stretch of euchromatin, a large heterochromatic knob followed by a euchromatin tip. Knobs are primarily composed of two repeat families: a 180bp repeat (Peacock, et.al, 1981) and an approximately 350bp repeat named TR-1 (Ananiev, et.al, 1998). The three chromomeres contain the TR-1 repeat only. The large Ab10 knob has both the 180bp repeat and the TR-1 repeat. With TR-1 as a marker for the three chromomeres we examined meiotic pairing between N10 and Ab10, as well as N10 and several Ab10 deficiency lines. FISH analysis demonstrates that TR-1 and the 180bp repeat have different localization patterns within functioning neocentromeres. In neocentromeres, the TR-1 repeat often extends toward the poles ahead of the 180b repeat regions forming long, linear strands of chromatin.

12:05 pm Saturday

T22 Genetic variation for phenotypically invariant traits detected in teosinte: implications for the evolution of novel forms

Lauter, Nick(1); Doebley, John(2) (1) University of Minnesota, St. Paul, MN 55108, USA; (2) University of Wisconsin, Madison, WI 53706, USA

Research in our laboratory has focused on the inheritance of the differences in inflorescence and plant architecture between maize and its wild ancestor, teosinte. In a previous experiment, we noticed that groups of F1 progeny derived from crosses between various teosintes and the same maize inbred line showed slight differences for a few traits that distinguish maize from teosinte. Two of these traits, inflorescence phyllotaxy and spikelet abortion in the ear, are of particular interest because they have discretely different character states in maize and teosinte and are invariant within teosinte. In order to investigate the possibility that teosintes harbor genetic variation affecting traits for which they have no phenotypic variation, we employed QTL mapping to analyze a testcross population that was created by crossing two teosintes, Zea mays ssp. parviglumis and Zea mays ssp. mexicana, and subsequently test crossing their F1 hybrid to a maize inbred line. Since the maize portion of the genome is identical among all testcross progeny, QTL differences among TC1 progeny can only be detected if genetic differences affecting the traits in question exist between the teosinte parents. Despite a lack of phenotypic variation between these teosintes, a total of 23 QTL affecting the aforementioned discrete traits and four other quantitative traits were detected. Both teosinte parents contributed QTL alleles that make TC1 progeny more maize-like. Moreover, the QTL that segregate in teosinte map to many of the same regions of the genome as the QTL involved in maize evolution from its common ancestor with teosinte. We discuss the implications of these results both for the evolution of maize and the evolution of discrete traits in flowering plants.

Session # 6 - THE PLANT (PART 2)

10:45 am Saturday

T23 Modifying flowering time through modulation of indeterminate1 (id1) expression

Muszynski, Michael G.(1); Wright, Deanne (2); Nelson, Kellie (1); Briggs, Kristen (4); Tomes, Dwight (1); Colasanti, Joseph (3) (1) Agronomic Traits, Pioneer Hi-Bred Intl., Inc., Johnston, IA 50131, USA.; (2) Associative Genetics, Pioneer Hi-Bred Intl., Inc., Johnston, IA 50131, USA.; (3) PGEC/Dept. of Plant and Microbial Biology, Univ. California-Berkeley, Albany, CA 94710, USA; (4) EPIcyte Pharmaceutical, Inc., San Diego, CA 92121, USA

Our goal is to modify the flowering time pathway in maize to produce early maturity inbreds for more rapid germplasm improvement and the development of ultra-early hybrids for the Canadian market. In Arabidopsis, mutations in more than twenty genes, that affect flowering time, have been identified, and of these, about six have been cloned. For some of those genes tested, modulation of their expression in transgenic plants results in changes of up to three-fold in flowering time. These studies reveal that even though time-to-flower is a polygenic trait, dramatic changes in maturity can occur by altering the expression of a single gene in the flowering time pathway. The only cloned maize flowering-time gene is indeterminate1 (id1). Plants lacking a functional id1 gene continue to grow vegetatively for an extended period of time and undergo the transition to reproductive growth very late in development. Thus the id1 gene product is a floral promoter; its function is required for a plant to transition normally to reproductive growth. The id1 gene encodes a zinc-finger protein with sequence similarity to transcription factors. Accumulation of id1 mRNA is specific to immature leaves with no transcript detected in the shoot apex. mRNA accumulation is low at the seedling stage but peaks near the time of floral transition and then diminishes as reproductive growth proceeds. We used several constitutive promoters to drive expression of id1 in transgenic maize to modify flowering time. Transgenic plants carrying a rice actin::Id1-genomic construct transition to reproductive growth earlier than their non-transgenic sibs. We will present efficacy data for several events in both the T1 and T2 generations. We will also show that two events tested are able to complement the null id1-m1 mutation.

11:05 am Saturday

T24 Mosaic Analysis of a dorsiventral leaf polarity mutant.

Nelson, Jennifer (1); Lane, Barbara(1); Freeling, Michael(1) (1) University of California-Berkeley, Berkeley, CA 94710, USA

The Rolled1 (Rld1) mutants in maize are characterized by several interesting phenes, many of which are associated with a partial reversal of dorsiventral (abaxial-adaxial) polarity in leaves. The switching of dorsiventral polarity involves both epidermal and ground tissue. Foremost, Rolled plants display a curled leaf phene in which the lamina of the leaf blade roll adaxially inward toward the midrib. Macrohairs and bulliform cells, characteristic of the adaxial epidermis in normal leaves, are seen on the abaxial epidermis in Rolled leaves. In addition, Rolled leaves often display an ectopic ligule flap at the adaxial blade-sheath boundary. More severe Rolled plants display regions with no transverse veins, unfused coleoptiles with additional veins, and ectopic sheath or sheath/blade flaps on the abaxial leaf surface. A mosaic analysis was performed to gain insight into the Rld1 phenotype. X-ray induced chromosome breakage generated clonal sectors of wildtype tissue in Rolled leaves. The observed sectors were scored based on epidermal characteristics, and suggest that Rld1 acts autonomously in the lateral

dimension, yet non-autonomously in the transverse dimension. Furthermore, the presence of Rld1 in the abaxial epidermal cell layer is necessary and sufficient to confer the Rolled phenotype. These results support a model for transverse signaling during the establishment of dorsiventral tissue identity. Perhaps one epidermis signals the other to adapt a default identity.

11:25 am Saturday

T25 indeterminate floral apex 1 is required for maintenance of meristem identity

Laudencia-Chingcuanco, Debbie(1,2); Hake, Sarah (1,2) (1) University of California-Berkeley, Berkeley, CA 94710; (2) Plant Gene Expression Center, Albany, CA 94710

The indeterminate floral apex1 (ifa1) gene is required for the maintenance of floral, spikelet, and branch meristem identity in both the male and female inflorescences. In ifa1 mutants, the floral meristem, after forming the floral organs, continues to proliferate, giving rise to a mass of pistillate material at the center of the flower. Plants homozygous for the ifa1 mutation also produce extra flowers and spikelets. Double mutants with zea agamous (zag1), which affects floral meristem determinacy, develop ectopic inflorescence at the center of the flower, indicating that ifa1 and zag1 play redundant roles in the specification of the floral meristem identity. Double mutants with indeterminate spikelet 1 (ids1), which affects the determinacy of spikelet meristems, form extra flowers and spikelets within spikelets, showing that ifa1 and ids1 play redundant roles in the determination of spikelet meristem identity. ifa1 is a recessive mutation that maps to chromosome 1S, within 3 cM from umc 76. No other developmental mutants map within this region. Preliminary evidence indicates that zmm14, a MADS-box gene that maps close to umc76, does not encode ifa1. We have four putatively tagged Mu alleles of ifa1, three of which are from targeted tagging experiments. We are currently investigating the linkage of Mu elements with the ifa1 phenotype.

11:45 am Saturday

T26 Characterization of xcl, a mutation affecting planes of cell division

Kessler, Sharon A.(1); Sinha, Neelima(1) (1) University of California-Davis, Davis, CA 95616, USA

Plant morphogenesis depends on the coordinated regulation of cell division, expansion and differentiation. During leaf initiation and development, cells divide in either the anticlinal plane (perpendicular to the surface of the organ) or in the periclinal plane (parallel to the surface of the organ). Cell divisions in the protoderm occur in the anticlinal plane in order to expand the surface of the developing leaf. The xcl (extra cell layers) mutation leads to the formation of additional cell layers below both the adaxial and abaxial epidermis of leaf blades without affecting the arrangement of mesophyll and bundle sheath cells. These extra cells arise from aberrant periclinal divisions in the protoderm and have epidermal features, indicating that they differentiate according to lineage and not position. The presence of the extra cells also affects the differentiation of the epidermis. Cells are square rather than rectangular, less crenulated, and

stomatal frequency is decreased, especially on the adaxial surface of the leaf. While overall plant morphology is not severely affected by the xcl mutation, xcl leaf blades are twice as thick but half as wide as wild-type leaves. Leaf sheaths are also narrow, with extra cell layers predominantly under the abaxial epidermis. xcl kernels have a multiple aleurone layer phenotype, indicating that the XCL protein is involved in controlling the orientation of cell division in cells which divide preferentially in the anticlinal plane.

12:05 am Saturday

T27 EPC, a gene controlling juvenile to adult phase change in maize

Sauer, Matt(1); Poethig, R. Scott(1) (1) Univesity of Pennsylvania, Philadelphia, PA 19104

Higher plants go through four distinct developmental phases during their life cycle: embryonic, juvenile, adult and reproductive. In maize, leaves produced during the juvenile phase of development differ markedly from those produced during the adult phase. The developmental phase of the plant determines the types of waxes produced by the leaf, with adult leaves appearing glossy compared to juvenile leaves. In addition, epidermal cell shape differs between juvenile and adult leaves, and adult leaves produce specialized cell types, such as hair cells and bulliform cells, not found on juvenile leaves. Mutations in several genes affect the timing of the transition from juvenile to adult phase indicating that this process, known as phase change, is under genetic control. For example, the number of leaves initiated during the juvenile phase of plants homozygous for the epc (early phase change) mutation is markedly reduced, resulting in the production of leaves with adult characteristics as early as the first leaf. In Arabidopsis thaliana, leaves produced during the adult phase have abaxial trichomes while leaves produced in the juvenile phase do not. Several mutations in Arabidopsis cause the premature production of abaxial trichomes, a phenotype comparable to epc. Two of the genes defined by these mutations, SQUINT and HASTY, have been cloned by our lab. We cloned maize orthologues of both the Arabidopsis SQUINT and HASTY genes. Using RI lines, ZMSQUINT was mapped to chromosome 6L, where there are no known phase change mutants, and ZMHASTY to 8L. Subsequent analysis showed that ZMHASTY is tightly linked to the epc mutation. The mRNA levels of ZMHASTY are greatly reduced in two epc alleles, epc-W23 and epc-1s2p. We are trying to identify mutations of the ZMHASTY gene in these, and other, epc alleles.

SESSION #6 THE GENE

10:45 am Saturday

T28 Diverse roles of required to maintain repression (rmr) factors in gene silencing

Hollick, Jay B.(1); Lisch, Damon(1) (1) Universtiy of California, Berkeley

Genetic analysis of paramutation at the purple plant (pl) locus, a regulator of the anthocyanin biosynthetic pathway, is ongoing to gain insights into general chromosome processes used in gene control. Paramutation describes a directed heritable alteration in gene regulation that appears to occur without DNA sequence changes. One particular pl allele, Pl-Rhoades (Pl-Rh), is unstable and can spontaneously change to a weaker transcription state called Pl'. The Pl' state is both mitotically and meiotically stable and invariably causes paramutation silencing of other Pl-Rh alleles present in the

same nucleus. Stable maintenance of the silenced Pl' state requires undefined interactions with a second Pl-Rh or Pl' allele and at least seven distinct trans-acting factors, mop1 (modifier of paramutation; J. Dorweiler, J. Hollick, V. Chandler), rmr1 and rmr4 (required to maintain repression; J. Hollick and V. Chandler), rmr6, rmr7, rmr8, and rmr9. At variable frequencies, Pl' can heritably revert to the fully active Pl-Rh state in homozygous rmr1, rmr4 and rmr6 mutant sporophytes but not in homozygous rmr7 mutants. Further characterization of mutant rmr alleles will be presented that reveals both mechanistic relationships and distinctions between paramutation at unrelated loci, plant development, and the regulation of Mu elements. The diverse effects of rmr functions are consistent with their proposed role in general regulation of genome homeostasis and function.

11:05 am Saturday

T29 Altered patterns of sucrose synthase phosphorylation and localization precede root tip death in anoxic maize seedlings

Chalivendra, Subbaiah C.(1); Sachs, Maritn M. (1, 2) (1) University of Illinois, Urbana, IL 61801, USA; (2)USDA-ARS

Root extracts made from maize (Zea mays L.) seedlings submerged for 2 h showed an increased 32P-labeling of a 90 kDa polypeptide, in a Ca2+-dependent manner. This protein was identified as sucrose synthase (SS) by immunoprecipitation and mutant analysis. Metabolic labeling with 32Pi indicated that the aerobic levels of SS phosphorylation were maintained up to 2 h of anoxia. In contrast, during prolonged anoxia, the protein was under-phosphorylated and by 48 h, most of the protein existed in the unphosphorylated form. In seedlings submerged for 2 h or longer, a part of SS became associated with the microsomal fraction and this membrane localization of SS was confined only to the root tip. In O2-deprived seedlings, the SS redistribution in the root tip preceded callose induction, a symptom of cell death. sh1 mutants showed a sustained rate of SS phosphorylation and lacked the anoxia-induced relocation of SS, indicating that it was the SS1 form of the enzyme that was redistributed during anoxia. sh1 mutants also showed less callose deposition and greater tolerance to prolonged anoxia than their normal siblings. EGTA addition to the submergence buffer led to an increased dephosphorylation as well as membrane localization of SS and greater callose accumulation, while Ca2+ addition decreased the proportion of membrane-bound SS and callose deposits. We propose that the dephosphorylation and membrane association of SS is an important early event in the death of the anoxic root tip.

11:25 am Saturday

T30 Cell cycle regulatory components in the endosperm endoreduplication cycle.

Dilkes, Brian P.(1); Dante, Ricardo A.(1); Coelho, Cintia M.(1); Woo, Young Min(1);

Leiva-Neto, Joao T.(1); Kretzschmar, Ellen(2); Larkins, Brian A.(1) (1) University of Arizona, Tucson, Arizona 85721, USA; (2) Luther College, Decorah, Iowa 52101, USA

During maize endosperm development, most nuclei engage in multiple rounds of DNA synthesis without intervening mitoses. This process, referred to as endoreduplication, is typical of tissues with high metabolic activity and is associated with high levels of gene expression. These cells replicate their nuclear DNA to completion without any signs of chromatin condensation, segregation or cytokinesis, resulting in multiple uniform copies of the genome. Thus, the initiation of DNA synthesis is uncoupled from mitosis but other operations, such as the maintenance of genome integrity, are preserved. While this process has been well documented and quantified, the molecular events inducing and maintaining this unique cell cycle are unknown. In the standard cell cycle, mitosis and DNA synthesis are associated with changes in the expression of phase-specific cyclins and changes in the activity of cyclin-dependant kinases (CDKs). As endoreduplication represents a simplified cell cycle, we predict that the CDK activities present in endosperm will comprise a G1- or S-phase form. We hypothesize that endoreduplication in maize endosperm is the result of inactivation of the M-phase CDK and oscillations in the activity of S-phase CDK in a given cell. We have identified two post-translational inhibitors of the M-phase CDK and tested their effects on partially purified S-phase CDK. In addition we have begun to describe the transcriptional program associated with endoreduplication in maize endosperm. Both the levels of activity for these inhibitors, and their transcript abundance are consistent with a role in modulating the activity of the S-phase CDK. In addition, we have developed a rapid transgenic bioassay for identifying cell cycle regulators via an overexpression phenotype. In combination with a transgenic approach plant we have investigated the roles of multiple, putative cell cycle regulators.

11:45 am Saturday

T31 *Ac* tagging and characterization of a terpenoid cyclase gene induced by herbivore damage

Shen, Binzhang(1); Dooner, Hugo K.(1) (1) Rutgers University, Piscataway, New Jersey 08854, USA

In the tritrophic interaction, caterpillar-damaged plants emit volatile chemical signals that attract natural enemies of the foraging herbivore. Among these volatiles, terpenoids are some of the most consistently released compounds across plant species. The elicitor of plant volatiles, designated volicitin, has been isolated from the oral secretion of beet armyworm (BAW) larvae, but little is known about the plant signal transduction pathway from the perception of volicitin to the production of defense terpenoids. Although biochemical analysis has identified terpenoid cyclase (synthase) genes as key plant defense genes, a lack of gene knockout mutations for the enzyme has precluded the elucidation of the role of these genes in the tritrophic interaction. We have cloned a maize sesquiterpenoid cyclase gene (*stc1*) by Ac tagging and have subsequently identified two deletion mutations of the gene. The *stc1* gene, located in 9S, consists of seven exons, which are conserved in number and size among plant terpenoid synthase genes. Though key amino acids are conserved, the nucleotide sequence is sufficiently divergent to preclude isolation of the maize gene on the basis of DNA homology. The single copy *stc1* gene is induced 15 to 30 fold in the damaged leaves of 10-day old maize seedlings by BAW damage or treatment with purified volicitin. In contrast, no *stc1* gene transcript can be detected in undamaged wild type seedlings or in damaged *Ac* insertion and deletion mutants. Volatiles from the volicitin treated maize seedlings were analyzed by GC-MS. A major peak corresponding to a naphtalene-derived sesquiterpenoid was present in wild type, but absent in both the insertion and deletion mutants. Among the volatiles emitted by caterpillar-damaged maize plants, sesquiterpenes are particularly attractive to parasitic wasps so it is highly likely that the compound missing in the mutants is a plant-released wasp attractant.

Such a possibility is being tested by using a two-choice flight tunnel sssay. The significance of the *stc1* gene in investigations of insect-induced plant signal transduction pathways is discussed.

12:05 am Saturday

T32 Regulation of the Maize *a1* Promoter in Transgenic Plants

Cocciolone, Suzy M.(1); Chopra, Surinder(1); Peterson, Thomas(1) (1) Iowa State University, Ames, Iowa 50011, USA

Anthocyanins and phlobaphenes are flavonoid pigments commonly produced in maize. The initial steps in the biosynthesis of both pigments are identical, but synthesis of each pigment is controlled by different regulatory genes. Anthocyanin production requires coordinate expression of two regulatory genes: one from the myb-homologous *c1/pl1* gene family and one from the myc-homologous *r1/b1* gene family. In contrast, activation of phlobaphene biosynthesis seems to require a single myb-homologous gene, *p1*. To develop an in vivo reporter for regulatory gene activity, we transformed maize plants with an *a1* gene promoter linked to the GUS reporter gene (*A1::GUS*); the maize *a1* gene encodes a structural enzyme that functions in both pigmentation pathways. Unexpectedly, the A1::GUS transformants exhibited two distinct patterns of transgene regulation. For three independent transformation events, the A1::GUS transgene is activated by *c1* and *r1* regulatory genes, but not by *p1* alleles. In contrast, another transformation event exhibits A1::GUS expression that is both c1/r1-responsive and *p1*-responsive. This event also expresses the transgene in the absence of a functional *p1* gene; however, transgene activity becomes *p1*-regulated in the presence of a functional *p1* allele, as demonstrated by the induction

of blue pericarp sectors by the variegating *P-vv* allele. An interpretation of these observations will be presented.

7:30 pm Saturday Plenary Talk

T33 Wheat, a model or commodity-chromosome pairing and polyploidy

Moore, Graham. John Innes Centre, UK

More than 70% of flowering plants are polyploids. The presence of additional sets of chromosomes possessing similar structures can create disruption at meiosis. To produce viable gametes, polyploids must behave as diploids during meiosis, only true homologues must pair. Surprisingly meiosis is shorter in polyploids compared to their diploid progenitors. Studying wheat and its wild relatives, we have shown that centromeres associate early during anther development in polyploid species (1). In contrast, centromeres in diploids species only associate at the onset of meiotic prophase (1). Sir Ralph Riley's wheat Ph1 locus affects chromosome pairing. A single mutant carrying a deletion of this locus has been used in wheat breeding for the last 25 yrs. We have shown that the deletion of this locus affects the early centromere association lengthening pre-meiotic interphase (2). Using comparative mapping approaches (3), we have reconstructed the rice equivalent region to the Ph1 locus; isolated a set of overlapping deletions of the Ph1 locus in wheat (4); and narrowed the locus to a 200 Kb region which was sequenced by Dupont. This sequence in conjunction with the deletion breakpoints is being used to identify a candidate gene for this locus, which will help elucidate the mechanism. References: (1) Martinez-Perez et al., 2000, Polyploidy induces Centromere Association. J Cell Biol 148 233; (2) Aragon-Alcaide et al.,1997, Association of homologous chromosomes during floral development. Curr Biol 7 905 (3) Moore et al., 1995, Grasses, line up and form a circle. Curr Biol 5 737 5 737; (4) Roberts et al., 1999, Induction and characterisation of Ph1 wheat mutants. Genetics 153 1909.

8:15 pm Saturday Plenary Talk

T34 Genetic evidence and the evolution of maize. Doebley, John. University of Wisconsin.

No abstract provided.

8:30 am Sunday

T35 Genomic approaches to seed development

Settles, A. Mark(1); McCarty, Donald R.(1) (1) University of Florida, Gainesville, Florida 32611, USA

The maize endosperm provides a genetically accessible model for plant development. As a direct product of fertilization, the endosperm does not require a functional meristem to show defects in organ formation, and pleiotropic mutants can be interpreted in the context of organ development. However, there are large numbers of seed mutants that affect endosperm development making these mutants difficult to sort and prioritize for cloning. We are taking a genomics approach to seed phenotypes and focusing on mutants that disrupt normal aleurone patterning. We have developed three essential tools for high through put cloning of Robertson's Mutator tagged seed mutants. First, we have generated a mutagenic inbred especially suited for accelerated cloning by introgressing Mutator stocks with the bz-mum9 marker into color converted W22 (UniformMu). By alternating a backcross generation with a self generation, we purge all existing seed mutants from UniformMu. Thus, our population has the following advantages: 1) mutants are identified in a homogenous genetic background aiding the interpretation of both quantitative and qualitative phenotypes, 2) the independence of allelic mutations is assured, 3) the direct progenitor of new mutations is known to aid cloning, and 4) Mu-inactive mutants can be selected using the bz-mum9 marker so that somatic insertions will not interfere with cloning strategies. Second, we have adapted TAIL-PCR to amplify germinal Mu insertion sites in maize (Mu-TAIL). Finally, we have developed an efficient in vitro subtraction of Mu-TAIL products based on representational difference analysis (RDA). Our general strategy is to identify novel germinal transpositions in seed mutants as candidates for causative insertions. By selecting Mu-off kernels from both the progenitor and new mutant, only germinal insertions will be amplified by Mu-TAIL. These are then subtracted with RDA to identify candidate transpositions prior to even generating a segregating population of mutants.

8:50 am Sunday

T36 Molecular Marker Mapping of Chromosomal Regions Associated with Carotenoids and Tocopherols in Maize

Wong, Jeffrey C.(1); Lambert, Robert J.(1); Rocheford, Torbert R.(1) (1) University of Illinois Urbana-Champaign, IL 61801, USA

We have detected QTL for total and specific carotenoids (a-carotene, b-carotene, b-cryptoxanthin, lutein and zeaxanthin) and tocopherols (a-tocoperhol, d-tocopherol, and g-tocopherol) in a 200 F2:3 family mapping population of the cross W64a x A632 (50 SSR markers thus far). QTL in bins 6.04 and 7.02, are noteworthy for carotenoids because of the presence of known mutants. Bin 6.04 contains yellow1 (y1), which is associated with phytoene synthase, and bin 7.02 is near yellow8 (y8)/viviparous9 (vp9), which influence carotenoid levels. The y1 SSR was probed on the population and accounted for 3% to 10% of the total variation for individual carotenoid compounds. The QTL in bin 7.02 near y8/vp9 also accounts for a significant amount of variation with 7% to 18% for individual carotenoids. For total carotenoids, seven markers were included in the multiple regression model which accounted for 39% of the total variation, of this 30% of the total variation is accounted for by two markers, y1-SSR and marker phi034 (7.02). For combined tocopherols, the multiple regression model included five markers and accounted for 48% of the variation, with the two markers in bin 1.08 and 5.04 accounting for 41% of this variation. The QTL in these bins account for different levels of variation when looking at individual tocopherols. In bin 1.08 the QTL explains 5% to 22% of the total variation and the marker in bin 5.04 accounts for 4% to 18% of the total variation for the individual tocopherols . Work done on the carotenoid pathway has allowed for the cloning

of two more genes with effect on key regulatory enzymes. Zeta-carotene desaturase (ZDS), which maps near 7.02, has been cloned along with phytoene desaturase (PDS) (both clones provided by E. Wurtzel) ,which is currently unmapped. Both of these genes are currently being probed on the population. The tocopherol pathway is not as well characterized, but a recent study has cloned the gene controlling the change from g-tocopherol to a-tocopherol. Using the genbank accession number an EST from the ZmDB maize genome database was found to have homology. This EST is being assayed on the population and the effect will be determined. We will continue to evaluate the effects of specific genes obtained from ZmDB regulating these biosynthetic pathways as relates to quantitative variation.

9:10 am Sunday

T37 Association tests of candidate genes regulating plant height and flowering time.

Thornsberry, Jeffry(1); Doebley, J(3); Goodman, MM(1); Buckler, ES(1,2) (1) North Carolina State University, Raleigh, NC 27695, USA; (2) USDA-ARS, Raleigh, NC 27695, USA; (3) University of Wisconsin, Madison, WI 53706, USA

Animal systems have taken advantage of association approaches to rapidly link nucleotide polymorphisms with traits. A similar approach was utilized to relate candidate gene sequence diversity with the quantitative traits of flowering time and plant height in maize. A putative transcriptional regulator of floral transition, indeterminate1 (id1), is thought to be a major player in controlling flowering time (Colasanti et al, 1998). The candidate gene dwarf8 (d8) is involved in the signaling pathway of gibberellin and influences plant height and maturity (Peng et al, 1999). Each candidate gene was PCR amplified and sequenced from 100 diverse maize lines. Flowering time and plant height for each line were scored in two environments. Estimates of population structure, derived from random markers throughout the genome, were used to eliminate non-functional associations. The effect of sequence polymorphisms on flowering time and plant height was tested with association tests. Understanding the effects of such nucleotide polymorphisms and their presence in the germplasm will improve selective breeding techniques for these quantitative traits.

9:30 am Sunday

T38 Insights from applying expression profiling to female development under stress.

Helentjaris, Tim (1); Habben, Jeff (1); Sun, Yuejin(1); Zinselmeier, Chris(1) (1) Pioneer Hi-Bred Int., Inc.

Genomic technologies are radically transforming many of our approaches to plant genetics and even many aspects of agricultural research. One of our newest tools is mRNA expression profiling, which represents a quantum increase in our capacity for measuring mRNA levels and what they infer about gene expression. Beyond just representing a more efficient alternative to Northern analysis, this technology provides researchers with an entirely new approach to dissecting what is happening within an organism by tracking its own gene responses. We have applied different variations of this approach to analyze changes in female development under the imposition of various abiotic stresses (limiting water, shade, high plant population) and have found this to be an invaluable tool for revealing how the ear and kernels react developmentally to stress. Collective gene expression patterns describe both common and distinctive elements of stresses X tissue responses that when combined with physiological analyses provide us with

unparalleled insights into plant stress response and tolerance. As the data from these individual analyses only become more powerful for gene discovery as they are collected with other similar analyses, a strong argument can be made for the maize research community to centrally organize these efforts via development of common templates and collation of the resulting data.

9:50 am Sunday

T39 Centromeres, Telomeres, and Meiotic Chromosome Pairing

Carlton, Peter(1); Ananiev, Evgueni(2); Friebe, Bernd(3); Gill, Bikram(3); Cande, W. Zacheus(1) (1) UC Berkeley, Berkeley, CA 94720; (2) University of Minnesota, St. Paul, MN 55108; (3) Kansas State University, Manhattan, KS 66505

The pairing of homologous chromosomes in meiotic prophase is a complex phenomenon involving large-scale chromosome reorganization. In many species, centromeres and telomeres display conspicuous behavior at or prior to the prophase substage of zygotene, when chromosomes approach each other and synapse. Telomere clusters, or bouquets, have been observed in members of each crown eukaryote kingdom, and centromere clustering or centromere-telomere polarization (the *Rabl configuration*) has been seen in meiosis of mice and many grasses. This conserved behavior indicates centromeres and telomeres may be playing roles in bringing homologs together.

This study used three-dimensional **f**luorescence **i**n **s**itu **h**ybridization (3d-FISH) to analyze centromere and telomere behavior in maize male meiocytes. The polarization of chromosomes was measured throughout all the stages of meiotic prophase by comparing centromere to telomere positions. A large proportion of leptotene nuclei were found to be semipolarized, with centromeres in one half of the nucleus but with telomeres distributed nearly randomly throughout the nuclear volume. This may indicate that meiotic nuclei never reach a state of complete randomness but retain some degree of order, which later chromosome reorganizations can build upon.

In addition, to further explore the role of telomeres in homologous pairing, we are studying lines of hexaploid wheat to which have been added pairs of rye chromosomes with or without terminal deletions. Previous studies showed that heterozygous, but not homozygous, terminal deletions caused marked reductions in meiotic pairing. 3d-FISH studies are being carried out to identify at what point in meiosis the failure occurs.

7:15 pm Friday, 17 March

WORKSHOP: Comparative genomics

W Comparative genetics in the Solanaceae

Jahn, Molly(1); Livingstone, Kevin(1); Grube, Rebecca(1); Paran, Ilan(1,2); Thorup, Troy(1) (1) Cornell University, Ithaca, NY 15853; (2) Volcani Institute, Bet-Dagan, Israel

We have recently published a detailed comparative genetic map that ties together three major crop genera in the Solanaceae: *Lycopersicon, Solanum,* and *Capsicum* (Livingstone et al, Genetics 152:1183-1202). From this map, we have drawn inferences about the types and numbers of karyotypic changes that occurred during the divergence of these lineages. Using this map, we have analyzed two major categories of phenotypically defined traits, disease resistance and fruit-related characters. The Solanaceae affords an unparalleled opportunity to analyze the comparative genetics of disease resistance because a wide array of pathogen species affect all three genera, nearly one hundred major resistance (R) genes or QTL have been mapped, and a number of R genes have been cloned. Our analysis reveals that genomic positions of R genes appear to be conserved, however, it is rare that the specificity of R genes occurring at corresponding locations has been preserved. The implications of this observation for the evolution of resistance gene structure and function and pathogen virulence will be discussed. We have also used this map to examine the extent of functional conservation of homologous loci that control fruit color and shape across the family. We observe correspondences between a number of fruit morphology traits, and have demonstrated using structural genes from the carotenoid pathway that candidate genes mapped in one genus may be useful in linking genetic variability across a plant family.

Gernot Presting, Clemson University

NO ABSTRACT

Andy Kleinnofs, Washington State University

NO ABSTRACT

4 pm Saturday, 18 March

WORKSHOP:Maize Genomic and Internet Resources

Functional genomics of chromatin genes Vicki Chandler, University of Arizona

NO ABSTRACT

Maize gene discovery project Virginia Walbot, Stanford University

NO ABSTRACT

Genomic sequence comparisons between maize BACs and orthologous regions of barley, rice, sorghum and wheat Jeff Bennetzen, Purdue University

NO ABSTRACT

W Comprehensive Genetic, Physical, and Database Resources

Coe, Ed(1,2); Cone, Karen(1); Gardiner, Jack(1) (1) USDA-ARS; (2) University of Missouri-Columbia, Columbia, Missouri 65211, USA

Comprehensive Genetic, Physical, and Database Resources Integrated genetic and physical maps; resources and tools for mapping; data management, analysis, and compilation; and effective dissemination of genetic and genomic information to users are targets of the Missouri Maize Project (MMP). RFLPs and new SSR markers are placed on the high-resolution Intermated B73/Mo17 Recombinant Inbred panel (IBM). Mapped markers are being used to anchor BACs in a 5X HindIII library produced at Clemson, and will soon be applied to a 5X EcoRI and a 5X MboI library. Goals are 1,000 RFLPs and 2,000 SSRs from maize mapped and anchored, plus 1,000 RFLPs from sorghum. A summary presentation of information on all publicly available SSR markers is in the Maize Genome Database (MaizeDB) at http://www.agron.missouri.edu/ssr.html. Many of the RFLP and SSR loci are based on cDNA clones. Anchoring of ESTs by the overgo method is planned. Fingerprinting of the BAC libraries is being initiated. The progress and relative efficacy of anchoring, fingerprinting, and other assembling methods will be assessed in stages. Progress is being made toward construction of a panel of Radiation Hybrids of maize genomes in CHO cells. Procedures have been developed for high-throughput mapping of mutants for visible traits, and mapping is progressing. Characterization and systematization of mutant and trait descriptions is under development toward enhancement in parallel with mapping and with developing programs in structural and functional genomics. Within MMP data undergo self-verifying entries at the LabDB level. An Object Protocol Management utility, which has a desirable combination of capabilities including table-making on demand and interoperability among databases between species, is in development and has reached testing stage. Comparative map viewing is in development. The data from MMP and other projects and publications are ported into MaizeDB and are disseminated in tables, front-ends, and search utilities from MaizeDB, where they are integrated and linked with all publicly available data at http://www.agron.missouri.edu/. The MaizeDB encyclopedia indexes and cross-indexes access to knowledge and understanding about genetic differences; processes of genetic change; gene function and expression; growth and development; physiology; and pests and stresses.

Functional genomics of maize centromeres Kelly Dawe, University of Georgia

NO ABSTRACT

Center for maize targeted mutagenesis Rob Marteinssen, Cold Spring Harbor Laboratory

NO ABSTRACT

An integrated map of cytological, genetic and physical information of maize Lisa Harper, University of California-Berkeley

NO ABSTRACT

Radiation hybrid and cloning system for the genetic and physical mapping of the corn genome Ron Phillips, University of Minnesota

NO ABSTRACT

W The International Rice Genome Sequencing Project

Messing, Joachim (1) (1) Rutgers University, Piscataway, New Jersey 08854-8020, USA

A position paper has been developed for sequencing the entire rice genome with international cooperation (www.staff.or.jp). The Plant Genome Initiative at Rutgers (PGIR) has initiated a US collaboration to sequence rice chromosome 10 in the next few years. Chromosome 10 has been selected because it is with 87.3 cM the smallest chromosome of rice and could be completed sooner than the other ones. A minimal tiling path protocol based on the end sequences of a BAC library has been tested. Contiguous sequence information has been used to analyze gene density, repeat element distribution, characteristics of recombination and nucleotide preferences. Although rice appears to be rich in repeat elements, its gene density appears to be very high and not much lower than Arabidopsis.

W Applied genomics: strategies for efficient molecular analysis of complex genetic systems.

McCarty, Donald R.(1); Settles, A. Mark(1); Tan, Bao Cai(1); Suzuki, Masaharu(1) (1) University of Florida

The emerging genomics resources enable new strategies for molecular analysis of complex genetic systems such as seed development. Seed mutants are very numerous and typically pleiotropic making it difficult to distinguish regulatory genes in the pathways of interest from so-called "housekeeping genes". By bringing sequence information into play early in the analysis of these mutants we will greatly facilitate experimental analysis of these genes. Toward this end, we have focused on developing methods for efficient molecular analysis of transposon-tagged mutants. We show that TAIL-PCR enables efficient extraction of germinal Mu-insertion sites from an individual genome or pool of genomes. We have explored three strategies based on this technology. 1) Sequencing of cloned Mu-TAIL products can be used to identify random gene knockouts. In a pilot study, we have sequenced 1700 Mu insertion sites from our Uniform-Mu population representing 350 unique insertion sites. 30% of the sequences give significant BLAST hits in genbank and/or EST databases. Hence, with minimum resources we have identified 100 putative gene knockouts. Most of the knockouts are in previously undescribed maize genes. The spectrum of genes is in line with the composition of the arabidopsis genome suggesting that sampling by Mu-TAIL is not biased in this respect. We estimate that with the existing Mu-TAIL methodology and Mu populations a cooperative sequencing effort could establish a comprehensive set of gene knockouts for maize in less than 3 years. 2) Mu-TAIL amplicons may be used to identify insertional mutations in genes represented in arrayed cDNA libraries. A deep cDNA library can be used as a filter to select insertions that occur within genes expressed in a particular tissue. By coupling this approach with a conventional mutant hunt, we propose to use the endosperm EST collection to analyze large numbers of endosperm mutations. 3) In a pedigreed tagging population, subtractive hybridization and representational difference analysis are used to identify new transposition events that are candidates for the insertions linked to specific mutations. This allows focused analysis of tagged genes that have rare transcripts. Together these approaches offer a comprehensive strategy for high-throughput transposon mutagenesis that may be targeted to any process or set of genes.

Posters -- Biochemical Genetics

P1 Preliminary characterization of the geranylgeranylpyrophosphate synthase (GGPPS) gene family of maize.

Cervantes-Cervantes, Miguel(1,2); Ratnayaka, Swarnamala(1); Wurtzel, Eleanore T.(1) (1) Lehman College and The Graduate School, The City University of New York; (2) Hostos Community College, The City University of New York

GGPPS is a member of the polyprenoid synthases, enzymes responsible for the production of the precursors in the biosynthesis of carotenoids and many other essential plant metabolites, including gibberellic acid, phytoalexins, and the chlorophyll phytol chain. Because of this metabolic diversity, several isoforms of the enzyme might be expected, namely, in the cytosol, the mitochondria and the plastids. Using a functional complementation technique, we isolated six clones encoding GGPPS out of 30,000 colonies of Escherichia coli doubly transformed with a cDNA library from maize B73 endosperm (14 DAP) and the plasmid pACCRT25-delta-crtE, which contains the gene cluster for carotenoid biosynthesis from Erwinia uredovora minus the gene encoding GGPPS. All six clones complemented the delta-crtE mutation, but were found to differ in size; the four larger clones seem to contain an untranslated region. With the purpose of establishing the gene copy number for the GGPPS gene in this variety of maize, one of the larger clones was used as a probe in Southern analysis and for screening of a maize B73 BAC library (5.6X genomic size). DNA from positive BAC clones was analyzed by Southern blotting. We present our results in the determination of the gene number for GGPPS in maize B73.

P2 Metabolic analyses of a double mutant of sucrose synthase (SuSy) genes in developing endosperm.

Scott, Paul(1); Chourey, Prem(2) (1) USDA-ARS, Iowa State University, Ames, Iowa 50011, USA; (2) USDA-ARS, University of Florida, Gainesville, Florida 32611, USA

The enzyme SuSy plays a major role in energy metabolism by catalyzing the reversible conversion of sucrose and UDP to UDP-Glc and fructose. Both biochemical and genetic data suggest that in vivo role of the SuSy reaction is the production of UDP-Glc, a precursor and a substrate for starch and cellulose biosynthesis, respectively. In maize, Sh1 and Sus1, loci encode two isozymes of this enzyme. Developing endosperm, 21 DAP, of the double mutant, sh1sus1-1, exhibits ~ 0.5% of the total activity as compared to the Sh1Sus1 genotype (Chourey et al., 1998, MGG 259:88-96). This mutant provides a unique opportunity to study carbon assimilation in developing maize endosperms. To do this, we quantified the levels of several key metabolites in the sh1sus1-1 endosperm, at various developmental stages, relative to Sh1Sus1, Sh1sus1-1, and sh1Sus1 genotypes. The double mutant was found to have reduced levels of UDP-Glc at all

developmental stages, but the reduction was not proportional to the reduction in sucrose synthase activity. Interestingly, we observed that the level of ATP in the double mutant showed a greater reduction than the level of UDP-Glc when comparing the double mutant to other genotypes. These results suggest that in the absence of sucrose synthase activity, an alternative pathway can be used to produce UDP-Glc, but this alternative pathway requires more energy that the standard sucrose synthase pathway.

P3 Analysis of a double mutant of sucrose synthase (SuSy) genes that shows evidence of a third SuSy gene.

Carlson, Susan (1); Helentjaris, Tim(2); Chourey, Prem(1) (1) USDA ARS & University of Florida, Gainesville, Florida 32611-0680, USA; (2) Pioneer Hi-Bred International, Inc. Johnston, Iowa 50131-1004, USA

Previous genetic analyses indicate that there are only two sucrose synthase genes, Sh1 and Sus1 on chromosome 9, in maize (Chourey, 1981, MGG 184:372; McCarty et al., 1986, PNAS 83:9099). In a recent study of a double mutant, sh1sus1-1, (Chourey et al., 1998, MGG 259:88), we have shown that the mutant sus1-1 allele encodes greatly reduced levels of a truncated sus1 transcript that may not lead to an active enzyme. However, the sh1sus1-1 endosperm exhibits ~ 0.5% of the total enzyme activity and greatly reduced levels of a normal sized SuSy protein as compared to the Sh1Sus1 endosperm. The results described here using a maize SuSy clone, not reported thus far and mapped to bin 1.04, suggest that there is indeed a third expressed gene, Sus2. In particular, Northern blot analyses, using the Sus2 clone as a probe on RNAs from sh1sus1-1 kernels, show a transcript similar in size to the Sh1 or the Sus1 RNA, albeit in greatly reduced levels relative to the either gene product. The Sus2 RNA is also seen in Sh1Sus1 and sh1Sus1 endosperm and embryo at nearly the same levels as in the sh1sus1-1 genotype. Comparative gene expression analyses for several genes of sucrose metabolism in the sh1sus1-1 endosperm show little or undetectable changes, except cell wall invertase2 (Incw2) that shows reduced levels of RNA. Specific activity of the cell wall invertase is ~2-fold less in the mutant than the wild type. The physiological significance of these data will be discussed.

P4 ADP-Glucose Pyrophosphorylase Activity from Maize-Potato Hybrids

Cross, Joanna MF(1); Greene, Thomas W(1); Shaw, Janine R(1); Clancy, Maureen (1); Okita, Thomas W (1); Schmidt, Robert R(1); Hannah, L. Curtis(1) (1) University of Florida, Gainesville, Florida 32611, USA

The allosteric enzyme, adenosine diphosphate glucose pyrophosphorylase (AGP) catalyses the synthesis of ADP-glucose and represents a rate limiting step in starch synthesis. The maize endosperm AGP is a heterotetramer composed of two small subunits encoded by brittle2 (Bt2) and two large subunits encoded by shrunken2 (Sh2). An E.coli expression system was constructed with Bt2 and Sh2 on two different and compatible vectors. The resulting E.coli-expressed maize endosperm AGP was partially purified and shown to have virtually identical properties as the native endosperm-expressed AGP. Hence the E.coli-expressed system can be used for further studies of the allosteric properties of AGP. Accordingly, hybrid AGPs composed of potato tuber and maize endosperm subunits were expressed in E.coli. While these proteins have long been separated by speciation and evolution, the potato and maize subunits were sufficiently similar to form active enzymes. Because the native maize endosperm and potato tuber AGPs have distinctly different allosteric properties, the E.coli expression system allowed us to ask if one subunit acted as the allosteric subunit while the other served as the catalytic subunit. The allosteric and kinetic properties of the resulting hybrid AGPs differed from those of both potato and maize AGP. These results show that each of the two subunits is important in determining both catalytic and allosteric parameters of plant AGPs.

P5 Biochemical and molecular characterization of the terpene synthase gene family in *Zea mays*

Degenhardt, Jörg(1); Köllner, Tobias(1); Schnee, Christiane(1); Gershenzon, Jonathan(1) (1) Max Planck Institute for Chemical Ecology, 07745 Jena, Germany

Herbivore attack induces the emission of volatile organic compounds from maize foliage. Since maize volatiles have been shown to attract herbivore enemies, it has been suggested that volatile release represents an indirect defense mechanism to protect the plant from further damage. When maize is attacked by armyworm larvae (*Spodoptera sp.*), the mixture of induced volatiles is dominated by monoterpenes and sesquiterpenes. Such terpenes are released not only by the attacked leaf but also systemically, by undamaged leaves of the same plant. In order to understand the function of volatile emission in maize and its regulation by herbivore damage, we are investigating the molecular genetics and biochemistry of volatile formation in this species. A key step in terpene biosynthesis is the conversion of acyclic prenyl diphosphates to the parent carbon skeletons of this class by terpene synthases. Biochemical studies have demonstrated a terpene synthase activity, nerolidol synthase, in cell-free extracts of herbivore-damaged maize that is closely correlated with the emission of a major volatile nerolidol metabolite. We are currently isolating genes for nerolidol synthase and other terpene synthases by homology-based PCR and screening of ESTs. These genes form a rather heterogeneous family that shows only a low homology to the terpene synthases of dicotyledonous plants and gymnosperms. Since sequence comparisons do not allow the deduction of the biochemical function of these genes, they are being expressed heterologously in *E. coli*. In future work, isolated genes will be employed to create transformants with altered volatile emission profiles that can be tested under natural condition to determine the ecological function of maize volatiles.

P6 Characterisation of zmKCS, a ß-Ketoacyl-CoA-Synthase from Maize possibly involved in Wax Biosynthesis

Frenzel, Karsten(1); Janke, Sabine-Annette(2); Brettschneider, Reinhold(1); da Costa e Silva, Oswaldo(3); Wienand, Udo(1) (1) Institute for General Botany, AMPI, Ohnhorststr. 18, 22609 Hamburg, Germany; (2) Eppendorf-Netheler-Hinz GmbH, Barkhausenweg 1, 22339 Hamburg, Germany; (3) BASF AG, ZHP/T, Building A30, 67056 Ludwigshafen, Germany

The epicuticular wax layer on plant leaves is a heterogeneous mixture of long carbohydrates synthesized in different biosynthetic pathways. To some extent components are derived from the fatty acid elongation pathway. Fatty acids are elongated by a fatty acid elongation complex of four enzymes which successively add two carbon units to fatty acids. The ß-Ketoacyl-CoA-Synthase(ß-KCS) is a condensing enzyme that plays a key role in the fatty acid elongation complex. Two different cDNAs zmKCS-1 and zmKCS-2 from maize with high homology to the Cut 1 gene from A. thaliana (Millar et al.1999) and various other plant ß-KCSs were isolated from a cDNA-library of germinating kernels and a cDNA-library of young seedlings. The two cDNAs show a high sequence similarity in the coding region but are differently expressed in the endosperm of germinating kernels, in young seedlings and in tassels. There is no expression in adult leaves and developing kernels. Yeast overexpression of zmKCS-1 did not lead to a change in long chain fatty acid. This observation and the fact of high homology to the wax biosynthesis related Cut 1 gene as well as the expression pattern suggests that the gene function of zmKCS is that of a ß-Ketoacyl-CoA-Synthase involved in biosynthesis of wax precursors. Antisense experiments in maize did result in small changes in leaf wax composition of young seedlings. The precise gene function, however, remains unclear and

has to be further examined. Reference: Millar, A. M., et al. (1999), The Plant Cell, Vol. 11, S. 825 – 838,

P7 Characterization of the camouflage 1 mutant of maize

Janick-Buckner, Diane(1); Cox, Justin (1); Mayberry, Laura(1); Buckner, Brent(1) (1) Truman State University, Kirksville, MO 63501 USA

Mutants of the camouflage 1 (cf1) locus of maize exhibit a zebra banding pattern of yellow-green and necrotic tissue on the leaf. Previous light and electron microscopy studies revealed that bundle sheath cells within the yellow-green tissue die via what appears to be a programmed cell death event. Mesophyll cells in this tissue appear stressed, but do not die. In this study, we have further characterized the events taking place in the yellow-green tissue. Nuclei were isolated from leaves of wild-type and cf1 sibling seedlings and subjected to a cell death ELISA (Boehringer Mannheim), which detects DNA fragmentation through the use of antibodies against DNA and histone proteins. Significantly more DNA fragmentation was found in the cf1 plants, further suggesting that cell death in the yellow-green sectors of cf1 plants may be a programmed event. Immunoblot analysis was carried out on proteins extracted from leaves of cf1 and wild-type plants using antibodies against phosphoenolpyruvate carboxylase (a mesophyll cell specific enzyme) and ribulose bisphosphate carboxylase (a bundle sheath cell specific enzyme). Both of these enzymes were reduced in the yellow-green sectors of cf1 leaves as compared to wild-type sectors of cf1 plants and wild-type plants, however, ribulose bisphosphate carboxylase levels were more drastically altered. Consistent with this we find that levels of carotenoids and chlorophylls are reduced in yellow-green sectors of cf1 leaves. Finally, we have determined that a light/dark cycle is required to initiate cell death, however, it is not responsible for establishing the zebra banding pattern.

P8 Differential response of pathogenic and non-pathogenic fungi to maize ribosome-inactivating protein

Nielsen, Kirsten(1); Payne, Gary A.(1); Boston, Rebecca S.(1) (1) NC State University, Raleigh, NC 27695-7612, USA

See Talk Abstract T10

11:20 am Friday 17 March

P10 Regulation of sugar production in cereal seedlings

Thomas, Bruce R(1); Rodriguez, Raymond L(1); Brandley, Brian K(2) (1) University of California, Davis, CA 95616-8535, USA; (2) Glyko Inc., 11 Pimentel Ct, Novato, CA 94949, USA

Cloning and characterization of the alpha-amylase genes of rice has led to breakthroughs in our understanding of cereal seedling development. Sequence analysis demonstrated that the genes could be classified into three subfamilies. Patterns of gene expression and isozyme function clearly demonstrate that the Amy3 subfamily has unique physiological roles that are distinct from those of the genes in the Amy1 and Amy2 subfamilies. The Metabolite Signal Hypothesis describes how sugar concentrations in the seedling effect changes in gene expression, thereby helping to regulate the growth and development of the seedling. Fluorescence Assisted Carbohydrate Electrophoresis (FACE) methods provide a sensitive assay for sugars and oligosaccharides. This will lead to improved assay methods for alpha-amylase enzyme activity in vitro and for production of sugar in seedlings. These methods will facilitate testing of the Metabolite Signal Hypothesis, particularly for testing whether changes in alpha-amylase gene regulation have the predicted effects on sugar production in the developing seedlings.

P11 Cloning of maize yellow stripe1 (ys1), an iron-regulated gene involved in high affinity Fe(III) uptake.

Panaviene, Zivile(1); Curie, Catherine(2); Loulergue, Clarisse(2); Dellaporta, Stephen L.(3); Briat, Jean-Francois(2); Walker, Elsbeth L.(1) (1) University of Massachusetts, Amherst, Amherst, MA 01003, USA; (2) Université Montpellier 2 et École Nationale Supérieure d'Agronomie, Place Viala, F-34060 MONTPELLIER cedex 1 (France); (3) Yale University, New Haven, CT 06520

See Talk Abstract T6

9:30 am Fri 17 March

P12 Functional Genomics of Hemicellulose Biosynthesis

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Cell walls are a defining feature of plants, yet little is known about the biosynthesis of their constituent polysaccharides. Compared to cellulose, the hemicelluloses are more varied and dynamic; there are several kinds, they change during development, and some show high turnover rates. Also in constrast to cellulose, all hemicelluloses except callose are synthesized in the Golgi apparatus. Dicots (Arabidopsis) and cereal monocots (maize) have distinct types of hemicelluloses; e.g., the primary walls of dicots contain xyloglucan and pectin, whereas cereals contain mixed-linked glucan, arabinoxylan, and only a small amount of pectin. We are taking a genomics approach to understand the biochemistry, and cell biology of maize hemicelluloses. Using public and private EST databases, we have identified genes encoding putative wall polysaccharide polymerases based on their similarities to the cellulose synthase (CesA) and cellulose-synthase like (Csl) genes of Arabidopsis. A preliminary assumption is that hemicellulose synthases will be Csl's, that is, similar to cellulose synthases yet clearly distinct. Based on the complexity of the Csl family in Arabidopsis, we predict that maize will have about distinct 30 Csl genes. Once candidate genes have been identified, they will be analyzed by sequencing, for their patterns of expression in different maize tissues, and for regulation by environmental factors known to influence wall composition. These studies will utilize subtracted and normalized cDNA libraries and microarrays. The cellular locations of puative hemicellulose gene products will be determined by the construction and heterologous expression of GFP fusions. The functions of putative hemicellulose genes will be further analyzed by the construction of specific mutants, using methodologies developed in other maize genomics projects. Funded by the NSF Plant Genomics Initiative. We thank Khanwarpal Dhugga (Pioneer Hi-Bred Intl.), and Virginia Walbot and the Zea mays Genome Data Base (ZmDB; Iowa State University) for their invaluable assistance.

P13 QTL mapping of elongation factor 1-alpha (eEF1A) content and characterization of eEF1A genes in maize endosperm

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The nutritional value of cereal grains is most limited by their protein quality, with lysine being the most limiting essential amino acid. In maize, the lysine content of the kernel can be significantly increased by the opaque2 (o2) mutation, which reduces prolamin synthesis and increases production of proteins that contain lysine. We recently showed that elongation factor 1A (eEF1A) is one of the lysine-containing proteins that is increased in o2 mutants; furthermore, the concentration of eEF1A is highly correlated (R2= 0.9) with the lysine content in the endosperm (Habben et al., 1995). To understand the biological basis of this finding, QTL mapping of loci influencing eEF1A content was conducted. Two crosses between inbred lines with different eEF1A content were made, and analysis of their F2 progenies showed that eEF1A content is genetically regulated. A linkage map based on 83 polymorphic SSR markers was created for OH51Ao2 and OH545o2 and two QTLs were identified. Interestingly, one QTL on the short arm of chromosome 4 is tightly linked with an a-zein gene cluster and measurement of a-zein protein suggested that the different content of this storage protein maybe underlie the variation in eEF1A level. The other QTL influencing eEF1A content is located on the long arm of chromosome 7. eEF1A is encoded by multiple genes

in maize and four of them are expressed in endosperm tissue. Two genes account for 80% of eEF1A mRNA transcripts. We have identified at least three forms of eEF1A protein in immature maize endosperm, and the relationship between these proteins and the mRNA encoding them is being investigated.

P14 Biochemical and reverse genetic analysis of the maize starch debranching enzyme ZPU1

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The structural organization of granular starch in maize kernels is determined by the coordinated activities of starch synthases, branching enzymes, and debranching enzymes (DBEs), although the specific roles of DBEs are not yet clear. Two distinct DBEs have been identified in maize. The sugary1 (su1) gene codes for one, an isoamylase-type DBE that also affects the activity of the other, a pullulanase-type DBE. Previously, our laboratory cloned a cDNA that codes for a maize pullulanase-type DBE, designated Zpu1 (for Zea mays pullulanase). Both ZPU1 and SU1 activities were shown to be deficient as a result of the su1 mutation (Beatty et al., 1999, Plant Physiol 119:255). Using the Pioneer TUSC system, four independent, heritable Mu insertion alleles of zpu1 have been identified. Mapping and sequence analyses indicate that three alleles have Mu elements inserted in the first intron of the gene, and the fourth has a Mu insertion in the first exon, downstream of the translation start site. Homozygous zpu1 kernels do not exhibit an obvious visual phenotype, although chemical analyses are underway to determine whether the mutation causes alterations in carbohydrate structure. To address the specific role of the pullulanase-type DBE in starch assembly, the biochemical activity of ZPU1 was characterized. Near full-length ZPU1 was expressed in E. coli and purified to apparent homogeneity. Determination of the specific activity of recombinant ZPU1 (ZPU1r) toward various substrates confirmed that it functions as a pullulanase-type enzyme to specifically hydrolyze a-(1Æ6) linkages in branched glucans. The pH dependence, reaction temperature, and thermal stability of ZPU1r were determined. Analysis of the kinetic properties of the enzyme revealed that ZPU1r exhibits a higher Vmax toward the b-limit dextrin of amylopectin, but has a higher affinity for pullulan, an ordered isomaltotriose polymer. These findings indicate that ZPU1r differs from SU1r with regard to substrate affinity, and suggest that the two DBEs hydrolyze differently branched substrates during starch synthesis in the endosperm.

P15 A heterologous system to identify strategic genes for metabolic engineering of the maize carotenoid biosynthetic pathway.

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Carotenoids, derived from plant food sources, are converted in humans to vitamin A and other important compounds needed for growth and development. Endosperms of food crops, such as maize and wheat, are low in carotenoid content, and are especially low in provitamin A as compared with nonprovitamin A carotenoids. In an effort to metabolically engineer this pathway in maize endosperm, we are screening for genes that either promote or interfere with carotenoid accumulation. We are using a heterologous system in which carotenoid genes from the bacterium Erwinia uredovora are introduced into Escherichia coli cells that are subsequently transformed with either test genes or with a maize cDNA expression library. We will report on the identity of some genes isolated and/or tested using this approach. Based on our results, we will discuss the possible impact of simultaneously manipulating the starch and carotenoid biosynthetic pathways.

P16 Highly polymorphic zeins of maize represent a useful source of genetic markers.
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Using high resolution system of acidic continuous polyacrylamide gel electrophoresis (acidic PAGE) extraordinary high polymorphism of zeins of maize was revealed (Zayakina & Sozinov, 1993). In this note we summarize the results of genetic analysis of zeins. It has been shown, that aside from the common feature of the genetics of zeins and other known the prolamins (occurence of multigenic multiallelic loci) there are essential differences. Besides three multigenic loci encoding the most zein polypeptides (Zep1, Zep2 and Zep3) there are many zeincoding loci that encode one or two minor components in some inbreds and that are inactive (have null alleles) in other inbreds (Zayakina & Sozinov, 1997). Sixteen hybrid combinations involving 19 inbred lines have been analyzed. 12 and 14 alleles of the Zep1 and the Zep2 loci, correspondingly, have been identified for 19 inbreds. Six alleles of the Zep3 locus were identified in five crosses. These data suggest that zeins are the most polymorphic biochemical markers available in maize and they are more polymorphic in comparison with other prolamins. Extraordinary high variability of zeins is very useful for identification and registration of breeding materials, and for breeders' rights protection for maize. The linkage test have resulted in localization of group of loci Zep5-Zep2-Zep4-Zep3 on the short arm of the chromosome 4 . The application of definite alleles allows the using of the zeincoding loci as markers of certain chromosome regions. A complit presentation of the cotalogue of alleles is in press now.

P17 Analysis of desynaptic 2 supports an involvement of the Rad51 recombination protein in homologous chromosome synapsis

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The identification and synapsis of homologous chromosomes is a key step in meiosis and is a prerequisite for meiotic recombination. How homology is identified between partner chromosomes remains one of the major unresolved issues within the meiosis field. We have performed light, fluorescence, and electron microscopy to characterize chromosome pairing, synapsis, and recombination in the maize meiotic mutant desynaptic2. Analysis of chromosomes at diakinesis/metaphase I revealed that most are present as univalents. However, the presence of three rod bivalents per nucleus, on average, indicates that dsy2 is not recombination deficient. The ten-fold reduction in recombination is due to a global failure in homologous chromosome synapsis. For example, the 5 S rDNA locus on 2 L is only paired in 25% mutant nuclei as determined by fluorescent in situ hybridization. Electron microscopy of silver-stained nuclear spreads revealed improper chromosome synapsis. Previous work on normal maize (Franklin, et al., Plant Cell, 11: 809-824) has correlated homologous chromosome synapsis with the presence and distribution of structures composed of the Rad51 recombination protein. Immuno-fluorescence of Rad51 in mutant nuclei during the chromosome synapsis stage of zygotene revealed ususually long Rad51 structures. These structures were observed wrapping around single chromosomes or stretching between two widely separated chromosomes. Therefore, improper chromosome synapsis is associated with improper Rad51 structures. Based on our evaluation of Rad51 structures in dsy 2 and normal maize nuclei, we propose that the homology search is DNA-based and utilizes some of the same machinery involved in recombination. Furthermore, the normal dsy2 gene product is a trans-acting factor that directly, or indirectly, is required for the normal behavior of Rad51 complexes during zygotene.

P18 The pam1 gene: Bouquet Formation and Homologous Synapsis

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The pam1 maize meiotic mutant (the plural abnormality of meiosis 1) was isolated after chemical mutagenesis of dry seeds and was first characterized in 1977 (Golubovskaya, 1989). Here we present an electron microscopy and three dimensional fluorescent in situ hybridization (3-D FISH) analysis of pam1 mutant chromosomes in meiotic prophase I which show that the pam1 gene plays an important role in the chromosome's search for homologous partners. Neither male nor female pam1 mutant meiocytes proceed beyond pachytene; most remain blocked at that stage. TEM examination of SC spreads of pachytene chromosomes reveals four types of incorrect synapsis: i) exchange of synaptic partners resulting in promiscous nonhomologous synapsis; ii) occasional univalent chromosomes intrasynapsed only with itself; iii) partial or complete chromosomes remain unsynapsed and iv) unresolved interlocks. Telomere regions on SC spreads at zygotene are located close each other. The bouquet formation during zygotene in

the pam1 mutant is normal, as shown by 3-D FISH. The coexsistance of normal bouquet formation and severe nonhomologous synapsis in the pam1 mutant suggests that the pam1 gene control a pathway of homologue recognition that is independent from the bouquet formation.

P19 Towards an Integrated Map of Cytological and Genetic Information

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The goal of this NSF funded genome research is to generate a cytogenetic map of maize which will integrate information from existing cytological and genetic maps with new cytological and physical data. Our contribution to this new map will be to use three-dimensional fluorescent in situ hybridization (3-D FISH), deconvolution light microscopy and computerized image analysis to place genetically mapped genes onto the cytological map. We are basing the map on the maize pachytene chromosomes and integrating existing breakpoint and genetically mapped marker data collated by Ed Coe over a 30 year period To this map we are overlaying our data obtained by using repetitive and single copy DNAs as probes to pachytene chromosomes. We have successfully visualized the gene probes su1 (4s), p (1s), phyA (1l and 5s), 22 kDa zein (4s) on pachytene chromosomes. We are cytologically mapping these loci with reference to various repetitive probes including 5 S rDNA (2s), the rDNA intergenic spacer region (6s), knobs, centromeres and telomeres. We also present our working FISH knob karyotype of A344, our progress towards developing a cocktail of FISH probes to unambiguously identify each chromosome, and a comparison of detection methods. This map is a work in progress and will eventually provide information on the distribution of genes and repetitive elements, on the position of recombination events, and possibly shed light on the process of meiotic homologous pairing. It will also be useful for ordering large insert clones, for integration of the many genetic maps of maize, and for the genetic placement of markers that can not be mapped genetically. We welcome any contributions from the community; especially any data that will correlate cytological features with genetically mapped marker, and we would like to solicit large (10kb) genomic clones to use as gene probes.

P20 Phosphorylation of histone H3 is correlated with changes in sister chromatid cohesion during meiosis in maize

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Meiotic chromosome condensation is a unique process, characterized by dramatic changes in chromosome morphology that are required for the correct progression of pairing, synapsis, recombination and segregation of sister chromatids. We used an antibody that recognizes a ser 10 phosphoepitope on histone H3 to monitor H3 phosphorylation during meiosis in maize meiocytes. H 3 phosphorylation has been reported to be an excellent marker for chromosome condensation during mitotic prophase in animal cells. In this study, we find that on maize somatic chromosomes only pericentromeric regions are stained with little staining on the arms. In addition, meiotic chromosome condensation from leptotene through diplotene occurs in absence of H3 phosphorylation. The changes in H3 phosphorylation observed at different stages of meiosis correlate with changes in sister chromatid cohesion. Just before nuclear envelope breakdown histone H3 phosphorylation is seen first in the pericentromeric regions and then extends through the arms at metaphase I; at metaphase II only the pericentromeric regions are stained. In afd1 (absence of first division), a mutant that is defective in many aspects of meiosis including sister chromatid cohesion and has equational separation at metaphase I, staining is restricted to the pericentromeric regions, and at metaphase II there is no staining at all. In el (elongate), a mutant that shows incomplete chromosome condensation and uncoiled chromatids at diakinesis, the phosphorylation pattern is normal. We conclude that changes in the level of phosphorylation of ser10 in H3 correspond to changes in the cohesion of sister chromatids rather than the extent of

chromosome condensation at different stages of meiosis.

P21 Development and characterization of maize-Tripsacum F1 hybrid population segregating for apomixis

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Apomixis represents a natural asexual process that allows clonal reproduction through seed, resulting in immediate fixation of any desired genotype, including F1 hybrids. In a program aimed to map and clone genes controlling apomictic reproduction in Tripsacum, a N 500 maize-Tripsacum F1 hybrid population segregating for apomixis and non-apomixis (sexual) has been generated by crossing diploid "Supergold" popcorn with heterozygous tetraploid T. dactyloides. The mode of reproduction in the F1 individuals was evaluated by seed set and MMC callose examination. A 1:1 segregation ratio of apomictics (lack of callose expression) and non-apomictics (callose expression = presence of meiosis) was obtained by the MMC callose analysis. Average percent of seed set in the F1 plants without callose is 12% whereas that in the plants with callose is only 0.38%. As expected, the average percent of seed in the apomictic F1's is significantly higher than that in the sexual ones. However, seed set can be higher in individual F1 plants with callose than in F1 plants without callose. Therefore, seed set should not be used alone for classification of apomictics and non-apomictics in the maize-Tripsacum F1 hybrid population.

P22 FRETM as an optical technique to determine molecular interactions in maize kinetochore/centromere on a sub-optical scale

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Fluorescence Resonance Energy Transfer Microscopy (FRETM) is a combination of microscopic and mathematical techniques that can show interactions between molecules separated by 10-100 angstroms, a scale not possible using ordinary light microscopy alone. Using maize root tips sections, and a combination of immunolocalization and fluorescence in situ hybridization (FISH), we studied the kinetochore/centromere complex using FRETM. Image sets generated by the microscope were analyzed for grey value content and a spreadsheet model based on ratios between values provided a quantifiable value of interaction. We have shown that CENP-C and a maize satellite DNA repeat (CentC) are interacting within a 50 angstrom separation limit. Combinations of antibodies to tubulin and CENP-C, and tubulin and centc DNA showed negative interaction, while combinations involving CENP-C or tubulin alone showed

positive interaction. We plan to use FRETM and antibodies to several kinetochore proteins as a way to interpret the three-dimensional organization of the maie kinetochore complex.

P23 Wheat, a model or commodity-chromosome pairing and polyploidy
Moore, Graham. John Innes Centre, UK

See Plenary Talk Abstract # T33

7:30 pm Saturday 18 March

P24 Towards an oat-maize radiation hybrid panel
Okagaki, Ron J.(1); Kynast, Ralf G.(1); Odland, Wade E.(1); Russell, Charles D.(1); Livingston, Suzanne M.(1); Rines, Howard W.(2); Phillips, Ronald L.(1) (1) University of Minnesota, St. Paul, MN 55108, USA; (2) USDA-ARS and University of Minnesota, St. Paul, MN 55108, USA

A complete series of ten maize chromosome addition lines in oats has been produced by crossing Zea mays cv. Seneca 60 to seven different Avena sativa lines. Among the crosses the germination frequency of in vitro rescued F1 embryos varied between 0 and 12 %. From more than 25,000 crosses, a total of 63 newly recovered F1 plantlets retained maize chromosomes; 37 with one of the ten maize chromosomes and 26 with multiple maize chromosome additions. The added maize chromosomes have been identified by genomic FISH and by PCR using chromosome arm-specific markers. Doubled haploid F2 offspring with one homologous maize chromosome pair ($2n = 6x + 2 = 44$) are being produced and characterized morphologically and by molecular and cytogenetic means. Seed and genomic DNA are available for chromosome addition lines 2, 3, 4, 6, 7, and 9. We used the series of addition lines to allocate new markers to chromosome. The use of these addition lines as a source for creating radiation hybrid lines further demonstrates their value as a maize genomics tool. Radiation hybrid lines have been generated by irradiating monosomic addition seed for chromosome 2, 4, and 9 with g-rays. Plants have been grown and selfed offspring harvested from each individual panicle. As a model, 38 offspring lines of chromosome 9 radiation hybrids have been selected to define the first radiation hybrid panel. PCR primers for 70 SSR, EST, and STS markers have been used to define the radiation hybrid lines by presence vs. absence of the markers. This radiation hybrid panel includes 17 lines with small deletions, 11 lines with translocations into the oat background, 2 lines where a chromosome arm has been lost, and 8 lines with more complex chromosome rearrangements. To date, 56 markers define over 40 physical segments of chromosome 9. This

work is supported by the National Science Foundation under Grant No. 9872650.

P25 Evidence For and Evolutionary Relationship Between Chromosome 4 and the B Chromosome, Based on Related Centromere Repeats

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Many plant centromeric sequences have been reported in recent years. There have been few if any plant centromeric sequences reported that are specific to one chromosome pair. We have isolated a repeat from chromosome 4 that has been cytologically mapped to the centromere and shares homology to the B chromosome centromere repeat and the knob repeat. The repeat was selected by using the B specific centromere repeat at reduced stringency to a library prepared from maize DNA without B chromosomes. We cytologically mapped this repeat to chromosome 4 using trisomic maize lines. Trisomic 4 revealed 3 centromeric signals, indicating that the repeat is specific to chromosome 4. Analysis of BAC clones selected with this repeat show that both Cent A and Cent C, two previously reported maize A centromere repeats, are present in some of the BACs along with the chromosome 4 specific repeat. This finding is consistent with our cytology and suggests that this repeat is centromeric in location.

P26 Comparison of the genetic map to the physical map of molecular markers related to QTLs for resistance against southwestern corn borer (Diatraea grandiosella D.) on pachytene chromosomes using in situ hybridization in maize (Zea mays L.)

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Resistance against southwestern cornborer (SWCB) was mapped as quantitative trait loci (q-swcb) on chromosome 9S and 9L in maize by molecular markers. The data did not reveal the physical positions of these markers and associated genes which will be essential for isolating genes by map-based cloning or microtechniques. Unique DNA sequences were used to localize markers and/or genes on chromosome 9 by fluorescent in situ hybridization (FISH). Pachytene chromosomes were analyzed to achieve a higher resolution. On chromosome 9, the locations of the centromere and the knob were determined with specific DNA probes as references. Molecular markers for SWCB resistance (umc105a, csu145a) were used to isolate homologous probes from a genomic cosmid library. After suppressing repetitive DNA sequences by chromosome in situ suppression hybridization (CISS), two fluorescent dyes and four probes were used to physically position SWCB resistance. These positions were correlated to the genetic map of chromosome 9. Genetic analysis positioned the two markers for SWCB resistance in a central interval of 50cM representing approximately 33% of the genetic length of chromosome 9. However, the physical distance between these probes was determined to encompass about 70% of its physical length. Comparison between

the genetic and physical map in pachytene chromosomes could elucidate mechanisms of recombination and rearrangement. An integrated physical and genetic map of individual chromosomes will be established.

P27 Effect of Abnormal Chromosome 10 on the Frequency of Recombination in maize
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A better understanding of the control of genetic recombination would have important applications in both the academic and corporate sectors. In maize (Zea mays L.), abnormal chromosome 10, a variant of chromosome 10, has been shown to affect recombination frequencies in several regions of the maize genome. All previous studies on the effect of abnormal 10 on recombination have employed morphological genetic markers, and only a very limited portion of the maize genome has been analyzed. By utilizing molecular markers, it is possible to determine the effect of abnormal chromosome 10 on recombination throughout the genome. An inbred line of W22 with normal chromosome 10s was crossed with the inbred, Black Mexican with normal chromosome 10s, to produce F1s that had normal chromosome 10s. A near-isogenic line of W22 with abnormal chromosome 10s was crossed with the inbred, Black Mexican with normal chromosome 10s, to produce F1s that were heterozygous for abnormal chromosome 10. Both F1s were then selfed to produce F2 segregating populations. The segregation of 52 RFLP loci in 100 individuals of each population was used to estimate linkage maps. The map for the control (N10/N10) population was 1364.5 cM and the map for the experimental (K10/N10) population was 1934.5 cM. The map lengths for chromosomes 1-9 of the two populations were compared using a paired-t test, and were found to be significantly different at the 95% confidence level ($P<0.042$). Chromosome 10 was excluded because it was morphologically different in the two popualtions.

P28 Epigenetic inheritance of an aneuploid induced phenotype
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Maize plants heterozygous for translocation T5-6b can produce aneuploid progeny due to adjacent I disjunction. Dp-Df plants in the B73 genetic background display several aneuploid-induced abnormal phenotypes, including reduced height, delayed development and severe knotting of the leaves. The 6^5 translocation chromosome is female, but not male, transmissible. Interestingly, the phenotypes associated with Dp-Df plants are observed in progeny of Dp-Df plants with a normal chromosomal constitution in some cases. The phenotypic syndrome is observed to segregate 1:3 in some F2 families in which all individuals have a normal chromosomal constitution. The phenotypic syndrome reverts to a less severe or wild type

condition spontaneously when maintained in plants with a normal chromosomal complement. The observations that 1) abnormal phenotypes are seen in plants with normal chromosome complements and 2) the abnormal phenotype can revert, indicate that the aneuploid condition heritably but reversibly alters expression of one or more genes controlling plant development.

P29 Maize-chromosome 9 rearrangements in progenies of oat-maize chromosome 9 radiation hybrids

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The irradiation of monosomic maize-chromosome 9 addition line seed of oat followed by self-pollination of surviving plants has resulted in the production of plants possessing different pieces of maize-chromosome 9 (maize-chromosome 9 radiation hybrids, M9RHs). The M9RHs included plants with apparently normal maize-chromosome 9 as well as plants with various maize-chromosome 9 rearrangements (intergenomic translocations, deletions, and a combination of both). Variations in the stability and transmission of maize-chromosome 9 rearrangements by self-fertilization of the M9RHs might be expected. Therefore, we analyzed progenies from 31 M9RHs to evaluate the transmission of the various forms of chromosome 9 derived from irradiation. The average transmission of the maize-chromosome 9 rearrangements is around 25%. In general, the normal or deleted versions of maize-chromosome 9 were transmitted at much lower frequencies than rearrangements involved in intergenomic translocations. These results are expected since maize-chromosome 9 additions transmit at about 10% frequency in control non-irradiated monosomic addition plants. The progenies of M9RHs have a molecular marker composition similar to the parental lines. In situ hybridization is being performed to study the actual organization of the chromosome-9 rearrangements. Lines with stable maize-chromosome 9 rearrangements are useful sub-chromosome stocks for DNA-based marker mapping and for the manipulation of specific regions of maize-chromosome 9.

P30 Use of the r-X1 Deficiency System to Recover Trisomics for Chromosome 8 in Maize

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The r-X1 deficiency is an X-ray induced deficiency in maize that includes the R locus on chromosome 10. Gametes with the R-X1 deficiency undergo a high rate of post-meiotic non-disjunction during embryo sac formation. When R/r-X1 deficiency heterozygotes are crossed as female parents by r/r male parents, over 10% of the colorless (r/r-X1) kernels contain monosomic embryos and a similar portion are trisomic. Multiple aneuploids and deficiencies are also recovered. Nondisjuncton of a chromosome caused by the r-X1 deficiency in the female parent will produce embryo sacs with eggs that are either nullisomic or disomic for that chromosome. A trisomic is produced when an egg disomic for a chromosome is fertilized by haploid pollen. Here, we describe the use of the r-X1 system to recover trisomics for chromosome 8. R/r-X1 female parents that were V16/V16 (on chromosome 8) were crossed by r/r; v16/v16 male parents. Colorless (r/r-X1) kernels from this cross were crossed as female parents by v16/v16 males. Kernels from 138 testcross ears were germinated and classified in a sand bench planting. Most of the ears had approximately equal numbers of green (V16/v16) and virescent (v16/v16) seedlings as would be expected if the female was diploid. However, six of the ears produced 3 or more times as many green seedlings as virescent seedlings, a ratio expected if the female parent was trisomic for chromosome 8 (V16/V16/v16). Trisomics for chromosome 8 were genetically and cytologically verified in one of these families,

and more are under analysis.

P31 CRP1: A Translational Activator in Maize Chloroplasts

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The proplastid to chloroplast developmental transition is characterized by a massive upregulation in the expression of both chloroplast genes and nuclear genes encoding chloroplast proteins. Previously, we identified a nuclear gene called chloroplast RNA processing *(crp1)*, whose activity is required for normal chloroplast gene expression. *crp1* mutants are deficient in the translation of the chloroplast *petA* and *petD* mRNAs, and in the processing of *petD* mRNA from its polycistronic precursor (Barkan *et al.*; EMBO J. 13:3170-3181, 1994). The *crp1* gene was been cloned by *Mu*-tagging (Fisk, Walker, and Barkan; EMBO J. 18(9):2621-2630, 1999). CRP1 is a 66 kD polypeptide that is part of a ~350 kD complex *in vivo* and contains 14 tandem repeats of a 35 amino acid motif, similar to the tetratricopeptide repeat (TPR) motif that mediates protein-protein interactions. CRP1 is related to the fungal proteins CYA5 in *Neurospora crassa* and PET309 in *Saccharomyces cerevisiae*, both of which activate mitochondrial *cox1* translation. We hope to characterize the mechanism of CRP1-mediated translation by identifying the other factors in this stable complex as well as any that interact more transiently with CRP1. We are using two approaches to isolate CRP1-associated factors: isolation of the complex with affinity chromatography and yeast two-hybrid screening. In addition, we are screening for other nuclear mutants deficient in the translation of components of the cytochrome b_6f complex. *Arabidopsis thaliana* has a large family of CRP1-related open-reading frames of unknown function. Among them is a gene product with an overall similarity of 66% to CRP1 and a similarity of 88% over the last two thirds of the sequence. We believe this product is an orthologue of CRP1. In collaboration with the Arabidopsis Knockout Facility, we have identified insertions within the putative CRP1 orthologue. Our goal is to determine whether the CRP1 orthologue in *Arabidopsis* also functions in both mRNA processing and translation and to use the mutants as recipients for mutant forms of CRP1 to address structure-function questions.

P32 Maize single-kinetochore chromosomes can align at the equator by tension-sensitive interactions with opposite spindle poles

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In meiosis, sister kinetochores segregate to the same spindle pole during meiosis I and then lose their cohesiveness to disjoin in meiosis II. However, in the previously described maize meiotic mutant *afd1* (absence of first division 1), sister kinetochores separate prematurely in the first meiotic division and meiosis II is carried out in the presence of single-kinetochore (SK) chromosomes. Here we verify the afd1 phenotype using anti-kinetochore antibodies and report in detail on the behavior of the single-kinetochore chromosomes in meiosis II. A living meiocyte culture system allowed us to demonstrate that SK chromosomes in afd1 meiocytes can align at the equator at metaphase II, but that they fail to segregate properly at anaphase II. A combination of CENPC and centromere staining suggests that congression occurs in part because single kinetochores can split into subunits and interact with microtubules from opposite poles. Single kinetochores interacting with both poles can be stretched up to 6 times their normal diameter at anaphase, while the underlying centromeric DNA is more resistant to distortion. As with the kinetochores on normal chromosomes, the kinetochores of single-kinetochore chromosomes stain positive for tension-sensing spindle checkpoint proteins (MAD2 and the 3F3/2 antigen) during prometaphase, but lose their staining for checkpoint proteins prior anaphase. Our data suggest that maize meiotic kinetochores have an inherent plasticity that allows them to mediate tension between spindle poles.

P33 Analysis of a small cluster of B specific repeat sequences in the long arm of the B chromosome

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The nondisjunction property of the maize B chromosome requires the B centromere and the distal euchromatic tip of the long arm. B specific repeat sequences have been previously cloned in the lab. They are derived almost exclusively from the centric region of the B chromosome, but a minor site is present near the end of BL. A series of B centromere derivatives demonstrated a strong correlation between certain features of the repeat cluster and meiotic transmission, indicating that the B specific sequence is heavily represented in the B centromere (Kaszas and Birchler, 1998). In this study we examined the nature of the minor BL cluster using hypoploids of B-A translocations that retain this site but remove the centromere. Restriction enzyme digestion of DNA isolated from hypoploids derived from TB-10Sc, TB-10La, TB-9Sb, TB-4Sa, TB-9Sd and TB-10L36 have the same or similar *Dpn*II banding pattern in Southern blots probed with the B specific repeat. TB-10L36 has a breakpoint in the middle of DH4 and extends to the euchromatic tip. The hypoploids of TB-8La, in which the A-B chromosome has the most distal BL breakpoint, showed that the banding pattern is different from that of TB-10L36 but a representation of the B specific sequence is still present. We are interested in exploring the possibility that this repeat cluster plays a role in B chromosome nondisjunction.

Posters -- Cytoplasmic Inheritance

P34 Transposon tagging of nuclear genes that regulate mitochondrial gene expression
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Hundreds of nuclear gene products are needed to maintain and express the mitochondrial genome and to assemble mitochondrial respiratory complexes. The S system of cytoplasmic male sterility (CMS-S) in maize presents a unique opportunity for the identification, cloning and functional characterization of nuclear genes regulating mitochondrial gene expression. The expression of a novel, chimeric gene in CMS-S mitochondria results in the collapse of developing pollen and a male-sterile phenotype. Loss-of-function mutations in nuclear genes required for mitochondrial gene expression behave as restorer-of-fertility (*Rf*) alleles, disrupting expression of both essential and CMS genes. These mutations are tolerated in pollen because late-stage pollen development and pollen germination do not require mitochondrial respiration. A screen of 1,241 Mo17-S plants carrying active *Ac* resulted in the recovery of 10 independent *Rf* alleles, predicted to result from mutations at different nuclear loci required for mitochondrial gene expression. *Rf* alleles arose in the Mo17-S *Ac* background at ten times the rate observed in the Mo17-S line without transposable elements. However, spontaneous *Rf* mutations are recovered at high rates from other CMS-S inbred lines. Twenty-four independent *Rf* alleles were recovered by screening 2,635 CMS-S Oh51A plants. This rate was comparable to that of our Mo17-S *Ac* stock. We have therefore initiated genetic tests for the presence of active transposable elements in Oh51A. Our ultimate goal is the cloning of *rf* loci by transposon tagging and the molecular characterization of *rf* gene functions within the mitochondria.

P35 Comparison of Seven Inbred Maize Lines with Their BC-3 Derivatives in Tripsacum Cytoplasm
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Our objective was to compare the relative growth rate of maize inbred lines with their third generation back-cross progeny in a Tripsacum cytoplasm. The F1 hybrid between Tripsacum pollinated by maize was obtained at Woodward, OK in 1995 and Tripsacum chromosomes were eliminated following 3 back-cross generations with maize testers, leaving maize chromosomes in the Tripsacum cytoplasm. These plants were crossed by specific maize inbred lines for 3 additional generations and seed were planted at weekly intervals at University of Galvan, Vera Cruz, Mexico in November 1999. Plant measurements were made in late January, 2000. The

maize inbred lines, 3 field corn lines, and 4 sweet corn lines were significantly later in maturity with less plant height and vigor than their third generation back-cross derivatives in Tripsacum cytoplasm. This indicates that the Tripsacum cytoplasm may have significant effects on maize characteristics.

P36 Genetic characterization of CMS-S restorer-of-fertility alleles in Mexican races of maize and teosinte

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The S-type of cytoplasmic male sterility (CMS-S) evolved in the Mexican highlands. We therefore surveyed Mexican races of maize (*Zea mays* ssp.*mays*) and teosinte (*Z. mays* ssp. *parviglumis* and *mexicana*) to identify those carrying nuclear restorer-of-fertility (*Rf*) alleles for CMS-S. Twenty-seven of 30 maize races and seven of 13 teosinte accessions carried *Rf* alleles. Two major classes of *Rf* alleles are known -- naturally occurring, homozygous-viable alleles and homozygous-lethal alleles that arise by spontaneous mutation. Forty-eight *Rf* alleles recovered from 25 races of maize were tested for homozygous viability, and none was homozygous lethal. Since *Rf* alleles arising in the course of our study are expected to be homozygous lethal, we have identified *Rf* alleles that occur naturally in Mexican sources. The *waxy* translocation series was used to map newly identified *Rf* alleles to chromosome. Most mapped to the long arm of chromosome 2 (2L). RFLP marker analysis demonstrated that three independently recovered maize *Rf* alleles and one teosinte *Rf* allele were linked to the *whp1* locus on 2L. Some of the *Rf* alleles identified in Mexican maize and teosinte may therefore be allelic to *Rf3*, a restoring allele linked to *whp1* in some US maize inbred lines. This was confirmed by genetic means for one of the newly isolated alleles. Because *Rf* alleles were prevalent in maize and teosinte with normal cytoplasm, we hypothesize that *rf* loci are involved in normal mitochondrial function, and that some alleles of these loci are also capable of restoring CMS-S plants to male fertility.

P37 Phenotypic analysis of corkscrew; a recessive mutation affecting shoot development
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The corkscrew (cks) mutation was identified in an Spm mutagenesis screen as a recessive mutation affecting shoot development. At a gross level mutant plants exhibit dwarfism due to reduced internode length, aberrant phyllotaxy and shortened, twisted leaves. Leaf phenotypes also include ligule and auricle displacement and 'pinched' midribs. Some plants also have extra leaves arranged in a decussate phyllotaxy. These mutant characteristics are evident at very early stages of seedling development, suggesting that cks has a role in both post-embryonic shoot development and during embryogenesis. The cks mutant phenotype varies in severity, ranging from plants which grow to almost half as tall as wild type and flower normally, to severe mutants which reach only 3cm in height and seldom survive to maturity. A number of severe mutants additionally exhibit curled mesocotyls and/or shoot positive gravitropism, suggesting that hormonal pathways may be disrupted. The general phenotype of cks mutants resembles that exhibited by rough sheath2 (rs2) mutant plants. However, allelism tests have confirmed that cks is distinct from rs2. The rs2 mutant phenotype is caused by a failure to correctly downregulate knox gene expression in leaves, thus it is possible that knox genes are also ectopically expressed in lateral organs of cks mutant plants. This idea is currently being tested via an RT-PCR approach.

P38 Characterization of nl*1179, a mutant affected in leaf development
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The narrowleaf*1179 mutation was isolated as a recessive mutation from a Mu-active background. In a B73 background, under summer field conditions, the nl*1179 mutation shows a suite of leaf abnormalities which become progressively more severe in the uppermost leaves of the plant, often resulting in the reduction or absence of a tassel. In most cases mutant plants also do not produce an ear, though husk leaves are always present. The phenotypes displayed in the leaves can be markedly different based on how affected the leaf is. In the lowest, most normal appearing leaves, the phenotype is observed in the leaf tips as small clearings and minor distortions in leaf shape. In more severe leaves, the quality of the entire blade changes, with lateral veins closer together than in nonmutant leaves, and with an increase in the number of clearings. In the uppermost leaves, where the phenotype is often the most severe, the leaf size becomes markedly decreased, most notably in the blade, and there is a displacement of presumed sheath into the blade, with distortions in the ligule line. SEMS are being done to further

investigate the nature of these leaf abnormalities. Several aspects of the nl*1179 mutation show a resemblance to the tassel-less1 mutation, which also shows a reduction or absence of the tassel or ear (Albertsen et al. 1993), and when grown under the same field conditions as nl*1179, gives rise to similar, if less severe, leaf abnormalties. Complementation crosses have been performed to test for allelism between the two mutations. Albertsen, MC, Trimnell, MR and Fox, TW. 1993. Description and mapping of the tassel-less (tls1) mutation. MNL 67:51-52

P39 ramosa2 affects a determinacy switch point in the developing maize inflorescence
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Development of the maize inflorescence is characterized by formation of several distinct meristem types. The inflorescence meristem initiates several files of branch meristems on its flanks. Branch meristems are then commited to either a determinate or indeterminate developmental pathway. Branch meristems which follow the determinate pathway produce short branches consisting of a single pair of spikelet meristems, each of which gives rise to flowers. Branch meristems which follow the indeterminate pathway form long branches which reiterate the main inflorescence and produce multiple pairs of spikelet meristems. Mutations in ramosa2 (ra2) disrupt this developmental switch and result in the production of an inflorescence which is highly branched at maturity. An analysis of the effect of ra2 on ear development has been initiated. Preliminary SEM results suggest that the ra2 defect may not be visible until after the branch-to-spikelet meristem transition in immature ears. The interaction between ra2 and Suppressor of sessile spikelet1 , ramosa1, ramosa3, and unbranched1 mutations is discussed and models of ra2 function are proposed.

P40 Analysis of embryo-specific mutants in Zea mays reveals that radial organization of the maize proembryo preceeds the establishment of the shoot apical meristem
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Analysis of embryo-specific mutants in Zea mays reveals that radial organization of the maize proembryo preceeds the establishment of the shoot apical meristem Bommert, P. & Werr, W. We have characterized mutant embryo development of six embryo-specific mutations, namely emb*-8506, emb*-8515, emb*-8518 emb*-8521, emb*-8537, and emb*-8542. The analysis of the mutant morphology by confocal laser scanning microscopy indicated that three of the recessive mutations cause an early developmental arrest in the proembryo/early transition stage. Homozygous emb*-8506, emb*-8518 and emb*-8521 mutant embryos fail to establish a shoot apical meristem and thus can not enter the morphogenetic phase of embryogenesis. In contrast, emb*-8515, emb*-8537, and emb*-8542 show a more variable phenotype, at least some mutant embryos establish a functional shoot meristem, that initiates leaf primordia. The morphological characterization of mutants has been substantiated by the analysis of marker gene expression: Lipid transfer protein 2 (LTP2), Zea mays Outer Cell Layer 1 (OCL1), and Knotted 1(KN1). Our data indicate, that the early blocked mutant embryos, emb*-8506, emb*-8518 and emb*-8521, restrict LTP2 transcription to the outer cell layer, but fail to confine OCL1 transcripts to the protodermal cell layer. The establishment of radial organization therefore is incomplete and KN1 transcripts are not detectable in these three mutants. The establishment of radial organization in the maize embryo therefore seems a prerequisite for establishment of the shoot apical meristem. In support of this conclusion emb*-8515, emb*-8537 and emb*-8542 mutant embryos restrict OCL1 transcripts to the outer cell layer and activate the KN1 gene. However, in mutant

emb*-8537 and emb*-8542 embryos KN1 transcripts are sometimes localized in large and vacuolated cells of aberrant transition stage embryos. Therefore activation of KN1 transcription relies more on positional information rather than on the meristematic character of cells.

P41 Genes controlling later events in leaf development: liguleless1 and tie-dyed1

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We have been studying mutations that disrupt the development of the ligular region of the maize leaf. The maize leaf can be divided into three regions: the proximal sheath, the distal blade, and the ligular region at the blade/sheath boundary. The ligular region is composed of the ligule, an epidermal fringe located on the adaxial surface, and the auricles, a pair of wedge-shaped structures that allow the leaf blade to angle away from the stock. liguleless1 (lg1) is a recessive mutation that abolishes the formation of the ligular region of all leaves of the plant. liguleless2 (lg2) is a recessive mutation that abolishes the formation of the ligular region on the most juvenile leaves of the plants; however, leaves produced later in development show a progressive recovery with the last produced leaves appearing wild type. To identify other genes involved in the development of the ligular region, we have characterized new liguleless mutations. We complementation tested fourteen lg* mutations: eleven failed to complement lg1 and three failed to complement lg2. In a second approach we screened for second site modifiers of lg1 or lg2. Plants were ethylmethane sulfonate (EMS) mutagenized and M2 families screened. An enhancer of lg1 (eol1) was identified and is being characterized. In a separate screen, a new, recessive mutation, tie-dyed1 (tdy1) was identified based on the non-clonal appearance of yellow sectors on the leaves of mutant plants. The sectors are not visible on the leaves until late in development; leaves emerge green and then fade to yellow in patches. The fading is progressive and in the proper genetic background ultimately results in anthocyanin accumulation in the sectors. The sectors cross cell lineage boundaries, can appear all along and/or across the leaf blade, and are present on both juvenile and adult leaves. The sectors sometimes appear zebra striped, though they generally do not have such regularity to their pattern. tdy1 was genetically mapped using T-B translocation crosses to the long arm of chromosome 6.

P42 Three maize root-specific genes are not correctly expressed in regenerated caps in the absence of the quiescent center

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The quiescent center is viewed as an architectural template in the root apical meristem of all angiosperm and gymnosperm root tips. In roots of Arabidopsis, the quiescent center inhibits differentiation of contacting initial cells and maintains the surrounding initial cells as stem cells. Here we further explore the role of the quiescent center in the development of the maize root cap. We have identified three maize root-specific genes. Two of these are exclusively expressed in the root cap and one of them encodes a GDP-mannose-4,6-dehydratase. Most likely these two genes are structural, tissue-specific markers of the cap. The third gene, a putative glycine-rich cell wall protein, is expressed in the cap and in the root epidermis and, conceivably is a positional marker of the cap. Here we present microsurgical and molecular data suggesting that

the quiescent center and cap initials may regulate the positional and structural expression of these genes in the cap and thereby control root cap development.

P43 Inflorescence development in Setaria

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Five species of Setaria (including the foxtail millet S. italica), were examined by light and scanning electron microscopy to identify differences in inflorescence morphology. These differences include changes in number of orders of branching and in the arrangement of spikelets and bristles. Such differences can be traced to early developmental events which, together with the density of branches within the spike, have the potential to become major determinants of yield. It is also possible to draw correlations between the different morphologies and those of several mutant phenotypes of maize, raising the prospect of using genes identified from maize to identify differences in inflorescence morphology in a comparative manner across species groups. Interesting mutants include indeterminate spikelet1, tassel seed6, and ramosa1 & 2. This is part of an on-going project to use recent advances in model plant genetics to understand natural morphological differentiation in plants.

P44 Maternal Gametophyte Effect Genes in Maize Seed Development

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See Talk Abstract # T8

10:40 am Friday, 17 March

P45 Activation of maize defense markers by Les9

Fridlender, Marcelo G.(1); Johal, Guri(1); Yalpani, Nasser (1) (1) Pioneer Hi-Bred International Inc, Johnston, Iowa 50131-0552, USA

Activation of maize defense markers by Les9 Marcelo G. Fridlender, Guri Johal and Nasser Yalpani Les9 is a partially dominant mutation belonging to the lesion mimic class of mutants. It is characterized by the formation of discrete lesions on maize leaves. Les9 lesions, which in appearance resemble symptoms associated with the hypersensitive reaction (HR) of plants with pathogens, are developmentally programmed. The timing of their appearance and severity are both influenced by genetic background. Les9 potentiates maize's response to pathogens, and the degree to which it happens also depends on genetic background. Les9-associated enhancement of disease resistance correlates positively with salicylic acid levels. The expression of some pathogenesis-related proteins, PR1 and chitinases, is also elevated in Les9 leaves exhibiting enhanced resistance. Enhanced resistance to Cochliobolus heterostrophus (CHE), however, does not depend on the presence of elevated levels of PR proteins or lesions at the time of inoculation. Instead, following inoculation with CHE, PR1 protein accumulated more rapidly in Les9 compared to wild-type sibs. RNA profiling of Les9 vs. WT leaves using both Curagen and Affymatrix platforms showed that a number of genes, which are typically induced during HR, were upregulated in Les9 leaves.

P46 Characterization of maize mutants affecting embryogenesis

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During plant embryogenesis a single-cell zygote develops into a highly organized multicellular embryo containing the basic components of the future plant. Numerous mutants with aberrations in this developmental program have been isolated in genetic model species such as Arabidopsis thaliana and Zea mays. Several of them have been analyzed at the molecular level after cloning of the corresponding genes. In some cases the genes code for putative regulatory proteins that potentially play key roles in embryogenesis, in others the molecular analysis suggests functions not readily explaining the mutant phenotype. In this context we have detected, following chemical and traspositional mutagenesis, several monogenic recessive mutants affecting seed morphology. We have focused our attention on a sample of mutants from this collection exhibiting defective early embryogenesis but either normal endosperm development (emb) or almost suppressed endosperm development (abs). Mutant embryos at different DAP (days after pollination) were hystologicaly analyzed: emb mutants appear specifically blocked at an early embryogenic step, while abs mutants maintain their morphogenetic potential with root and shoot primordia recognizable. Immature embryos, between 13-25 DAP, were excised and transferred to basic or enriched culture media. After a culture period of about two weeks, the cultures were evaluated. The mutant embryos are able to germinate and differentiate shoot and root. This experiment doesn't show a promoting effect of enriched media on mutants growth, but is informative since it indicates that the abs and emb mutants tested, even though dramatically disrupted in development, maintain their morphogenetic potential as proved by their capacity as immature embryos to develop root and shoot. The majority of these mutants were generated by Mu or Ac trasposon mediated mutagenesis. At the molecular level, the presence of a co-segregating polymorphism provides a means by which the disrupted gene may be cloned. Co-segregation analysis has been performed on individual seedlings obtained by outcrossing heterozygous +/emb (or +/abs) male parents to W64A inbred females or F2 segregating progenies with the aim of identifying molecular fragments segregating with the mutant phenotype. Restriction length polymorphism was observed in the case of abs7065. This result is based on the analysis of 110 individuals obtained by outcrossing +/ abs7065 male parents to W64A females and it was confirmed on a sample of homozygous mutant seedlings recovered through in vitro embryo rescue. The Mu3-containing genomic DNA fragment was cloned by screening a size fractionated genomic library. Abs7065 was mapped on chromosome 1L via TB-A crosses.

P47 Characterization of the dominant leaf mutation Wab (Wavy Auricles in Blades).

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Wab is a dominant leaf mutation that arose spontaneously in an androgenesis tissue culture derived line (from J. Widholm & T. Rocheford, Univ. of Illinois). This mutation is characterized by auricle tissue that is found in the blade portion of the leaf. This extra auricle often extends distally from the normal auricle but also occurs in the blade surrounded by normal blade tissue. This ectopic auricle is often associated with membranous tissue that extends up the blade on either side of the midrib. Wab leaf blades have a more pronounced horizontal posture, possibly due to the extra auricle tissue. Blades are also narrower and the margins are often disrupted, possibly due to the extensions of membranous tissue. It has also been noted that there are one to three more leaves in Wab mutants than normal siblings. We crossed Wab mutants into a

liguless1 (lg1) mutant background to determine the effect on the ectopic auricle. The normal and ectopic auricle of Wab mutants disappeared in the lg1 background and the leaves were more narrow than the leaves of lg1 single mutants. In addition, the margins of the Wab;lg1 mutants at the sheath/blade border were membranous. Waxy translocation stocks were used to map the mutation to chromosome 2. Linkage was found with both T2-9b and T2-9d. Further mapping showed tight linkage with umc98a. The displacement of proximal cell fates to more distal positions in the leaf is reminiscent of dominant knox mutations. Therefore, we analyzed the expression of class 1 knox genes in Wab leaves by immunolocalization and RT-PCR. We found no misexpression in Wab leaves, suggesting that this mutation affects processes downstream of knox regulation or involves an alternative mechanism of altering cell fates in the leaf.

P48 Epidermal cell differentiation and radial pattern formation in grass embryogenesis.

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In plant embryogenesis, the cell fate determination of the epidermal cell layer may be one of the earliest events of the radial pattern formation. The early embryogenesis of grasses, including rice and maize, differs from that of Arabidopsis mainly in the following two points: (1) cell division occurs at random, and (2) the protodermal cell layer is morphologically formed at the relatively later stage. Based on these observations, radial pattern formation in grass embryogenesis may occur at the later stage than that in Arabidopsis. To elucidate the mechanisms of the epidermal cell fate determination and the radial pattern formation in grass embryogenesis, we have isolated a GL2-type homeobox gene Roc1 (Rice outermost cell specific gene1) from rice, which is specifically expressed in the protodermal cell layer of embryo. In the early rice embryogenesis, the cell division occurs at random and morphologically distinct layer structure of protoderm can not be observed until the embryo size reaches more than 70ƒÊm. Nevertheless, the in situ hybridization analyses have revealed that the outermost cell specific expression is established at the moment when the embryo cells can be physically classified as the inner or outermost cells at the very early stage of rice embryogenesis. The Roc1 gene is also expressed in the outermost cells of callus without any tissue formations. We also investigated the expression of Roc1 in ligule, which is one of the unique organs in grasses. While all cells of the ligule (not only the outermost cells but also inner cells) are derived from leaf epidermis, the Roc1 gene is expressed only in the outermost cells (epidermal cells) of ligule but not in the inner cells. These findings suggest that the Roc1 expression in the outermost cells may be based on the physical-positional information of the embryo or callus. In other words, the Roc1 expression caused by the physical information may be prerequisite for the differentiation of the epidermal cell and radial pattern formation during the rice embryogenesis. Based on these analyses, we will discuss the mechanism of the radial pattern formation in grasses.

P49 laminate coleoptile is required for coleoptile identity and normal leaf pattern
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We are interested in understanding the processes by which organs initiate, aquire identity, and become patterned. The coleoptile, the first leaf-like structure to emerge from the germinating maize seedling, offers several advantages as a model genetic system to investigate questions of organogenesis. First, morphological mutants of the coleoptile can be easily and rapidly detected in the greenhouse. Second, because the coleoptile facilitates soil penetration and arrival of the plumule at the soil surface, mutatations that might otherwise be lethal to the growing seedling can be isolated by planting seeds shallowly in loose soil. We are screening M2 families of EMS and Mutator-mutagenized material and have isolated a number of novel recessive mutants affected in coleoptile patterning and morphology. Here we describe our best-characterized mutant, laminate coleoptile, in which the normally tubular coleoptile is transformed into a leaf. The laminate coleoptile mutation also affects patterning of the vegetative leaves and both inflorescence and flower development. Consistent with the leaf phenotype, which resembles that of dominant knox (kn1-like homeobox) mutants, laminate coleoptile developing leaves display ectopic knox gene expression. The study of coleoptile morphological mutants should bring light to processes and genes involved in organ identity determination and patterning during development.

P50 rgo1 and ids1 interact to control spikelet meristem identity and new spikelet identity mutants.
Kaplinsky, Nick J(1); Freeling, Mike(1) (1) UC Berkeley

Ear morphology is determined by a series of branching events and meristem identity changes. The infloresence meristem branches to give rise to spikelet pair meristems, which branch to produce spikelet meristems. Each spikelet meristem produces two floret meristems. reverse germ orientation 1 (rgo1) and indeterminate spikelet 1 (ids1) both increase the number of floret meristems produced by each spikelet. ids1/+ ; rgo1/+ plants have a reversed germ phenotype. In the selfed progeny of these plants, a more severe phenotype affecting branching of spikelet pair meristems is seen. These data suggest that rgo1 and ids1 have overlapping functions. SSR mapping places rgo1 on 9L. Several new mutants that affect ear morphology have been identified in standard Mu lines. Selfed ears were screened for the reversed kernel phenotype, and the progeny of some of these families contain mutants that affect ear development.

P51 Characterization of clear spot-1 (csp1), a disease lesion mimic mutant of maize.
Kolomiets, Mikhailo V. (1); Johal, Guri (1) (1) Pioneer Hi-Bred Int., Inc., Johnston, Iowa 50131,

Characterization of clear spot-1 (csp1), a disease lesion mimic mutant of maize. Mikhailo V. Kolomiets and Guri Johal Disease Resistance Group, Pioneer Hi-Bred Intl., Inc., Johnston IA 50131 The clear spot mutant (csp1) is characterized by the formation of tiny to medium-sized elliptical, nearly transparent spots that are randomly scattered on the leaf blade. Like other lesion mimic mutants of maize, csp1 is developmentally programmed. The csp1 lesions first appear at the 4- to 6-leaf stage, and they continue to form untill the plant reaches maturity. The development of csp1 lesions appears to be independent of light. While this mutation inherits in a semi-dominant fashion in B73 background, its penetrance and expressivity are severely hindered in all other backgrounds tested. Scanning electron microscopy studies have revealed that leaf tissues affected by this mutation contain deformed trichomes, and have a reduced number of apparently nonfunctional stomata. Ultrastructural studies showed that mutant mesophyll and bundle sheath cells have an irregular shape and size, and are often devoid of cellular contents and organelles except nuclei. However, DNA fragmentation has not been observed in csp1 cells. In addition, mutant cells also seem to be able to respond defensively (as suggested by synthesis of anthocyanins) to infection with Cochlibolus carbonum race 1. Taken together, these studies suggest that the mutant csp1 cells may have undergone some form of programmed loss of organelles reminiscent of events associated with tracheary element differentiation. Our objective for this project is to isolate and characterize the gene responsible for this mutation. Toward this goal, several alleles of csp1 gene have been tagged with Mu elements and studies are underway to clone the gene by co-segregation analysis.

P52 To understand how plants sensesce
Lee, Yew (1); Johal, Guri(1) (1) Pioneer Hi-Bred International, Johnston, IA 50131

Senescence is the last stage of plant development. From a functional viewpoint, this process has been thought to be involved in the recruitment of nutrients from the senescing tissues to the developing reproductive parts. Although numerous studies involving transcriptional changes in senescing tissues have been performed, it is still not known what gene(s) plays a critical or pivotal role in the senescing process. In an effort to understand the senescence mechanism at the genetic and molecular level and to exploit it biotechnologically, we have chosen the premature senescence mutant of maize (pre1). pre1 is a recessive mutant which senesces earlier than wild-type plants. This mutant was first observed in a randomly Mu-tagged population at Pioneer. The

senescing phenotype of pre1 is apparent at least 2-3 weeks before anthesis. A wave of diffused chlorosis and necrosis begins at the tip and margins of the leaves and spreads toward the leaf base. Like natural senescence, pre1 phenotype develops from the lowermost leaves and then spreads to the top of the plant in a progressive fashion. The pre1 plants become fully senescent within weeks after anthesis. Our immediate aims are to clone the gene responsible for this mutation and to probe into the cellular nature of the defect in pre1.

P53 Radial patterning during regeneration of the root apical meristem in maize

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Maize and Arabidopsis root apical meristems differ in several aspects of their radial organization and ontogeny. Despite the large evolutionary distance and differences in root radial patterning, analysis of the putative maize orthologue of the Arabidopsis patterning gene SCARECROW (SCR) revealed expression localized to the single endodermal cell file similar to that found in Arabidopsis. Expression in maize extends through the quiescent center (QC), a population of mitotically inactive cells formerly thought to be undifferentiated and lack radial pattern information. Zea mays SCARECROW (ZmSCR) was used as a molecular marker to investigate radial patterning during regeneration of the root tip after either whole or partial excision along either the apical-basal axis or a portion of the radial axis. Analysis of the dynamic expression pattern of ZmSCR indicates that previously patterned tissues can be re-specified in accordance with new positional values probably set by new boundaries, suggesting the involvement of positional information as a primary determinant of regeneration of the root radial pattern.

P54 Discerning the function of liguleless3 in leaf development: a search for downstream targets

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Development occurs through the coordinated temporal and spatial regulation of various genes and genetic programs. In recent years our laboratory has focused on leaf development and has described several genes that are involved in this process. Work from our laboratory and others has demonstrated that the knotted1-like homeobox (KNOX) gene family plays an important role in leaf development. The protein products of these genes are thought to be DNA binding proteins

and are presumably involved in transcriptional regulation. Mutants that ectopically express KNOX genes in the developing leaf cause proximal cell identities such as sheath cells to occur in more distal boundaries such as the blade. This phenotype manifests itself as a displaced ligule-auricle boundary with sheath extending into the blade region. A molecular analysis of this phenomenon revealed possible interactions between the KNOX gene product of liguless3 and other KNOX gene products. Additionally, we have used a modified yeast screen to look for downstream targets of liguleless3 and have identified a putative binding sequence. A working model for the role of liguleless3 in leaf development is presented as well as a strategy for identifying downstream targets of LG3.

P55 barren inflorescence2 (bif2), barren stalk1 (ba1), Barren inflorescence1 (Bif1) and Suppressor of sessile spikelet1 (Sos1): multiple pathways for axillary meristem development in the maize inflorescence

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Previous phenotypic, histological and genetic analysis has shown that barren inflorescence2 (bif2) is required for axillary meristem initation and maintenance in the maize inflorescence. Here we describe genetic interactions between bif2 and three mutants with similar phenotypes: barren stalk1 (ba1), Barren inflorescence1 (Bif1) and Suppressor of sessile spikelet1 (Sos1). ba1 is a recessive mutant which has the same suite of phenotypes as bif2: no ear shoot and fewer branches, spikelets, florets and floral organs. Moreover, double mutant analysis shows that ba1 has a similar interaction with tasselsheath1 (tsh1) as bif2 implying that ba1 acts at a similar stage of development as bif2. To test whether ba1 acts in the same pathway as bif2, double mutants were constructed. The ba1;bif2 double mutant has a more extreme phenotype than either single mutant implying that bif2 and ba1 act in separate pathways. Bif1 and Sos1 are semi-dominant mutants that have similar phenotypes as bif2 and ba1. Analysis of Bif1;bif2 and Sos1;bif2 double mutants shows that there are multiple pathways for axillary meristem development in maize.

P56 Cloning and Characterization of Maize Lazy-1

Moon, Jennifer(1); Meeley, Bob(2); Arbuckle, John(2,3); Estelle, Mark(1) (1) University of Texas-Austin, Austin, TX 78712, USA; (2) Pioneer Hi-Bred, Intl. 7300 NW 62nd Ave, Johnston, IA, 50131-1004 USA; (3) Novartis Seeds Inc. 317-330th St. Stanton, MN 55018, USA

The maize gene Lazy-1 (La1) appears to control a developmentally regulated response to gravity by maintaining the normal, upward growth of the adult shoot. The recessive la1-Ref mutation, first characterized by Jenkins and Gerhardt in 1931, causes the adult stalk to grow towards rather than away from gravity. Seedling and juvenile shoot growth, as well as the roots and all other features of the plant remain unaffected. The La1 gene was cloned in 1997 at Pioneer Hi-Bred Intl. Although many gravitropism mutants have been identified that are deficient in sensing or responding to gravity, mutant lazy plants are competent to do both. It is the direction of growth that is aberrant. This unusual phenotype and the apparent novelty of the La1 gene product suggest a unique role for this protein. Perhaps as a component of the elusive signal transduction

pathway, Lazy-1 may interpret the signal of gravity perception. As such, characterizing the role of Lazy in gravitropism would have a major impact on our understanding of how plants interpret environmental signals. This poster will provide an overview of what is known about Lazy and discuss some of the avenues taken to determine what proteins interact with Lazy, where the protein and transcript are localized and how perception and response elements of gravitropism are affected as a result of the la1 mutation.

P57 Diurnal Response of Selected Chlorophyll Mutants Under Different Combinations of Light and Temperature

Neuffer, M.G.(1) (1) University of Missouri-Columbia

M2 progenies of 18 unique chlorophyll seedling mutants were planted in 4" pots and grown in growth chambers at four different light and temperature regimens: 1) constant low temperature (22C), 16 hours light, 8 hours dark 2) constant high temperature (32C), 16 hours light, 8 hours dark 3) constant light, 16 hrs at high temperature (32C), 8 hours at low temperature (22C) 4) 16 hours light at high temperature (32C), 8 hours dark at low temperature (22C). The purpose of the experiment was to determine the relationship of light and temperature to diurnal aspects of phenotypic expression common to most of the mutants. The plantings were germinated at such a time as to provide mutant seedlings at the 3- to 4-leaf stage for most mutants. Normal seedlings were pulled, when recognizable as such. Notes were taken periodically and a photographic record comparing responses was made. This poster is a presentation of the photos taken showing unique and differing responses to the different light-temperature regimens. In some cases variations in light are essential, in some cases variation in temperature is the key, in some both, and in others a pre-set response was in place before treatment. This work, consisting of photographs taken in 1974, came to light as a consequence of reviewing mutant images for the mutant collection for MaizeDB (www.agron.missouri.edu). Much clearer representations and other photos, with excellent detail, can be obtained from this web site.

P58 Analyses of genes involved in the lateral organ formation from the shoot apical meristem

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We have been studying the molecular mechanisms of the lateral organ formation from the shoot apical meristem (SAM) through characterizing the function of genes that are involved in the processes. It has been considered that the class1 kn1-type homeobox genes are responsible for meristem maintenance and/or lateral organ formation from the SAM. In fact, their expressions are observed in the SAM, but down regulated in the predicted position of lateral organ primordia or developing lateral organs. We have previously isolated several kn1-type class1 homeobox genes from rice, OSH genes, and clarified the regulated expression patterns in the SAM. In order to understand further molecular mechanisms of lateral organ formation, we attempted the isolation of other genes that may be involved in the event. An Arabidopsis PINHEAD (ZWILLE) gene is thought to play an important role in the SAM formation and/or maintenance. We isolated the homologous gene in rice, called OsPNH, and investigated the expression pattern around the SAM by in situ analyses. In wild type, the strong expression of OsPNH was observed in the region that would generate the vascular tissues and the developing vascular of leaf primordia. In the early stage of primordia development, OsPNH was expressed throughout the center of leaf primordia, and then its expression was focused into the expected regions of midvein and large vascular bundles. The earliest expression of OsPNH was observed in the specific region of the SAM where would generate the next primordia (P0). In sho1 (shoot organization 1) mutant leaves, which are narrow like tendril, the OsPNH expression was observed only in the midvein and few vascular bundles throughout the primordia development. Based on these expression analyses, we will discuss the function of OsPNH gene in lateral organ formation from the SAM.

P59 Genetic dissection of nuclear endosperm development

Olsen, Odd-Arne (1); Lid, Stein Erik (1); Meeley, Bob (2); Chamberlin, Mark (2); Brown, Roy C. (3); Lemmon, Betty E. (3); Olsen , Karin S.(1); Nichols, Scott E. (2) (1) Agricultural University of Norway, Ås, Norway; (2) Pioneer Hi-bred International, Johnston, Iowa , USA; (3) University of Louisiana at Lafayette, Lafayette, Louisiana, USA; (4) (6) ; (7) ; (8)

See Talk Abstract # T12

12:00 noon Friday 17 March

P60 Tissue-specific Expression of AUX1 in Maize Roots

Park, Woong June(1); Hochholdinger, Frank(1,3); Schwall, Michael(2); Feix, Guenter(1) (1) Institut fuer Biologie III, Universitaet Freiburg, Germany; (2) Suedwestdeutsche Saatzucht, Rastatt, Germany; (3) Iowa State University, Ames, USA

Towards an analysis of the genetic basis of the role of auxin in root development, we isolated the cDNA of a maize homologue of Arabidopsis AUX1. The deduced amino acid sequence of ZmAUX1 showed 73% identity to the corresponding AtAUX1 and displayed 7 to 10 putative trans-membrane domains. Northern experiments revealed ZmAUX1 expression in the tips of all maize root types including the primary-, lateral-, lateral seminal- and crown-roots. In situ hybridisation experiments with tissue sections of the primary root showed that ZmAUX1 expression was highly tissue-specifically confined to the endodermal and pericycle cell layers of the primary root along the border of the central cylinder as well as to the epidermal cell layer. This expression pattern makes ZmAUX1 useful as a new marker for studies of root development.

P61 The phase-specific identity of a leaf is specified after leaf initiation

Poethig, Scott(1); Orskwiszewski, Joseph(1) (1) University of Pennsylvania, Philadelphia, PA 19104 USA

The ABC model of floral development proposes that the identity of floral organs is determined by the expression pattern of genes in the shoot meristem before organ initiation. According to this model, floral morphogenesis is primarily problem in the spatial patterning of the shoot apical meristem. Is the production of different types of vegetative organs regulated in the same way? To address this question we examined whether the phase-specific identity of maize leaves is determined before or after leaf initiation. This was accomplished in two ways. First, we induced somatic sectors in pre-existing transition leaves (maximum size = 800 μm) in order to determine when the juvenile and adult domains in these leaves become clonally distinct. This experiment produced several leaves with sectors that spanned juvenile and adult domains in the leaf blade, demonstrating that these cell types were undetermined at the time of irradiation. Second, we examined the susceptibility of adult leaf primordia to rejuvenation. Shoot apices bearing 5-6 adult leaf primordia of known size were placed in culture in order to induce rejuvenation. All leaves 3 mm or less in length were partially or completely rejuvenated by this treatment. The distribution of juvenile and adult tissue in partially transformed leaves was reversed relative to the pattern in normal transition leaves, and was correlated with the age of the leaf. This observation supports the hypothesis that vegetative phase change is regulated by factors that affect the identity of both the shoot meristem and pre-existing leaf primordia. We also observed that vegetative regeneration was not associated with a change in the reproductive phase of the shoot. Excised shoots produced exactly the same number of additional leaves in culture (2-3) as they would have had they remained in situ. This result is consistent our analysis of the Tp mutant phenotype and suggests that the reproductive and vegetative phase of the shoot are regulated independently.

P62 CHARACTERIZATION OF KNOX CLASS HOMEOBOX GENES FROM RICE

Postma-Haarsma, A. Dorien(1); Rueb, Saskia(1); Lamers, Gerda E.M.(1); Hoge, J. Harry C.(1); Meijer, Annemarie H.(1) (1) Institute of Molecular Plant Sciences, Leiden University, Clusius Laboratory, P.O. Box 9505, 2300 RA Leiden, The Netherlands.

Three types of KNOX (maize Knotted1-like) class homeobox genes were identified in libraries of early zygotic embryos (Postma-Haarsma et al., 1999, Plant Mol Biol 39: 257-271). In situ hybridizations showed that during the early stages of embryo development, all three KNOX genes, Oskn1, Oskn2 and Oskn3 are expressed in the region where the shoot apical meristem (SAM) is organizing, while these patterns diverge at later stages. Transgenic overexpression of Oskn2 and Oskn3 in tobacco supported their possible involvement in control of meristem formation or maintenance, as cells in various organs appeared to adopt meristem-like identities, like previously reported for Knotted1 and OSH1 ectopic expression. Ectopic expression of Oskn2 and Oskn3 in rice results in a blade-to-sheath transformation, i.e. a more proximal cell fate, as described for the dominant mutations KN1-O, RS1-O and Lg3-O in maize. Genomic clones of Oskn2 and Oskn3 were isolated and a reporter gene approach in transgenic rice plants

is used to analyze the expression patterns in more detail.

P63 Esr genes show different levels of expression in the same region of maize endosperm
Bonello, Jean-Francois(1); Opsahl-Ferstad, Hilde-Gunn(1); Perez, Pascual(2); Dumas, Christian(1); Rogowsky, Peter M(1) (1) RDP, ENS-Lyon, F-69364 Lyon, France; (2) Biogemma, Campus Universitaire des Cezeaux, F-63177 Aubiere, France

Esr genes share high homology among each other, code for small hydrophilic proteins and are expressed in a restricted region of maize endosperm surrounding the embryo. We show here that not only Esr2 but also Esr1 and Esr3 are expressed in maize and that the relative contribution of Esr1, Esr2 and Esr3 to total Esr mRNA is 17%, 55% and 28%, respectively. DNA sequence analysis of putative promoter fragments ranging from 0.53 kb to 3.54 kb revealed the presence of retrotransposons related to the Zeon and Cinful families in the distal parts of the promoters. The proximal parts show high homology that extended over 504 bp between Esr2 and Esr3 and 265 bp between Esr1 and the other two genes. The most conspicuous potential cis element is a fully conserved tandem repeat of the sequence CTACACCA close to the respective ORFs. By analysis of transgenic maize plants carrying promoter-Gus fusions it was shown that all three cloned upstream fragments contain functional promoters, that the spatial activity of all three Esr promoters is identical and that the cis element(s) responsible for the expression in the embryo surrounding region reside in the 265 bp upstream of the respective ORFs.

P64 Mutations in thick tassel dwarf 1 affect meristem function
Running, Mark P.(1,2); Vollbrecht, Erik (1,2); Hake, Sarah(1,2) (1) University of California-Berkeley; (2) USDA-ARS Plant Gene Expression Center, 800 Buchanan St., Albany CA 94170

Plant meristems are responsible for initiating organs and other meristems on THEIR flanks. Indeterminate meristems, such as inflorescence meristems, continually maintain a pool of undifferentiated cells throughout the life of the meristem, while determinate meristems, such as floral meristems, initiate a fixed number of organs before ceasing growth. Recessive mutations in thick tassel dwarf 1 (td1) show a failure in the restriction of proliferation in inflorescence and floral meristems. Both male and female inflorescences show a dramatic increase in size and sometimes show line or ring fasciation. Male flowers sometimes contain extra stamens, and female flowers often show extra carpels and indeterminate proliferation of the meristem. Branch and spikelet meristems are not affected in the single mutant. The penetrance and expressivity varies greatly among different inbred backgrounds, with some inbreds completely masking the

phenotype. Double mutant studies using td1 and other mutants affecting meristem function and flower development have been helpful in assigning tentative regulatory relationships between td1 and other maize genes. Several td1 alleles have been isolated from targeted tagging experiments, and progress in cloning will be reported.

P65 EPC, a gene controlling juvenile to adult phase change in maize

Sauer, Matt(1); Poethig, R. Scott(1) (1) Univesity of Pennsylvania, Philadelphia, PA 19104

See Talk Abstract # T27

12:05 pm Saturday, 18 March

P66 Bundle Sheath Defective2 (BSD2); a novel protein required for the accumulation of RuBisCO

Sawers, Ruairidh J. H.(1); Brutnell, Thomas P.(2); Langdale, Jane A.(1) (1) University of Oxford, UK; (2) Boyce Thompson Institute for Plant Research, Ithaca, NY 14853-1801, USA

BSD2 is a member of a growing family of small, novel proteins apparently derived as truncations of the DnaJ class of chaperone. The maize gene encodes a peptide of 130 amino acids and contains a DnaJ-like zinc-finger domain and an N-terminal plastidic targeting sequence. Mutant plants in which the bsd2 gene is disrupted demonstrate a specific faliure to accumulate the enzyme RuBisCO. Despite the lack of RuBisCO, the transcripts encoding the large and small subunits (SSU and LSU) of the enzyme accumulate to normal levels in mutant plants. This result suggests that BSD2 is specifically required for the accumulation of RuBisCO and that it acts at the post-translational level. The SSU and LSU proteins that constitute the RuBisCO holo-enzyme are encoded in the nucleus and chloroplast genomes respectively. Given that the BSD2 protein is localised to the chloroplast stroma we have investigated the requirement for BSD2 in the regulation of the rbcL gene that encodes the LSU protein. We have demonstrated that translational initiation of rbcL and early elongation of LSU are not perturbed in mutant plants. Furthermore, we have shown that BSD2 is associated with translating polysomes in wild-type leaves. From these results we propose that BSD2 acts at the co-translational level in the synthesis of LSU.

P67 Clonal analysis of NS1, cloning of ns2?: progress toward elucidating the function of the narrow sheath duplicate genes during maize leaf development.
Scanlon, Michael J(1) (1) University of Georgia, Athens, GA 30602

The narrow sheath duplicate genes (ns1 and ns2) perform redundant functions during maize leaf development. Plants homozygous for mutations in both ns genes fail to develop wild type leaf tissue in a lateral domain that includes the leaf margin. Previous studies implicated a model in which the NS gene product(s) functions during recruitment of leaf founder cells in a lateral, meristematic domain that contributes to leaf margin development. A mosaic analysis was performed in which the ns1-O mutation was exposed in hemizygous, clonal sectors in a genetic background already homozygous for ns2-O. Analyses of mutant, sectored plants demonstrate that NS1 function is required in L2-derived tissue layers for development of the narrow sheath leaf domain. NS1 function is not required for development of the central region of maize leaves. Furthermore, the presence of the non-mutant ns1 gene outside the narrow sheath domain cannot compensate for the absence of the non-mutant gene within the narrow sheath domain. NS1 acts non-cell autonomously within the narrow sheath-margin domain, and directs recruitment of marginal, leaf founder cells from two discrete foci in the maize meristem. Loss of NS1 function during later stages of leaf development resulted in no phenotypic consequences. These data support our model for NS function during founder cell recruitment in the maize meristem. A modification of the AIMS technique of Mutator transposon-display was used to isolate a MuDR-tagged genomic clone that is linked to the ns2-Mu77 mutation. The authenticity of the putative ns2-Mu77 clone is currently being assayed in DNA and RNA gel-blot analyses of all known ns1 and ns2 mutations.

P68 A role for the rice homeobox gene Oshox1 in provascular cell fate commitment
Scarpella, Enrico(1); Rueb, Saskia(1); Hoge, J. Harry C.(1); Meijer, Annemarie H.(1) (1) Insitute of Molecular Plant Sciences, Leiden University, Leiden, The Netherlands

See Talk Abstract # T17

9:50 am Saturday, 18 March

P69 Expression analysis of ZmMADS1 and ZmMADS3 in different tissue cultures of maize

Hansen, Susanne (1); Heuer, Sigrid(1); Dresselhaus, Thomas(1); Loerz, Horst(1); Brettschneider, Reinhold(1) (1) Center for Applied Plant Molecular Biology, AMP , University of Hamburg, Institute of General Botany, D-22609 Hamburg, Germany

In higher plants, MADS box transcription factor genes have been shown to control flower meristem and flower organ identity. We have isolated two novel MADS box genes, ZmMADS1 and ZmMADS3, which are differentially expressed during male and female flower organ development. They probably function in flower, gametophyte and zygote development. The aim of this study was to investigate the role of ZmMADS1 and ZmMADS3 during somatic embryogenesis and their potential as markers for measuring the embryogenic capacity of callus and suspension cultures in maize. We have characterised the expression pattern of ZmMADS1 and ZmMADS3 in RT-PCR approaches, Northern blot analyses and in situ hybridisation experiments in different tissue cultures. ZmMADS1 is lowly expressed in type I callus. The expression level increases in competent type II and type II callus with somatic embryos, but is low in isolated somatic embryos of different stages. In situ hybridisation shows signals in the outer cell layers of competent type II callus and after the appearance of somatic embryos in globular and suspensor-like structures of type II callus. ZmMADS3 is higher expressed in type I callus than ZmMADS1 and the expression decreases in competent type II and type II callus with somatic embryos. In contrast to ZmMADS1, ZmMADS3 shows a relatively high expression level in isolated somatic embryos of different stages. These results are not detectable in Northern blot or in situ hybridisation experiments, because the expression level of ZmMADS3 in tissue cultures is in general much lower than that of ZmMADS1. Both genes do not show any signal in non-embryogenic suspension cultures, which make them appropriate as marker for embryogenic tissue cultures. ZmMADS1 is more suitable to examine the embryogenic capacity of type II callus, while ZmMADS3 marks type I callus.

P70 Observing the predictability of random events during leaf development: Analysis of *rli1-warty*and other cell pattern mutants.

Sylvester, Anne W.(1) (1) University of Wyoming, Laramie, Wyoming 82071, USA

Maize leaves can tolerate a high degree of cellular disorder. Cells in a leaf primordium apparently adjust to local growth requirements by modulating the rate or orientation of division and expansion. We are interested in understanding the mechanism of modulation and the spatial signals involved. We screened EMS-mutagenized M2 families and *Mutator*-active families, selecting for mutants with disorganized epidermal cells but relatively normal leaf development. We identified ten mutants with randomly distributed clusters of disorganized cells including *rli1-warty,chaos1-0,turbulent1-0,*and seven others currently designated *cells*-2,-38,-53,-60,-61,-80*and *Cells*-15.* Complementation tests demonstrate that *cells*-80*is also allelic to *rli1,*in addition to the previously identified alleles *gritty leaf,bumpy*and *reference.*We generated four *Mu*-insertion alleles in a directed tagging of *rli1-warty.*One of these (*rli1-m3)*was cloned and sequenced, identifying *Mu3*inserted in the promoter of a gene with 98% nucleotide identity to *Rab–2b,*which encodes a small GTP binding protein in the RAS family. Sporadic cellular defects observed in the mutants include arrested mitosis, endoreduplication, incomplete cytokinesis, altered cell expansion and various cytoskeletal abnormalities. Mutant cells experience problems in division and expansion during a restricted time of leaf development. Assuming these are null mutations, as is likely for *rli1-warty*and *rli1-m3,*the sporadic phenotype could be explained by developmental non-uniformity of a leaf primordium combined with

progressive expression of genes regulating cell division or cytokinesis. Analysis of these mutants confirms the coordinated and interactive behavior of leaf cells during development.

P71 Characterization of genes involved in organ development in maize

Theodoris, George(1); Freeling, Mike(1) (1) University of California, Berkeley, California 94720, USA

Our interest lies in understanding the genetic networks involved in initiating organ development from the meristem. The focus of our studies has been understanding the role of the rough sheath2 (rs2) gene in organ development. Mutations in rs2 result in severe alterations in leaf and floral development. Expression of knox (KN1 related homeobox) genes, another developmentally important gene class, is perturbed, usually derepressed, in rs2 mutants. rs2 belongs to a distinct subfamily of myb genes including the Antirinum phantastica gene as well as homologs from Arabidopsis and tomato. Because the myb domains of these genes are highly diverged compared to other myb genes, a fundamental question in determining the function of these genes is the elucidation of their DNA binding properties. We will summarize our findings regarding the DNA binding properties of RS2. We have used genetic approaches to identify functionally related genes and new mutations effecting organ initiation. The leaf development phenotype of rs2 , while dramatic, typically manifests itself when plants are past the juvenile stage. When rs2 is combined with mutations in the genes narrow sheath1 and narrow sheath2 (duplicate genes which also perturb leaf development after the juvenile stage), a hastening and synergistic interaction is observed. We are working on understanding the basis of this genetic interaction. A genetic screen for mutations which manifest the phenotype of rs2 in the seedling stage has also been undertaken, and we will present our preliminary characterization of seedling ear1 (se1), a recessive mutant affecting both leaf development and floral initiation in seedlings.

P72 Maize beta-glucanases - multiple genes and multiple roles in plant development

Thomas, Bruce R(1); Inouye, Masahiro(2); Simmons, Carl(3); Nevins, Donald J (1) (1) University of California, Davis, CA 95616, USA; (2) Ehime University, Matsuyama 790, Japan; (3) Pioneer Hi-Bred International, Inc., Johnston, IA 50131-1004, USA

The plant endo-glucanases in glycosyl hydrolase family 17 include endo-1,3;1,4-beta-glucanase (E.C. 3.2.1.73) and endo-1,3-beta-glucanase (E.C. 3.2.1.39). These enzymes hydrolyze polysaccharides found in the plant and fungal cell walls, thus functioning in plant development and in defense of the plant against fungal diseases. A novel glucanase gene family GnsN is expressed in the maize coleoptile and other growing tissues throughout the plant. Analysis of two maize coleoptile endo-glucanase ESTs shows that these sequences are not related to any other previously known family of glycosyl hydrolase. Thus, the coleoptile glucanase enzyme has now been classified in a new group as E.C. 3.2.1.XX. The Hydrolysis Hypothesis for hormone regulation of growth in plants is based on the simultaneous hydrolysis and incorporation of new glucans into the cell wall observed in growing plant tissues. Cell elongation in response to auxin is accompanied by a decline in a specific glucan polymer in the cell wall and by the accelerated activity of certain glucanases. The inhibition of growth in coleoptile tissues treated with glucanase antibodies provides direct evidence for a role of glucanase in control of plant growth. Analysis of glucanase isozymes in cereals and dicot plants suggests that glucan hydrolysis may be a conserved mechanism for growth regulation throughout the plant kingdom. These discoveries enable new initiatives for further investigation of the glucanase role in control of plant growth.

P73 Developing systematic descriptors and containment hierarchies for maize

Vincent, P. Leszek D.(1); Coe, Edward H.(1,2); Cone, Karen(3); Polacco, Mary(1,2); Kross, Heike(1) (1) Department of Agronomy, Univ. of Missouri-Columbia; (2) USDA-ARS Plant Genetics Research Unit, Univ. of Missouri-Columbia; (3) Division of Biological Sciences, Univ. of Missouri-Columbia

The Systematic Descriptors component of the Missouri Maize Project is aiming to provide descriptors in appropriate ‘containment hierarchies' (biological terms, their relationships and associations) for maize. We wish to design a structure that has wide contribution from scientists studying maize and many other plant species. Databases comprising all sorts of information on monocots and dicots will need to be increasingly interoperable to facilitate future concurrent searches of these databases and so be helpful to a wide range of end-users. Developing systematic descriptors in containment hierarchies which accommodate maize data is a challenge on its own. But developing a structure that can accommodate present and future data from other monocot and dicot taxa, is another very relevant and stimulating challenge. An overview has been developed and the framework is forming. We are seeking feedback on the framework and content. NSF award 9872655.

P74 Characterization of ramosa1, a gene regulating indeterminacy in the maize inflorescence

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In maize plants containing recessive mutations of ramosa1 (ra1) the ears and the tassel are many-branched relative to normal, and have a conical appearance. In the tassel, branch length tapers acropetally. In the ear, the ra1 phenotype varies with genetic background, from branched only near the base to highly branched along the length of the axis. Extra, long branches arise in locations normally occupied by short branches, without the concomitant gain or loss of any tissue types, indicating that ra1 acts as a "switchpoint" during inflorescence development. We have generated or collected at least ten mutant alleles of ra1. In B73, the ra1 phenotype is typical in the tassel and strong in the ear. A set of seven alleles converged into B73 suggests an allelic series, with most alleles classified as strong. The weakest allele affects only the tassel. Our collection includes three alleles (ra1-m1, ra1-m2 and ra1-m3) derived from targeted Spm mutageneses. These alleles show somatic and germinal mutability that correlates with Spm copy number. Moreover, for each of ra1-m2 and ra1-m3, genetic and molecular data argue strongly for distinct, autonomous Spm insertion at the ra1 locus. We have cloned one of these Spm elements and are in the process of cloning the second and ascertaining whether or not flanking

DNA identifies the ra1 gene. We also present results of a double mutant analysis with liguleless2. Our data corroborate observations involving unbranched1 (S. Moose, G. Chuck and R. Schmidt, pers. comm.), implying that normal branching in the tassel involves genetically separable pathways that promote branching at the base of the tassel and inhibit branching in the central spike. Finally, we present heritability data from sectored ra1-m1 and ra1-m2 tassels, which indicate that the ra1 gene product can function non cell autonomously.

P75 A candidate gene for the Tunicate1 locus

Wingen, Luzie U. (1); Werth, Susanne (1); Muenster, Thomas(1); Deleu, Wim(1); Faigl, Wolfram(1); Saedler, Heinz(1); Theissen, Guenter(1) (1) Max-Planck-Institute for Breeding Research,Carl-von-Linne-Weg 10, 50829 Koeln, Germany

See Talk Abstract #T16

9:30 am Saturday, 18 March

P76 Glucocorticoid Inducible cr4 Transcription in Transgenic Maize

Zhang, Yuan (1); Becraft, Philip(1) (1) Iowa State University, Ames, IA 50011, USA

The maize cr4 gene encodes a receptor-like kinase which is important in epidermis development and aleurone differentiation of kernels (Becraft, 1996). Little is known about this CR4 signal transduction pathway. Here we report the establishment of a chemical-induced cr4 transcription system in maize. It consists of two molecular components. One is a cr4 gene under the control of a promoter containing 6 copies of the Gal4 upstream activating sequence. The other is a constitutively expressed chimeric transcription factor, GVG, which contains the DNA-binding domain of yeast Gal4, the transactivating domain of the herpes viral protein VP16, and the hormone-binding domain of the rat glucocorticoid receptor (Aoyama, 1997). The system uses a commercially available glucocorticoid, dexamethasone (DEX) as an inducer chemical. Treatment of transformed maize calli with DEX resulted in the rapid and high induction of cr4 transcription. The transgenic plants generated will be very useful in our investigation of the CR4 signal transduction pathway. Our results suggest that the glucocorticoid system is a viable system for inducible gene transcription in maize.

Posters -- Genome Structure/Synteny

P77 Mutant Mapping in the Missouri Maize Project

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The mutants of maize are an undeniably valuable resource. They provide a rich source of variation accessible for identifying gene function. We have a goal to determine the bin locations of the many relatively uncharacterized mutants at the Maize Genetics COOP and from internal resources created at the University of Missouri, the Missouri Maize Project. While most of these mutants (70%) are unplaced and require a larger set of markers per mutant to identify map location, we are currently working with a set of mutants (~500) that were created and placed to chromosome arms using B-A translocations by Dr. M.G. Neuffer at the University of Missouri. F1 materials were produced from crosses between mutant stocks and up to four inbred lines: A619, A632, B73, and Mo17. F2 materials have been grown out and we have collected samples from 67% of them. We are presently mapping mutants to SSR-loci detected by PCR and SFR-agarose electrophoresis. Stable-crude extracts of sample tissues (endosperm, seedling, maturing adult leaves, and immature ear buds) are prepared by a simple, inexpensive method that is effective and safe. Dilutions are used directly in PCR. Our strategy involves first determining the best polymorphic markers from pools of segregating F2 samples. Then we analyze 18-24 F2 homozygous individuals from each mutant family with as many arm-specific polymorphic markers as necessary (6-12 polymorphic markers per mutant family, depending on the availability of markers per chromosome arm). We are currently optimizing the process to maximize our rate of mutant mapping. We will provide a report describing our method, rate of progress, successful mutant mappings, and future strategies.

P78 Progress On Maize Whole-Genome Radiation Hybrids

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Radiation Hybrid (RH) mapping blends aspects of physical and genetic mapping to produce detailed maps of whole or partial genomes. To use this technique, a "panel" (analogous to a mapping population), numbering 80-90 cell lines, must be developed. Each of these cell lines stems from fusion of a gamma-irradiated donor species nucleus (Zea mays L.) with the nucleus of a recipient species (Cricetulus griseus, Chinese hamster) cell. The extent of donor species chromatin fragmentation can be controlled by varying the irradiation dose. Cell lines derived from ovary tissue of the recipient species (designated "CHO" for Chinese Hamster Ovary) are capable of stably integrating broken pieces of foreign chromatin into their own chromosomes. Previous studies show that these same cell lines also retain part of the donor chromatin as extra-chromosomal fragments. Mapping panel resolution may be controlled by altering the amount of donor chromatin breakage. Only a portion of the donor genome is present in each member of the panel, and co-retention frequencies of STS marker pairs among the panel are used to produce a map. Polyethylene glycol-mediated fusions are currently being made between gamma-irradiated maize protoplasts and monolayer cultures of CHO cells, with the aim of producing a whole-genome radiation hybrid panel for maize mapping.

P79 A High-Resolution Genetic Map of the B73 x Mo17 Population.

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A high-resolution maize molecular map is being produced using RFLP and SSR markers. The mapping population is derived from a cross of B73 x Mo17 which underwent random mating for 4 generations. The random mating process increased the average number of recombination events per individual by 3-fold compared with F_2 or RI derived mapping lines. The map presented here represents the initial phase of a large-scale mapping effort aimed at producing a dense, high resolution reference map for maize. To date more than 570 markers have been mapped. The total map length is 2.9-fold greater than the UMC 1998 Maize Genetic map. The RFLP markers have been pre-selected for low copy number. The loci they identify will serve as a bridge to prior map information and anchors for contig assembly of fingerprint data on a 10X maize BAC library. More than 750 cDNA or genomic clones have been screened for use in RFLP mapping. Images of the screenings are available in the Maize Genome Database. The SSR markers provide a bridge from this map to other SSR maps and as additional anchor points for BAC contig assembly. Screening images of the SSR markers are also available in the database. All map data and associated marker information will be available through the Maize Genome Database (http://www.agron.missouri.edu).

P80 Announcing: The "Grass Hybrids" Public Database and Website

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We are interested in updating Irving Knoblock's "Grass Hybrids Checklist," a 1968 list of interspecies hybrids in the grass family. Toward that end, we will unveil the Grass Hybrids Database, and will advertise its web address at the Maize Meeting 2000. Please contribute your information on grass hybrids to this public resource. We will immediately disseminate your information to the public using this website. If your hybrids are proprietary, then listing on this database should help advertise your product. If your hybrids are reasonable deductions but not

proved, or are growing in the wild, please contribute this information anyway. It seems clear that, if you can map it to the ancestral grass genome, you can probably obtain the sequence of the allele you mapped. Fertile hybrids permit mapping.

P81 Genomic organization of the highly recombinogenic bz region of maize

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Meiotic recombination within bz is more than 100 times higher than in the average DNA segment of maize (Dooner and Martínez-Férez, 1997, Plant Cell 9: 1633-1643). To elucidate the possible basis of this high level of recombination , we are analyzing recombination in the immediate vicinity of the bz gene. Our objective is to determine the relationship between genetic and physical distance outside of (proximal and distal to) the bz gene, correlate it with the repetitive vs. unique nature of the flanking DNA, and compare it with the relationship already determined inside of the bz locus. The closest genetic markers proximal and distal to bz are Ac elements that have transposed from bz-m2 (Dooner and Belachew 1989, Genetics 122: 447-457). In order to determine the physical location of these transposed Ac (tac) sites, we have isolated two adjacent BAC clones from the Bz-McC progenitor allele of bz-m2: a 130-kb proximal and a 110-kb distal clone. Five closely linked tac sites (0.06-0.3 cM) that mapped either proximal or distal to bz were placed within the two BAC clones and their physical location was compared with their genetic location. We find a large difference in recombination levels on either side of bz. Whereas recombination immediately proximal to bz is at least 100-fold lower than within bz, immediately distal to bz it is of the same order of magnitude as within bz. The poorly recombinogenic proximal segment is made up mostly of repetitive, methylated DNA. Sequence analysis reveals at least one retrotransposon in this region. The highly recombinogenic distal segment is made up almost exclusively of single copy, hypomethylated DNA and has a high gene density. Four genes (including bz) are located next to each other in a 14.5 kb stretch of DNA, corresponding to a density (1 gene/3.8 kb) that is even higher than the Arabidopsis average. These data strongly suggest that recombination in maize is confined to the single- or low-copy, hypomethylated DNA component, which is where most genes reside.

P82 Gene discovery using the maize genome database ZmDB

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Zea mays DataBase (ZmDB) is a repository and analysis tool for maize sequence, expression and phenotype data. The data accessible in ZmDB are mostly generated in a large collaborative NSF funded project of maize gene discovery, sequencing and phenotypic analysis using a transposon tagging strategy and expressed sequence tag (EST) sequencing. We present the analysis of 50,000 ESTs in the database, which were derived from 12 different libraries from diverse tissues and developmental stages. The ESTs were assembled into ~15,000 tentative unique genes (TUG) based on sequence similarity. Overlap between libraries is less than 10% on average. Currently, the annotation of the TUGs is for the most part restricted to reporting of highly significant sequence similarity of predicted translation products to known proteins. Intriguingly, ~70% of TUGs do not bear significant similarity to any sequences in the public

repositories, indicating that our EST collection may be valuable for gene discovery. Novel tools for gene discovery by spliced alignment have been implemented as ZmDB web services. ZmDB can be accessed at http://zmdb.iastate.edu. ZmDB also provides web-based ordering of materials generated in the project, including EST and genomic DNA clones, seeds of mutant plants, and microarrays of amplified EST and genomic DNA sequences.

P83 Maize Targeted Mutagenesis: A Knockout Resource for the Maize Community. **May, Bruce P.(1); Vollbrecht, Erik W.(1); Rabinowicz, Pablo D.(1); Liu, Hong(1); Stein, Lincoln(1); Freeling, Michael(2); Senior, Lynn (3); Alexander, Danny(3); Martienssen, Robert A.(1)** (1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA; (2) University of California Berkeley, Berkeley, CA 94720, USA; (3) Novartis, Research Triangle Park, NC 27709, USA

Objective: to insertionally inactivate every gene in maize and detect individuals carrying insertions in any sequence of interest. **Design and Setup**: Maize lines containing active *Mutator* elements were crossed to a line carrying a dominant *Mu* repressor activity to establish a collection of stable insertions. Progeny of this cross, the F1 generation, were entered into 48x48 plant grids and tissue samples from each row and column were pooled. Insertions into a given sequence can be identified by PCR analysis of DNA prepared from the tissue pools. F1 plants were selfed to recover seed carrying the insertions. **Status**: A PCR method based on nested gene-specific primers and a *Mu* primer has been optimized to screen the present collection of 46080 F1 plants with a minimum of reactions. The conditions of PCR have also been adjusted to recover longer products (up to 3 Kb) and traverse GC-rich regions. Additionally, 12000 F2 families have been screened phenotypically and a web site (http://mtm.cshl.org) has been established to provide access to information generated by the project. We anticipate that requests for screening will be accepted starting in March 2000. Requesters will retain rights to any invention arising from an identified insertion.

P84 Current and future uses of oat-maize addition and radiation hybrid lines **Odland, Wade E.(1); Kynast, Ralf G.(1); Okagaki, Ron J.(1); Russell, Charles D.(1); Livingston, Suzanne M.(1); Rines, Howard W.(2); Phillips, Ronald L.(1)** (1) University of Minnesota, St. Paul, MN 55108, USA; (2) USDA-ARS and University of Minnesota, St. Paul, MN 55108, USA

Radiation hybrid mapping is a powerful method to physically map chromosomes. We are developing a system to map maize sequences using oat-maize addition and radiation hybrid lines. ESTs and other sequences are rapidly allocated to chromosome using addition lines and mapped at a higher resolution with radiation hybrids. This allows individual members of gene families to be mapped without the dependence of sequence polymorphisms within the mapping parental cross. We are evaluating new technologies that increase the throughput of mapping. The use of these lines for mapping makes them a vital tool for genomics in maize. Since these lines carry a single chromosome or chromosome pair of maize and a complete set of oat chromosomes, their usefulness can extend far beyond mapping. This material permits gene expression and interactions to be studied at an intergenomic level. Maize chromosome 3 carries the liguleless3 (lg3) gene. Oat-maize addition lines carrying chromosome 3 express lg3 and exhibit elements of the liguleless3 phenotype. Other addition lines exhibit phenotypes that correlate with the

presence of a maize chromosome. Associations that have been observed include hypersensitivity to stress with chromosome 9, lesion phenotypes with chromosome 6, and a bluish leaf color with chromosome 2. Chromosome sorting is also possible because of the size difference between maize and oat chromosomes. Fractions highly enriched for a maize chromosome could be recovered, allowing for the possiblity of chromosome specific libraries to be made. Details of the meiotic process can be examined by chromosome painting the maize chromosome pair in the oat chromosome background. These are only a few ways in which the oat-maize radiation hybrids and addition lines could be used in the science community. In the future this material will allow for experiments to be done on maize and oats that were previously not feasible. This work is supported by the National Science Foundation under Grant No. 9872650.

P85 The Arabidopsis Information Resource (TAIR)

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On October 1, 1999, The Arabidopsis Information Resource (TAIR) replaced the Arabidopsis thaliana Database (AtDB). TAIR is being developed as a collaboration between the Carnegie Institution and NCGR. Our goal is to provide the most accurate and up-to-date Arabidopsis genomics resources for the scientific community. We are in the process of developing a new database, interfaces and curation tools to enhance the information contained in AtDB. This first phase of database development is due to be implemented by the spring of 2000. This phase includes: 1) design of the Sybase data model; 2) transfer of data from AtDB to TAIR; 3) a map visualization tool that enables the viewer to display/align multiple maps; 4) reference sequence maps. Our database and interfaces will integrate sequence, gene, genetic marker, polymorphism, reference and community data. During the second phase of development, we will enhance the database to include stock, gene expression, and mutant phenotype data. Concurrently, we will develop controlled vocabulary terms to describe anatomical parts, developmental stages, environmental conditions, plant-specific processes, and nomenclature for molecular objects. We will work with the MaizeDB (http://www.agron.missouri.edu/)to develop controlled vocabulary terms that are shared between monocots and dicots. Controlled vocabulary is essential for accurately describing gene functions, mutant phenotypes, gene expression patterns and otherfeatures of the database. We will also collaborate with the Gene Ontology project (http://www.geneontology.org) to integrate our vocabulary with the yeast, mouse, and fruit fly databases.

P86 EVALUATING DISEQUILIBRIUM AMONG POLYMORPHISMS WITHIN AND BETWEEN CANDIDATE GENES IN MAIZE

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Association methods are a powerful tool for evaluating effects of candidate genes on quantitative traits. Disequilibria between genetic polymorphisms and traits within populations are generally limited to extremely close linkages, often within a single gene. A disadvantage of association methods, however, is that uncontrolled effects of population structure can create disequilibria between unlinked loci, resulting in false positive associations. This is a serious concern in maize, due to the effects of domestication and recent breeding efforts on population structure. We are evaluating linkage disequilibria among DNA sequence polymorphisms within and between four candidate loci (tb1, d8, id1, and d3) with various degrees of linkage to one another. This will provide information on disequilibrium at four levels of genetic linkage: within-locus, between tightly linked loci (~1 cM), between loosely linked loci, and between unlinked loci. Preliminary results indicate that disequilibrium breaks down rapidly, even between regions of a single gene, and there is little evidence for disequilibrium between loci. We discuss the potential implications for identifying polymorphism-trait associations in maize.

P87 MaizeDB -- Gateway to All Public Maize Genome Data

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MaizeDB http://www.agron.missouri.edu is aWeb-based internet resource whose missions are to integrate all maize genome information and to deliver up-to-date access. Integration challenges range from harmonizing gene and trait nomenclature for all maize researchers to insuring interoperability with databases useful for comparative genomics. Current data content and strategies for integrating data from the new maize genome projects will be described. Access has been by various browsable or text-searchable gateways, or by robust, forms query. Graphical access has been provided since 1993 in an ACEDB format, which is served opn the Web at Cornell, http://genome.cornell.edu, along with other USDA-ARS Plant Genome Databases . An ongoing challenge being addressed is to improve access to the broader science community, to include K -12 students and any researchers with an interest in maize biology. Progress in new access interfaces will be presented, including a strategy for interoperability

with a rice genome database with distinct location, schema and database management system

P88 Data Management in the Missouri Maize Project

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Data handling is the key to the success of the Missouri Maize Project. Management of all generated data must be performed to ensure that data integrity and quality is maintained. To this end a laboratory information management system (LIMS) is being developed for use by the MMP. The system consists of several components including a relational database, user interfaces, and various analysis/processing programs. Currently there are several forms of project data being handled: 1) sequence data from libraries (genomic and enriched) and primer sequences (SSR) and 2) mapscores from RFLP and SSR based mapping experiments. Data from genotyping, AFLP analysis and radiation hybrids are also in the process of being supported. Data entry into the system occurs either via custom user interfaces (Java and Visual Basic entry forms) or via automated batch processing (primarily Perl scripts). The backend database which stores all data provides validation and quality control as well as means for data tracking. Additional processing and analysis can then be carried out via applications that have direct access to the database - either presenting results to the user or storing them back to the database. These programs include those that process sequence data from traces to finished sequence as well as those that identify SSRs. Another feature of the system is the transfer of finished data into MaizeDB expediting information dissemination.

P89 Comparison of nucleotide substitutions and multi-base insertions in *b* alleles from *Zea mays* ssp and *Zea luxurians* suggests a relatively recent origin for most insertions.

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Comparison of nucleotide substitutions and multi-base insertions in *b* alleles from *Zea mays* ssp and *Zea luxurians* suggests a relatively recent origin for most insertions.

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The *b* locus of maize controls the expression of anthocyanin pigments by regulating, in concert with other genes, the transcription of the genes encoding the biosynthetic proteins needed for pigment synthesis. The *b* gene displays extensive allelic diversity; over thirty alleles have been recognized by phenotype. We previously determined the phylogenetic and phenotypic relationships between various *b* alleles from *Zea mays* ssp *mexicana*, ssp. *parviglumis*, and cultivated maize (PNAS **96:**15007-15012). This analysis revealed that the alleles fall into three distinct haplotype groups. The sequences in a haplotype group are very closely related within the same group, but are highly divergent from alleles in other groups and from alleles from *Zea mays* ssp *huehuetenagensis*. Using alleles from *Zea luxurians* we have extended these results and find that there appear to be five haplotype groups represented by the *Zea mays* and *luxurians b* alleles. Interestingly the alleles from ssp *huehuetenangensis* cluster with the *luxurians* alleles. We had previously noted a considerably higher frequency of nucleotide substitutions in *b* alleles than had been found at other maize loci. Because we found a discrepancy between the frequency of nucleotide substitutions and insertion/deletions in the *b* alleles relative to other maize genes, we were interested in determining when the various insertions had occurred. Using phylogenetic relationships and evolutionary distances determined solely from nucleotide substitution data, we mapped the alleles that had multi-base insertion/deletion polymorphisms (MBIs). Of the 15 MBIs in the 496 bp region we analyzed, 9 of the MBIs are polymorphic in alleles which differ by four or fewer nucleotide substitutions; the average number of nucleotide differences between alleles is 29. Because many of the MBIs are complete or partial direct repeats of adjacent sequence, they are likely to be relics of transposition events. Our finding that half of the insertions are polymorphic in closely related alleles suggests that they are recent in origin, implying that transposition frequency in *Zea mays* may have increased significantly in the recent past.

P90 Microsatellites in maize - development and mapping.

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Simple sequence repeats (SSRs, microsatellites) are a class of tandem repeats that involve a base motif of 1-6 base pairs. In plants SSRs are abundant, highly informative and easy to use markers. Genome mapping using these sequence-derived markers will facilitate integration of physical and genetic maps in maize. One of Missouri Maize Project goals is to develop and map SSR markers and to release this information through MaizeDB. We are developing SSR markers from

both microsatellite-enriched genome libraries and systematic searching of publicly available DNA sequences. Three mapping populations are exploited to map as many SSR polymorphisms as possible: IBM, F7:8 recombinant inbred population of Mo17 X B73 intermated through 4 generations, 360 individuals; TxCO IF2, immortalized F2 population of Tx303 X CO159, 54 individuals; and CEW6, immortalized F2 population of T218 X GT119, 93 individuals. Common SSR markers are mapped on all three populations to integrate information. Mapping on the IBM population will provide enhanced resolution for tightly linked loci while employment of IF2 and CEW6 will allow consolidation of newly developed maps with current information.

P91 Use of the transposon Ac as a gene-searching engine in the maize genome

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The aim of our Maize Genome project is to use the transposon Ac (Activator) as a gene identification and isolation tool, as well as a mutagen. Genes comprise a small percentage of the genome and are found in hypomethylated CpG islands. One can take advantage of the tendency of Ac to insert in hypomethylated DNA to identify genes as sites into which Ac transposes (tac sites) and, then, to sequence the DNA adjacent to the transposon. An advantage of this approach is that, in addition to a sequence that can be compared to the existing databases, it generates an insertion library. A collection of over 1200 independent Ac transposants from the mutable allele wx-m7(Ac) has been generated and all transposed Acs have been mapped relative to the wx donor locus. In parallel, PCR methods have been adapted for the isolation of DNA adjacent to the insertion (tac sites). To date, more than 250 putative tac sites have been isolated and sequenced, of which about 35 % are homologous to sequences in the databases. At least 20 of them have been confirmed to be bona fide tac sites by Southern blots, mapped to chromosomal locations by using recombinant inbreds or oat-maize addition lines, and analyzed for expression by Northern blots. Because Ac tends to transpose preferentially to closely linked sites, we have initiated transformation experiments of wx--converted Hi2 embryogenic lines with a wx-m7(Ac*) construct modified to facilitate the PCR isolation of tac sites. Many (300) putative transformants have been generated and are currently being tested for the presence of an active Ac . These lines should carry the wx-m7(Ac*) allele at multiple chromosomal locations and should serve as ideal starting materials in future Ac mobilization experiments designed to cover most of the maize genome. # Supported by NSF grant DBI 98-13364

Posters --Molecular Genetics

P92 Nuclear expression of T-urf13 in the tapetum mimics male sterility in CMS-T maize.
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In maize the URF13 mitochondrial protein found in genotypes containing T-cytoplasm is thought to impart sensitivity to host-specific toxins produced by the fungal pathogen Bipolaris maydis race T and the carbamate insecticide, methomyl, and also to cause cytoplasmic male sterility (CMS-T). Studies by others in bakers' yeast, fall armyworm cells and tobacco have shown that URF13 confers sensitivity to the fungal toxins and methomyl. In tobacco no correlation between expression of URF13 and male sterility was observed. Here we examine the effect on sterility of expressing URF13 as a nuclear gene in transgenic maize. We modified the coding sequence of the URF13 gene (T-urf13) for nuclear expression by introducing a 5' mitochondrial targeting pre-sequence and fusing the chimeric gene, called prebT-urf13, to the maize MFS14 tapetum-specific promoter. prebT-urf13 was introduced into maize cells and transgenic plants regenerated. In the backcrossed T1 (glasshouse-grown) and T2 (field-grown) families of 3 transgenic events male sterility was always associated with the presence of prebT-urf13. When compared with their fertile sibs, the tassels of the sterile plants were smaller and the anthers, which were shrivelled, failed to exsert. In these lines the frequency of plants inheriting prebT-urf13 was less than that predicted for a single locus, indicating that the transgenes were poorly transmitted by the female gametes. In contrast, a sterile line containing a MFS14::barnase fusion showed perfect Mendelian ratios, ruling out promoter expression in the embryo sac as the cause of prebT-urf13 instability. Sterile plants from the T2 field-grown families were either backcrossed or pollinated with a CMS-T restorer line. Sterile plants again appeared in the T3 backcross, whereas all plants in the restorer cross were fertile suggesting that nuclear expression of prebT-urf13 in the tapetum accurately mimics the male sterility in CMS-T maize.

P93 Nuclear dosage effects on mitochondrial gene expression
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Each mitochondrion possesses a genome that encodes many of its own components. Even so, the nucleus encodes the majority of mitochondrial proteins and it is reasonable to expect that nuclear factors regulate mitochondrially encoded genes. We tested whether nuclear gene dosage affects organelle expression in the same manner as occurs with nuclear genes. We were also interested to what extent coordinate regulation was operating for functionally related genes. B-A translocations were used to create dosage series for fourteen different chromosome arms in maize plants with normal cytoplasm. We identified chromoscome arms whose dosage caused variation in mitochondrial gene expression. Such an effect indicates that a chromosome arm possesses one or more factors involved in regulating mitochondrial gene transcript level. We present data for six genes obtained from quantitative northern analysis. Three of these genes are

mitochondrially encoded components of the cytochrome oxidase complex (*cox1*, *cox2* and *cox3*). One is a nuclearly encoded cytochrome oxidase component (*coxVb*). We also present data for two mitochondrially encoded genes that are not part of the cytochrome oxidase complex: ATP synthase alpha subunit and an 18S ribosomal RNA. Two tissues, embryo and endosperm, were compared in this study; most effects appeared to be tissue specific. In addition to a number of individual effects, several chromosome arms possessed factors that may involve the coordinate regulation of the cytochrome oxidase genes.

P94 Transcript profiling of the maize defense response to a fungal pathogen and its toxin, a histone deacetylase inhibitor

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HC-toxin from the fungus Cochliobolus carbonum is detoxified by resistant maize lines that successfully defend against infection by this leaf and ear pathogen. Susceptible inbreds are sensitive to the toxin and develop disease lesions upon inoculation with toxin-producing strains of the fungus, but resist infection by strains that lack HC-toxin. The observation that HC-toxin is a potent inhibitor of histone deacetylases suggests that chromatin remodeling directed by this enzyme may have a role in the maize disease defense response. Results are presented from two strategies to investigate this role: Affymetrix and CuraGen RNA profiling to identify genes that are transcriptionally regulated during the defense response and are affected by HC-toxin, and characterization of cloned histone deacetylases to understand how they interact with co-regulator protein complexes. Steady-state transcript profiles show few differences between early defense responses to C. carbonum(tox-) in the absence or presence of exogenous HC-toxin. More differences are seen when comparing C. carbonum(tox-) to C. carbonum(tox+) inoculations. Cloned histone deacetylase genes from families representing two classes have been expressed in E. coli. Members of both classes show rapid association kinetics with a peptide substrate, but the dissociation rate may be somewhat different. Neither class showed altered substrate binding in the presence of HC-toxin, supporting an earlier observation that the mechanism of inhibition may be non-competitive. In vitro transcription/translation products from some of the histone deacetylase clones interact with components of the maize retinoblastoma regulatory complex, and yeast two-hybrid library screening has identified new putative interactors that may form additional complexes that contain histone deacetylases.

P95 SNP discovery using the maize EST database.

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As part of our coordinated effort to identify genes involved in oil deposition in maize, we have produced a cDNA from RNA sampled from four stages of developing embryos from Illinois High Oil (IHO) - 14, 21, 28 and 35 days

after pollination. Thus far, 4000 ESTs from this library have been sequenced as part of the Maize Gene Discovery Project at Stanford. These cDNA clones are being used for the production of DNA microarrays for gene expression studies in developing embryos from IHO and other maize varieties (Illinois Low Oil & B73). We have begun building non-redundant EST contigs using all the raw sequencing traces generated by the Maize Gene Discovery Project (41,398 as of 1/3/00) with the PhredPhrap alignment programs from Phil Green's lab at the University of Washington. The contigs generated by these alignments will be used as the starting point for the discovery of single nucleotide polymorphisms (cSNPs). These cSNPs and a set of simple sequence repeat (SSR) markers will used in QTL mapping studies. As of Feb.1, 2000, 27485 EST sequences have been incorporated into the current project. Of these, 22,185 sequences have aligned into 4740 multimember contigs. Contigs are being edited if necessary, then screened for candidate SNPs. Consensus sequences from each contig are also blasted against the "nr" & "est" databases using either blastn or blastx at the National Center for Biotechnology Information (NCBI) in Bethesda, Maryland. As we identify SNPs we will post the contig consensus sequence, the list of EST sequences which make up the contig, and the location of the polymorphism at our website (http://genetics.mgh.harvard.edu/goodman/). We also plan to post the primers used to generate markers from these SNPs, the PCR conditions used to amplify the region and any mapping data obtained. We will be in close contact with the 2 database design groups, MaizeDB (E.Coe et al) and ZmDB (V.Walbot et al), to arrange for incorporation of our SNP data into their databases

P96 Sequence analysis of a recessive allele of the y1 gene of maize

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The y1 gene encodes phytoene synthase, the first enzyme committed to carotenoid biosynthesis. A standard dominant allele of y1 was previously isolated, sequenced and shown to contain two transposable elements, Stowaway and INS2, immediately upstream of the y1 coding sequence. Later studies identified a Tekay retrotransposon upstream of these transposable elements. These three mobile elements reside in a 1865-bp region upstream of the transcription initiation site of y1. This standard dominant allele of y1 was shown to be expressed in leaf, embryo and endosperm as a 1.8-kb mRNA transcript while a standard recessive allele was expressed in the leaf and embryo, but not the endosperm, as a 2.0-kb mRNA transcript. We have now cloned and sequenced most of this standard recessive allele of y1. When comparing the dominant and recessive alleles, approximately five-fold more nucleotide substitutions occur in intronic and upstream sequences than in exonic sequences. The exonic substitutions give rise to 5 amino acid substitutions 3 of which are nonconservative. Small insertions and deletions are common in the introns and upstream sequences, but are not found in the exons. In addition, all exon/intron splice sites within these two alleles appear identical. A notable difference between the two alleles is that the INS2 and Tekay mobile elements are not found upstream of the recessive allele. It remains to be determined if any of the observed sequence polymorphisms or the presence or absence of the mobile elements influence the expression pattern or mRNA length differences that exist between these two alleles.

P97 Characterization of two Novel Arginine/Serince-Rich Splicing Factors that are Differentially Spliced in Maize

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Regulation of many plant and animal genes are governed by alternative splicing. SR proteins are highly conserved RNA binding nuclear phosphoproteins that are involved in the regulation of alternative splicing. We have identified several expressed sequence tags (ESTs) in the maize genome database (ZmDB) with significant sequence similarity to vertebrate and plant SR proteins. Here we report two novel putative SR proteins from maize, designated zmSRp1 and zmSRp2. The deduced protein sequences reveal that zmSRp1 and zmSRp2 share ~90% sequence similarity at the amino acid level. Both sequences contain the characteristic RNA binding motifs RNP-1 and RNP-2, including the highly conserved peptide RDAEDA, which is diagnostic of

several important splicing factors. Northern blot analysis indicated the presence of several transcripts hybridized to zmSRp1 and zmSRp2 cDNA probes from RNA extracted from root and shoot tissues. RT-PCR analysis using primers designed from the 5¡¯and 3¡¯UTR regions of the cDNAs resulted in the amplification of two and five transcript for zmSRp1 and zmSRp2, respectively. The sequencing of several RT-PCR products and their corresponding genomic region revealed their origin via alternative splicing. Intriguingly, we also noted the utilization of several non-canonical splice sites during alternative splicing of zmSRp2.

P98 Identification and Characterization of Seven Rop GTPases in Maize

Christensen, T.M.(1); Vejlupkova, Z.(1); Albright, C.(2); Quatrano, R.S.(2); Meeley, B. (3); Sharma, Y.(3); Duvick, J.(3); Fowler, J.E.(1) (1) Oregon State University, Corvallis, Oregon 97331, USA; (2) UNC-Chapel Hill, Chapel Hill, North Carolina 27599, USA; (3) Pioneer Hi-Bred International Inc. Johnston, Iowa 50131, USA

Regulation of cell division and expansion are critical for plant development. The mechanisms that control these processes are not clearly understood in higher plants. In other eukaryotic models it has been shown that the highly conserved Rho family GTPases play a crucial role in these mechanisms. One distinct subset of the Rho GTPases, designated Rop, is present in higher plants and may play a role in cellular morphogenesis. At present we are conducting a preliminary characterization of seven rop genes in maize. Experiments involving multiplex RT-PCR assays are indicating the relative levels of gene expression in different plant tissues thus providing clues for phenotypic observations; in addition they have provided preliminary evidence of alternative transcripts from rop6. Additional experiments to elucidate the role of these GTPases utilize a genetic approach. Through the use of TUSC methodology (Pioneer Hi-bred International Inc.) we have obtained several rop mutants in Zea mays. We have also developed a rapid PCR-genotyping assay to identify and produce mutant families carrying these heritable Mu insertion alleles. From these groups we are attempting to identify mutant phenotypes of individuals and families homozygous mutant for rop1, rop6, or the rop1 /rop6 double mutant.

P99 Maize shrunken1 first intron-mediated enhancement of gene expression

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Certain plant and animal introns have the ability to increase expression of both cognate and heterologous genes. However, the mechanisms of enhancement have not yet been defined. Among plant introns, one of the most effective in stimulating gene expression is the 1028 bp first intron of the maize shrunken1 gene (Sh1). We have used reporter gene fusions in a transient

assay system to identify Sh1 intron 1 sequences that are necessary and sufficient for enhancement. Expression of the reporter gene was monitored by enzyme assays, and RT-PCR was used to examine the transcripts and determine splicing efficiencies. Deletion analysis defined the minimum intron derivative that confers maximum stimulation of gene expression. The presence of a 35 bp fragment contained within the intron is required for maximum levels of enhancement, although its removal does not affect transcript splicing efficiency. The important characteristic of this 35 bp element is AT-content rather than the specific sequence. Mutation of intron splice sites blocked splicing and abolished enhancement. Translation initiation codons contained within derivatives of the intron were found to have no affect on enzyme activity. An interesting preliminary observation suggests that the increase in mRNA may be significantly less than the increase in reporter enzyme activity. Based on our current findings, we conclude that intron splicing is required for enhancement, and hypothesize that splicing may serve to link the transcript with the trafficking system of the cell.

P100 Construction of a consensus SSR map for maize using a high-throughput marker screening system

Clayton, Kathryn A(1); Mickelson-Young, Leigh A (1); Ren, Ruihua(1); Frank, Carren(1); Robideau, Carol R(1); Lee, Warren S(1); Mason, Monique (1); Ernst, Cynthia A (1) (1) Trait Development Dept., Dow AgroSciences LLC, Indianapolis, IN

An efficient and robust high throughput molecular marker program using simple sequence repeats (SSRs) has been established at Dow AgroSciences LLC. We incorporated new DNA isolation technologies, performed in-PCR and post-PCR multiplexing, maximized gel electrophoresis capacity, and implemented a data analysis system to streamline our process. Because of this, we analyze hundreds of SSR based marker assisted selection and introgression projects each year. Data from several of these projects were combined to produce a maize consensus map which contains over 200 SSR markers, spans all 10 chromosomes, and provides greater than 95% genome coverage.

P101 Genetic control of endosperm endoreduplication and modes of maternal control

Coelho, Cintia M. (1); Dante, Ricardo A.(1); Dilkes, Brian P. (1); Leiva-Neto, Joao T.(1); Kretzschmar, Ellen(1,2); Larkins, Brian A.(1) (1) University of Arizona, Tucson, Arizona 85721, USA; (2) Luther College, Decorah, Iowa 52101, USA

Nuclear polyploidization occurs widely in metabolically active tissues of plants and animals, and the most common method by which it takes place is through endoreduplication. Endoreduplication is fairly extensive in maize endosperm, where it begins around 9 days after pollination (DAP), concomitant with the onset of starch and storage protein synthesis. Endoreduplication initiates in the central endosperm and radiates toward the aleurone, and by 20 DAP many starchy endosperm cells may have undergone as many as five cycles of genome amplification. This process is not synchronous, and it results in nuclei with ploidy values ranging from 6C to more than 96C. Kowles et al. (1997) showed a maternal effect influencing the pattern of endoreduplication in the endosperm. The degree of endoreduplication in F1 endosperm reflects that of the maternal rather than the paternal parent. F2 endosperms showed little variation in endoreduplication and were generally similar to the F1 pattern. Only in the F3 was variation in endoreduplication detected. Using a single kernel flow cytometric assay we found no significant difference in endosperm mean ploidy for the majority of common maize inbred lines. To extend this study, we surveyed a wider variety of maize genotypes to try and identify more extreme variation in endoreduplication and examined whether a maternal effect was manifested. At 19 DAP the Sg18 popcorn inbred and the dent inbred B73 had an average ploidy level of 14C and 10.5C respectively. We analyzed the degree of endoreduplication in F1 reciprocal, reciprocal BC1 and F2 progeny of Sg18 and B73 by flow cytometric analysis of single kernels at 19 DAP. Mean ploidy for individual endosperms was

determined and statistical analyses of the results were performed. ANOVA analysis showed a statistically significant difference ($p<0.01$) in the mean endoreduplication ploidy among all crosses and generations tested. Mean ploidy differences were found to be under the control of the maternal genotype, in the BC1 crosses. The expected ploidy values for the parents and the various generations were calculated based on the coefficient of the mean components specific for each model of inheritance. Models for inheritance via an effect of the maternal sporphyte and/or maternal gametophyte are favored by these data.

P102 Chromosome Arm Aneuploidy Causes Dosage Effects on *sucrose synthase1* and *shrunken1* RNA levels in maize plants

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To better understand the mechanism by which aneuploidy reduces vigor and viability, we are studying how changes in chromosome arm copy number modify gene expression. In maize, chromosome arm number can be varied from one dose (hypoploid) to three doses (hyperploid) and compared to the normal two doses (euploid). We are currently examining the RNA levels of *sucrose synthase1 (sus1)* and *shrunken1 (sh1)* in two-week old aneuploid maize plants. Because *sus1* and *sh1* are similar genes, it is possible that they are regulated by some of the same factors. For example, in a dosage series of the long arm of chromosome 6, both *sus1* and *sh1* RNA levels were increased in the hypoploid and decreased in the hyperploid when compared to euploid RNA levels. This result suggests that there is at least one regulator of both *sus1* and *sh1* on this chromosome arm that causes the same effect on both genes. The RNA levels of *sus1* and *sh1* did not always show identical responses to chromosome arm dosage. For instance, varying the long arm of chromosome 9 did not affect *sh1* RNA levels, but did result in higher *sus1* RNA levels in the hypoploid and hyperploid than in the euploid. This complex dosage effect is probably the result of multiple factors. The data we have accumulated on these and other chromosome arms indicate that *sus1* and *sh1* RNA levels are subject to regulation by several factors present throughout the maize genome.

P103 Characterization of the maize gl8 gene family and its role in the fatty acid elongase complex.

Dietrich, Charles R (1); Perera, Mahapatabandige A(1); Meeley, Robert B (2); Nikolau, Basil J(1); Schnable, Patrick S(1) (1) Iowa State University, Ames, Iowa 50011, USA; (2) Pioneer Hi-Bred Intl., Johnston, Iowa 50131, USA

Cuticular waxes are a complex mixture of very long chain fatty acids and their derivatives that are synthesized and secreted by epidermal cells. These waxes serve in a variety of physiological roles to protect the plant from stresses such as drought, cold, UV radiation, and pathogen attack. In maize, waxes are most prominently deposited to the surface of juvenile leaves but are also deposited to a lesser degree on adult leaves, silk and pollen. Seventeen mutations affecting the accumulation of juvenile leaf waxes in maize, called "glossy" (gl) mutants, have been described in the literature. A mutation at the gl8 locus allowed for the cloning and subsequent demonstration that the gl8 gene encodes the beta-keto acyl reductase component of the fatty acid elongase (FAE) complex. A second beta-keto acyl reductase (termed gl8b) has been identified which is 97% identical at the DNA level throughout the coding region to the original gl8 gene (now termed gl8a). Expression studies have revealed that the two genes are expressed in a nearly identical manner. Characterization of a collection of 146 gl mutants has, so far, identified four new loci; gl27, gl28, gl29, and gl30, but has failed to identify a mutation at the gl8b locus. A reverse genetic approach, TUSC, has identified alleles that contain Mutator transposons in the gl8b-coding region. GC and GC-MS analysis of waxes extracted from juvenile leaves from B73 and near-isogenic gl8a mutant seedlings has revealed the presence of compounds not previously reported as cuticular wax components. Similar analysis of waxes from gl8b mutant plants will

P104 Cell cycle regulatory components in the endosperm endoreduplication cycle.
Dilkes, Brian P.(1); Dante, Ricardo A.(1); Coelho, Cintia M.(1); Woo, Young Min(1); Leiva-Neto, Joao T.(1); Kretzschmar, Ellen(2); Larkins, Brian A.(1) (1) University of Arizona, Tucson, Arizona 85721, USA; (2) Luther College, Decorah, Iowa 52101, USA

See Talk Abstract #T30

11:25 am Saturday, 18 March

P105 Dosage dependent control of heterosis
Dogra, Anjali(1); Coe, Edward H.(1); Birchler, James A.(1) (1) University of Missouri-Columbia, Columbia, Missouri 65211 USA

See Talk Abstract # T19

11:05 am Saturday, 18 March

P106 *Mediator of Paramutation2* is a dominant inhibitor of the establishment of paramutation

Dorweiler, Jane E.(1); Kubo, Kenneth M.(1); Pilcher, Karen (1); Chandler, Vicki L. (1) (1) University of Arizona, Tucson, Arizona 85721, USA

We isolated a dominant mutation that disrupts the establishment of paramutation. Paramutation is a heritable reduction in expression of one allele when heterozygous with another specific allele. We initially termed this mutation *Frequency of paramutation1 (Fop1-1),* because initial studies suggested it reduced the frequency of directed paramutation. In non-mutant plants, when *B'* and *B-I* are heterozygous, *B-I* is always changed to the lower expressed *B'* state. In contrast, when individuals homozygous for *B'* and heterozygous for the *Fop1-1* mutation are crossed with homozygous *B-I* individuals, roughly 30-35% of the progeny are dark (*B-I*-like). Because 50% of the individuals inherit the *Fop1-1* mutation, but not all of those individuals are dark, it was presumed that this mutation was not fully penetrant in preventing paramutation, but rather had an effect on the frequency with which paramutation occurs. Results from subsequent experiments suggest that this mutation is capable of disrupting the directed interaction between the paramutagenic and paramutable alleles, but does not inhibit spontaneous changes of paramutable to paramutagenic alleles. Spontaneous changes of *B-I* to *B'* occur regularly in standard stocks and *Fop1-1* stocks, and could easily account for the ~15% discrepancy (35% observed vs. 50% expected) observed in the original experiments. Data will be presented in support of this interpretation. We have renamed the locus defined by this mutation to *mediator of paramutation2,* and thus this mutant allele from *Fop1-1* to *Mop2-1*. Mapping data place *mop2* near the telomere of 2S. *Mop2-1* has been shown also to disrupt *pl1* paramutation, suggesting *Mop2-1* acts in *trans* on two different loci. Recent results suggest that *Mop2-1* behaves as a recessive mutation with respect to maintenance of the reduced expression state associated with paramutation. When this mutation is homozygous, pleiotropic developmental effects are often seen. Experiments are in progress to test whether *Mop2-1* affects *r1* paramutation, and whether homozygous *Mop2-1* heritably alters the stability of the low expression state in *B'* alleles. Further mutant characterization should provide insights into the mechanism of paramutation.

P107 Utility of marker assisted selection for introgression of commercially important genes into elite germplasm

Ernst, Cynthia A(1); Clayton, Kathryn A(1); Ren, Ruihua(1); Mickelson-Young, Leigh A(1); Frank, Carren(1); Robideau, Carol R(1); Gupta, Manju(1); Thompson, Steven A(1) (1) Trait Development Department, Dow AgroSciences LLC, Indianapolis, IN 46268

Theoretical strategies for utilization of marker-assisted selection in crop improvement are well established. Successful application of these strategies remains largely undocumented. Dow AgroSciences, LLC has successfully incorporated marker-assisted selection (MAS) using simple sequence repeats (SSR) into its QTL and transgenic introgression programs. Our MAS strategy optimizes the selection intensity on linkage drag and recurrent parent percent at each generation analyzed. The theoretical expectation of this MAS strategy is to identify plants with minimized linkage drag and approximately 95 percent recurrent parent 95 percent of the time at the second backcross generation. Realized results from application of this strategy in transgene introgression

programs demonstrate that the theoretical expectation is attainable.

P108 Heritable allelic interaction between *P-pr* and *P-rr*

Goettel, Wolfgang(1); Messing, Joachim(1) (1) Waksman Institute, Rutgers University, Piscataway, NJ 08854

Paramutation is defined as an allelic interaction between an inducing (paramutagenic) and sensitive (paramutable) allele that leads to a meiotically heritable reduction in gene expression of the sensitive allele. We have found that two alleles of the *p1* gene encoding a transcriptional regulator of the phlobaphene pigmentation pathway participate in paramutation. The paramutable *P-rr* allele confers dark red pigmentation in pericarp and other floral organs, whereas the paramutagenic *P-pr* allele, a spontaneous derivative of *P-rr*, causes reduced pigmentation in the same tissues. *P-pr/P-rr* heterozygotes display either a *P-pr* phenotype or an intermediate level of parental pigmentation. However, the progeny of the F1 x *P-ww* (a null allele) testcross carrying the *P-rr* allele predictably retains the pigmentation level of the F1 parent. *P-rr'*, the *P-rr* allele after exposure to *P-pr*, is phenotypically and molecularly indistinguishable from the *P-pr* allele. *P-rr'* acquires paramutagenicity and is able to paramutate naïve *P-rr* alleles. Reciprocal crosses reveal no maternal-paternal differences in *p* paramutation. *P-rr* and *P-rr'* share the same nucleotide sequence. Expression of *P-rr'* inversely correlates with cytosine methylation at a proximal enhancer site. Preliminary results show that the *P-pr* allele when heterozygous with *P-rr* remains silent. The *p* transcript level in *P-pr/P-rr* is attributed to the expression of the *P-rr* allele.

P109 A CHLOROPLAST PROTECTIVE FUNCTION FOR *lls1 (lethal leaf-spot 1)* IN PLANTS ?

Gray, John(1); Janick-Buckner, Diane(2); Kim, Woo-Yang(1); Greenberg, Jean(3); Johal, Guri S.(4) (1) Dept. of Biology, Univ. of Toledo, Toledo, OH 43606; (2) Div. of Science, Truman State Univ., Kirksville, MO 63501.; (3) Mol. Genetics and Cell Biology Dept, Univ. of Chicago, Chicago, IL 60637.; (4) Pioneer Hi-Bred Int. Inc. Johnston, IA 50101.

We previously isolated the *lls1* gene from maize and its ortholog (*acd-1*) from Arabidopsis that encodes a novel cell death suppressing function in plants. The *lls1* lesion mimic gene is recognized by its recessive mutations, the phenotype of which mimics the disease (lesions) caused *Cochliobolus carbonum* on susceptible maize. The molecular function of the LLS1 protein is not yet known. Sequence analysis of the cloned *lls1* gene revealed the presence of two non-haem iron-binding motifs in the LLS1 protein. Such motifs are conserved in bacterial aromatic ring-hydroxylating enzymes (a biochemical function not described in plants) and more recently in chlorophyll a oxygenases from plants. Although *lls1* itself does not appear to be a chlorophyll *a* oxygenase it does appear to have a chloroplast related function from our studies. The expression of the *lls1*phenotype is exhibited in tissue in a developmental gradient that approximately parallels chloroplast maturation. In addition light is required for the progression of lesion formation, which can be triggered by wounding or infection. More significantly lesion

development is significantly reduced in a photosynthetically defective oil-yellow 700/lethal leaf-spot double mutant (*Oy700/+, lls1/lls1*). Lesions also did not form in the albino sectors of an iojap/lethal leaf-spot double mutant (*ij1/ij1 lls1/lls1*). Most recently we have found that a basal level of *lls1* gene expression occurs in green leaf tissue but not in albino tissue sectors. In the light, this basal level of expression increases almost one hundred fold in response to physical wounding of the green leaf tissue. Electron micoscope studies suggest that ultrastructural changes occur in the chloroplasts of wounded *lls1* plants prior to any other cellular changes. From these observations we hypothesize that LLS1 is required to protect the plant from a chloroplast derived intermediate which becomes a free radical due to excess light or by oxidative stress during wounding. In this manner LLS1 would provide an important cell death protective function in plants.

P110 Identification of a gene at the syntenic sh2-a1 region in maize acting as a QTL affecting silk maysin synthesis

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Comparative genomic analysis at the level of genetic-map has demonstrated the extensive conservation of the synteny of genes in different species. We report our investigation of the microcolinearity at sh2-a1 region and identification of a gene in maize in comparison with sorghum and rice. We synthesized a pair of primers based on the homologous sequence region of sh2 and a1 loci in sorghum and rice. Using this pair of primers, we amplified and cloned a PCR product with molecular weight of 1.4 kb from maize leaves. The DNA sequence of the cloned PCR product had more than 90% homology with the 5'-end sequence of the putative transcriptional regulator gene in sorghum and 80% to the X-gene in rice. We detected a gene transcript (1 kb) in leaves, cobs, and silks using RT-PCR with this primers. Northern analysis also revealed the X-gene transcript of about 2.3 kb in leaves, cobs, and silks. This X gene identified in maize is a single copy gene and mapping of maize genome revealed that this X gene is closely linked with sh2 and a1. Therefore, the X gene homologous to rice and sorghum exists in the maize genome. The position of the X gene in maize is closely linked with sh2 and a1 on chromosome 3 and this gene may act as a recessive QTL linked more close to sh2 and may affect silk maysin contents as evidenced in F1 backcross population.

P111 Insights into Molecular Basis of Heterosis: mRNA Profiles of Maize Hybrids and Inbred Parents

Guo, Mei(1); Rupe, Mary(1); Smith, Howie(1); Yang, Sean(1); Bowen, Ben(2); Crasta, Oswald (3) (1) Pioneer Hi-Bred Int. Inc., Johnston, IA 50131, USA; (2) Lynx Therapeutics Inc.,

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See Talk Abstract # T18

10:45 am Saturday, 18 March

P112 Imprinted genes in maize endosperm

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Endosperm is the product of a double fertilization process that occurs in most angiosperms. The egg and one of two sperm cells fuse to produce the embryo, while the two polar nuclei of the megagametophyte fuse with the other sperm to generate the triploid primary endosperm nucleus. Development of the endosperm in most angiosperms is required for the viability of the embryo and it has been demonstrated that the genomic ratio 2:1 (2 maternal:1 paternal) can be crucial for the successful development of this tissue. Any divergence from this ratio normally results in abortion of the endosperm. The molecular and cellular consequences for parental genomic interactions are thus highly important.A molecular mechanism must exist to sense the balance between the contribution of both parental genes, in that when an incorrect balance is detected, development is arrested. There is accumulating evidence that this molecular mechanism involves a system of gametic imprinting. Gametic imprinting is a unique form of epigenetic inheritance by which expression of certain genes, from generation to generation, is governed by their parental origin. Perhaps the most striking feature of imprinted genes is that the active and inactive parental alleles coexist within individual cells. In plants, current evidence suggest that the embryo is less susceptible to the effects of gametic imprinting, than the endosperm. To date, only four different genes have been found to be imprinted in the plant endosperm, and in every case, maternally-inherited alleles are undermethylated and highly expressed. In order to explore the role that gametic imprinting plays in endosperm development, and to reveal the molecular mechanisms involved, we have attempted to identify and characterise a range of imprinted genes expressed in the maize endosperm.

P113 Maize/Gibberella ear rot- maize genes induced in the plant/pathogen interaction

Harris, Linda (1); Allard, Sharon (1); Saparno, Audrey (1); Dadej, Kasia(1); Koul, Anju (1); Ouellet, Therese(1) (1) Eastern Cereal & Oilseed Research Centre, Agriculture & Agri-Food Canada, Ottawa, Ontario Canada K1A 0C6

We have isolated a number of maize cDNA clones induced or up-regulated upon silk channel inoculation with Fusarium graminearum using differential display PCR. F. graminearum causes infection in the reproductive organs of many monocots including maize (Gibberella ear rot), wheat and barley (Fusarium head blight). Focussing on a few genes, we have used 5'-RACE

PCR to obtain near full-length clones and conducted Northern analyses to further characterize their gene expression. These analyses have included time course studies, tissue specificity, and testing for induction by other fungal pathogens and inducing agents. A summary of our results will be presented.

P114 Maize genomics at ECORC

Harris, Linda (1); Ouellet, Therese(1); Robert, Laurian(1); Simmonds, John(1); Singh, Jas (1); Tinker, Nick(1) (1) Eastern Cereal & Oilseed Research Centre, Agriculture & Agri-Food Canada, Ottawa, Ontario Canada K1A 0C6

Maize genomics efforts at ECORC are focussed on two aspects of prime importance to Canadian corn and food producers: 1) Development of resistance to Fusarium graminearum (gibberella ear rot) Fusarium graminearum causes gibberella ear rot in maize and has been identified by the Canadian corn industry as the most important factor affecting global competitiveness. Crop quality is often reduced by ear rot and associated mycotoxin contamination. Sources of resistance in the maize silk channel and kernel have been identified in maize inbreds developed through conventional breeding at ECORC. However, the cellular and molecular biology of the plant/pathogen interaction has not been well defined. Understanding the basis of the natural resistance as well as pyramiding this resistance with additional biotechnological resistance mechanisms will avoid future susceptibility caused by environmental influences and evolution of the pathogen. 2) Development of cold and frost tolerance The Canadian corn industry has identified cold and frost as major impediments to the expansion of corn cultivation in Ontario, Québec and other parts of Canada. Although germplasm with differing degrees of chilling tolerance are reported to exist, the genetic basis for these differences are not known. In maize, seedling vigour under low spring temperatures, susceptibility to frost and the effect of low temperature on photosynthesis during grain fill are all factors in plant performance and yield. ECORC scientists have considerable experience in the study of cold tolerance (physiology, molecular biology, agronomy) and resistance to gibberella ear rot (breeding, epidemiology, molecular biology, mycology, toxin chemistry). Initial genomics efforts at ECORC (1999-2002) are directed towards developing EST databases, acquiring valuable toolbox strategies, gathering microarray analyses data, and implementing functional analyses including promoter evaluation.

P115 Insights from applying expression profiling to female development under stress.

Helentjaris, Tim (1); Habben, Jeff (1); Sun, Yuejin(1); Zinselmeier, Chris(1) (1) Pioneer Hi-Bred Int., Inc

See Talk Abstract # T38

9:30 am Sunday, 19 March.

P116 Genome-scale RNA profiling of parentally imprinted genes in maize endosperm

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Parental imprinting refers to differential gene expression that is dependent on the transmission of the allele from the male or female parents. We are interested in identifying imprinted genes in maize endosperm tissue, understanding the mechanisms involved, and its importance to endosperm development. We have taken genomic approaches to examine RNA expression differences in hybrids generated by reciprocal crosses and their inbred parents at three developmental stages of maize endosperm: 10, 14, and 21 DAP. RNA samples were analyzed using CuraGen profiling technology, which displays cDNA fragments of corresponding transcripts for most of the expressed genes in a given tissue. These samples generated approximately a half million RNA expression data points. Using these data, we employed both biological and computational strategies to search for paternally and maternally expressed genes. cDNA fragment that is expressed in male parent and its corresponding F1 progeny but not expressed in the female parent and the reciprocal F1 of the above F1 progeny was considered to be paternally expressed. Similar strategy was applied to the selection of maternally expressed genes. Preliminary data from eight reciprocally crossed hybrid progenies indicate that the number of paternally and maternally differentially expressed cDNA fragments varies from 1.2% to 7.1% and 3.4% to7.8%, respectively, out of the total expressed cDNA fragments depending on genotypes and developing stages. On average, 3.0% of the profiled CuraGen cDNA fragments were preferentially expressed or elevated through the paternal parent and 5.5% through the maternal parent. The number of maternally expressed cDNA fragments was consistently higher than that of paternally expressed genes in all genotypes and developing stages analyzed. We also found that a number of cDNA fragments exhibited maternally or paternally preferential expressions throughout all three developing stages within individual genotypes. Some of these genes are currently under characterization. The preliminary data from genome-wide RNA profiling suggests that significant number of genes may be imprinted in maize endosperm.

P117 Stable expression of the high methionine storage protein gene in transgenic progenies of various maize inbred lines

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The coding region of the high-methionine storage protein gene dzs10 from maize has been inserted into an expression cassette of the 27 kDa zein gene promoter and the CaMV 35S 3' polyA signal. This chimeric gene has been introduced into maize Hi-II lines. T0 plants were crossed with non-transgenic parents in the greenhouse. T1 transgenic seeds showed increased levels of 10-kDa gene expression compared to the non-transgenic seeds. Seeds of five independent events produced comparable levels of 10-kDa prote in to inbred BSSS53. T1 plants of each event were crossed to 6 different inbreds for further analysis of transgene inheritance and expression studies. Each event behaved as a single mendelian factor and produced consistent high level of 10-kDa protein regardless to the genetic background. This differs from the endogenous gene in many maize strains that have dominant negative alleles. Seed increase for a

feeding trial did not show any adverse effects of event 10K001 in Mo17 in seed production. Corn meal from 10K001 was able to replace a synthetic methionine supplement and support normal growth rates of 2-day old Petersen chicks. Event 10K001 appears to be stable in its performance in consecutive transmissions (T4). 0

P118 Rooting the Kinesin Tree: A Phylogenomic Analysis

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Kinesins constitute a diverse, anciently derived superfamily of microtubule-based motor proteins. We attempt to reconstruct the evolution of unique functions within the kinesin superfamily by building phylogenetic trees and mapping function onto monophyletic clades. Rooting the tree are four kinesins we sequenced from Giardia lamblia, an anciently diverged amitochondriate protist. In addition to classifying previously described kinesins from protists, fungi, and animals, we classify kinesins from flowering plants including 12 kinesins we sequenced from the monocot Zea mays and many dicot kinesins recently submitted to GenBank. Using sophisticated alignment and treebuilding methods, we resolve phylogenetic trees which allow us to make the following new inferences about kinesin evolution: (1) minus end-directed kinesins form a monophyletic clade whose representatives all possess the C-terminal arrangement of domains within the heavy chain, suggesting that a single evolutionary event accounts for the origin of reversed motor directionality, (2) all presumed Chromokinesins group together (including Drosophila nod), and (3) the CENP-E and MCAK/Kip3 families are closely related and may be the result of a duplication event predating the radiation of the crown eukaryotes. We also show that flowering plants possess both the conventional and C-terminal arrangements of domains within the kinesin heavy chain and are represented in all kinesin families except Chromokinesin and Kinesin II.

P119 Site-selected Mutagenesis of the rad51b Gene in Maize

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In yeast, the RAD51 protein play a crucial role in meiotic and mitotic recombination. Although, the role of RAD51 has not yet been fully established in plants, it appears that it plays a role in homology search (Franklin et al, 1999). In maize there are two closely related rad51 homologs (rad51a and rad51b). To provide resources for determining the unique functions of the RAD51A and RAD51B proteins, the Pioneer TUSC system was used to generate Mu insertions in each of the corresponding genes. Multiple insertion alleles were obtained for each gene. However, none of the rad51b alleles contained an exonic insertion. Because these intronic insertion may not

confer a mutant phenotype, a PCR-based, site-selected mutagenesis method (Das and Martienssen, 1995) was utilized to obtain derivative alleles from rad51b-98E7 that contains a Mu transposon insertion in intron 6. From a population of 1000 plants, four carried alleles that included deletions of genic sequences adjacent to the Mu transposon in rad51b-98E7. Because these alleles lack portions of exon 7, they represent a useful resource for determining the functions of the rad51a and rad51b genes.

P120 Translation of the chloroplast *atpB/E* mRNA requires a nuclear gene in maize.
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To elucidate mechanisms that regulate chloroplast translation, we sought nuclear mutations in maize that disrupt the translation of subsets of chloroplast mRNAs. Here we describe a nuclear gene, *atp1*, whose function is required for the translation of the chloroplast *atpB/E* mRNA. A mutation in *atp1* results in a failure to accumulate the chloroplast ATP synthase complex due to a reduced synthesis of the AtpB subunit. This decrease in AtpB synthesis does not result from a change in *atpB* mRNA structure and abundance. The *atpB* mRNA is associated with abnormally few ribosomes in *atp1-1* mutants, indicating that *atp1* function is required during translation initiation or early in elongation. Previously, only one nuclear gene that is required for the translation of specific chloroplast mRNAs had been identified in a land plant. We are in the process of cloning the *Mu*-tagged *atp1* gene and developing in vitro translation assays for this and other translational activators.

P121 Genomic Organization of the Fatty Acid Desaturase-2 (FAD-2) EST's in Maize.
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Lipids in the form of oils are a major form of energy. The value of oils depends upon fatty acid composition, which determines use as a food source or for industrial applications. Corn oil is primarily composed of Palmitic (11.5%), Stearic (2.2%), Oleic (26.6%), Linoleic (58.7%), Linolenic (0.8%) and Arachidic (0.2%) fatty acids. Increased levels of Oleic acid may enhance the nutritional and functional value of corn oil. The biochemical pathway for fatty acid biosynthesis indicates that a single gene, omega-6 desaturase, converts oleic (18:1) to linoleic (18:2) by inserting a double bond at the omega-6 position. EST's provided by Pioneer Hi-Bred from different tissue library sources (embryo, shoot, leaf and tassel) were sequenced, map positions determined, QTL associations were assessed, and Northern analysis was performed. FAD-2 EST's showed variable sequence identities with one another (60-95%) and with FAD-2 sequences from various plant species (40-80%). Northern analysis of Embryo and Endosperm RNA with embryo derived FAD-2 EST's detects a transcript size of 1.8 KB for all the clones assessed which suggests that the FAD-2 isoforms may have the same transcript size. Transcript levels were similar at all stages of development. This pattern was consistent with the temporal levels of oleic acid accumulation in developing kernels measured by Jellum in 1970. Mapping of FAD-2 EST's revealed a distribution throughout the genome. Mapping information along with

sequence identities confirms the presence of multiple isoforms of FAD-2 in maize. Some FAD-2 clones mapped to regions with minor oleic/linoleic acid concentration QTL, however, no FAD-2 clones mapped to the umc65 region, which has a major oleic/linoleic acid ratio QTL. This suggests that the QTL linked to umc65 may not be an allelic variant of FAD-2 and might be a regulatory locus of some nature.

P122 Chromatin polymorphism dependent gene expression in maize

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Chromatin polymorphism dependent gene expression in maize S. Mladenovic Drinic, K. Konstantinov and G. Milojevic Maize Research Institute, Belgrade 11080, Yugoslavia For our investigation we choosed two models: existing chromatin genes interaction by the analyses of total soluble protein complex of parental inbreds and its hybrid combination different time after pollination and transgenic maize plant, obtained by NPTII marker gene interaction as parental inbred. In several hybrids specific polipeptides have been identified. Hybrid specific polypeptides appeared 45 days after pollination and have been identified in embryo tissue too. Hybrid specific polypeptides are different in different hybrid combination. The studies is mRNA are different too are in progress. Transgenic hybrids expressed changes in izozyme pattern induced by bacterial gene integration in orginal inbred line genome. Different polypeptides are synthesized also in transgenic embryo tissue. It seems that bacterial gene integration changed orginal gene expression and if so, it would be good system for further experiments. By use of two exploited experimental models we are expecting to collect more information on the importance of genetic polymorphism at the chromatin level and its contribution to genome expression.

P123 Genetic analysis of thylakoid protein targeting

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The transformation of a proplastid into a chloroplast is a complex process that involves the assembly of the abundant and protein-rich thylakoid membrane system. Four of the major protein complexes involved in photosynthesis are located in the thylakoid membrane and are composed of both nucleus and chloroplast-encoded proteins. We have used Mu-transposons in both forward and reverse genetic screens to identify mutants with defects in translocating proteins across the thylakoid membrane. These mutants, together with biochemical assays in other

laboratories, defined three distinct translocation mechanisms: the delta-pH, cpSec, and cpSRP pathways.

The delta pH system was first characterized in chloroplasts, and the only known components, *hcf106* and *tha4*, were initially identified genetically in maize. Even though a homologous system was recently discovered in bacteria, the composition and mechanism of the delta-pH machinery is unknown. We have identified new delta-pH pathway mutants. *tha8-1* represents a new gene and has an ~6 kb Mu1 fragment which co-segregates with the mutant phenotype; this DNA is being cloned.

The cpSec pathway in chloroplasts consists of at least two proteins, THA1 and CSY1, which are homologues of the bacterial secA and secY proteins, respectively. We have isolated several new cpSec mutants that are not allelic with either *tha1* or *csy1*. They are currently being analyzed to determine if they are mutants in a maize gene encoding a homologue of the bacterial *secE* gene, or if they define novel components of cpSec. Preliminary results indicate that the *tha5-1* mutant identifies a new locus involved in cpSec-mediated protein targeting.

cpSRP consists of a 54 kD SRP receptor, a unique 43 kD protein, and a homologue of the bacterial ftsY protein, cpftsY. In collaboration with the Nakai laboratory (Osaka, Japan), we successfully screened our reverse genetics resource of non-photosynthetic, pigment deficient maize DNA pools for a *cpftsY* mutant, whose phenotype is now being analyzed. This reverse genetics resource will be useful for isolating maize mutants of biochemically identified targeting components.

P124 Expression of a wheat high molecular weight glutenin in transgenic maize: A comparison of seed-specific promoters.

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The high molecular weight glutenin of wheat is partly responsible for the elastic properties of dough. Corn flour does not contain this protein and therefore has limited dough quality. In order to improve the flour quality of corn and hence expand its value to the baking industry, we have placed two different gene constructs into the crop, both housing the coding region for the high molecular weight (HMW), glutenin subunit 1Dx5, from wheat but driven by either the 1Dx5 wheat promoter or by the 27kDa gamma zein promoter from corn. Both plasmids were co-bombarded with the plasmids containing the selectable marker gene, bar, for selection and regeneration of transformed plants on the herbicide ammonium glufosinate. In our preliminary investigations we have examined the expression of the 1Dx5 protein in the seed of stably transformed corn and shown that both promoters function to direct 1Dx5 protein accumulation to the endosperm. Using both PCR and leaf painting with the herbicide we can demonstrate a high correlation of co-integration of the marker gene and the 1Dx5 gene. Both types of transgenic plants have entered our breeding program for studies in transgene inheritance, protein processing and evaluation of the effects of the protein on flour quality.

P125 ANALYSIS OF THE ORGANIZATION OF THE ABNORMAL-10 CHROMOSOME OF MAIZE

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The Abnormal chromosome10 (Ab10) of maize possesses a large additional region of chromatin that is not present on the normal chromosome10 (N10). Genes located in the additional chromatin result in meiotic drive (or preferential segregation) of knobbed chromosomes. Three independent functions, mapping to different parts of Ab10, are thought to be required for meiotic drive: 1) neocentromeric activity of knobs, 2) increased recombination of structural heterozygotes, and 3) a poorly understood distal tip function that is required for drive but not neocentromere activity or increased recombination. The Ab10 chromosome is known to contain a transposed and inverted segment of the normal chromosome10 (that includes the l13, o7 and w2 genes) along with a differential segment of unknown function, a large heterochromatic knob,

and a distal tip of euchromatin. We are in the process of identifying RFLP markers which are specific to the Abnormal 10 chromosome, and mapping the location of those markers with respect to the Ab10 deficiency series generated by Rhoades. This will allow us to identify RFLP markers linked to the neocentromere, recombination and distal tip functions. We are also carrying out three-point crosses to better resolve the N10 map of this region by integrating the genetic and RFLP maps. Integration of the N10 maps will allow us to better resolve the differences between these two versions of chromosome 10.

P126 Characterization of five maize *hsp101-m-::Mu* lines obtained by reverse genetics
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Maize HSP101 protein belongs to a growing family of proteins called HSP100/ClpB. The best characterized member of this group of proteins is HSP104 from yeast, a chaperone involved in the renaturation of aggregated proteins. In addition to being induced by heat shock, in plants they are also accumulate in the absence of heat stress during seed development and germination. We are interested in the study of the function, both in yeast and in maize, of maize HSP101. In yeast, we have complemented a *delta-hsp104* mutant that displays a deficiency in induced thermotolerance. In maize, we have taken a reverse genetics approach to analyze its role during heat tolerance and development. Screening of a large F1 maize population mutated with the transposable element Mutator *(Mu)* yielded 10 lines with insertions in the *hsp101* gene. Analysis of the F2 generation of these mutant lines indicates that only five of them maintain *Mu* insertions in *hsp101*. Following a backcross to a non-mutator line (A63), we carried self-pollinations of those plants containing *hsp101-m-::Mu* alleles. The phenotypic characterization of these plants is pendant.

P127 The Maize Tousled-Like Kinase Gene Family
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The Tousled-like Kinase (TLK) genes of maize are members of a novel class of Ser/Thr protein kinases. Tousled, a single copy gene in Arabidopsis, enocdes a nuclearly localized protein kinase necessary for the correct partitioning of the floral meristem into organs [Roe et al., Cell 75:939-

950,1993]. Database searches based on protein alignments indicate that TLK genes are present in vertebrates and invertebrates but not in prokaryotes or S. cerevisae. In maize, three genes show high homology to the catalytic domain of TOUSLED. These can be grouped into two classes based upon amino acid differences in their catalytic domains. We are in the process of determining whether their regulatory domains are also diverse. Both classes of the maize TLK genes are expressed in all tissues examined. To learn the role of the different maize TLKs, we have identified five putative TLK mutants from a TUSC screen provided by Pioneer Hibred International

P128 dinf1 and nope1, two mycorrhiza-specific mutants in maize
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For the elucidation of plant factors important for the arbuscular mycorrhizal symbiosis a genetic approach in maize was undertaken. Mutator tagged M2 lines were individually screened for their mycorrhizal phenotype. Among 1250 M2 lines 27 putative mutants were identified. Until now for 2 of these 27 lines their true mutant nature could be confirmed since the respective mutant phenotype was reproducibly recovered in the following three generations. The phenotype of the first mutant resembled a delayed infection (dinf1: delayed infection). Preliminary data from timecourse experiments indicated that appressoria formation on mutant roots was delayed which consequently retarded the establishment of the interaction. These "late" appressoria appeared normal in structure and distribution. The further steps in development of the symbiosis proceeded in the same fashion as in the wild-type. The mutation segregated as a monogenic recessive trait. In the second mutant rare appressoria formation was observed but penetration of maize roots by the fungus was blocked (nope1: no penetration). Segregation patterns suggested that nope1 is a suppressible mutant.

P129 Expression and inheritance of a wheat endosperm storage protein in maize
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SDS PAGE and western blot analysis were used to characterize expression of the wheat high molecular weight glutenin gene 1Dx5 in maize. When F1 transgenic plants were crossed with an inbred line (B73), the 1Dx5 subunit was detected in endosperm of progeny only when the transgenic plant was used as a female parent. These progeny had 1:1 (presence: absence) ratios of 1Dx5 expression. In addition, half of the F2 transgenic families resulting from self-pollinating F1 transgenic plants had 1:1 (presence: absence) ratios of 1Dx5 expression, which fit a model in which the transgene is only transmitted through the female. However, when these plants were crossed as males to B73, 0-11 % of the resulting progeny showed 1Dx5 expression, depending on the family studied. These families represent four separate transformation events. These results suggest that transmission of the 1Dx5 transgene through the pollen is possible, but occurs with low frequency. In a preliminary effort to elucidate the mechanism of this inheritance pattern, we examined the pollen of the plants. In vitro germination was lower in transgenic plants than in non-transgenic plants. Collectively, these data are consistent with the hypothesis that the 1Dx5

transgene is inefficiently transmitted through pollen.

P130 Knock-out the knox genes

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The class 1 type knotted1-like homeobox genes (knox genes) are thought to be involved in developmental processes operating at the shoot apical meristem (SAM). According to analyses of in situ mRNA or protein accumulation pattern and loss of function mutations at knotted1, genes in this class play a role in maintenance of SAM activity and/or establishment of the boundary between site of leaf initiation and indeterminate cells in the SAM. In maize, nine class 1 type knox genes have been reported (Kerstetter et al., 1994). Although these genes may have partially redundant functions, we hypothesize that some of these genes act co-operatively in regulatory processes involved in morphogenetic events at the SAM. We will report on the progress to isolate and characterize plants with Mutator (Mu) element insertions into class 1 type knox genes. We have isolated five Mu insertions into knox4 (gnarley1) and two into knox3. Three Mu insertions into knox4 were found by screening for revertants of the dominant Gnarley1 mutation at the knox4 locus. The other Mu insertions were screened by PCR from Pioneer's Trait Utility System for Corn. All the insertions found were in the introns or 5' untranslated regions. At least two of the insertions into knox3 and knox4 decrease mRNA accumulation in the SAM. Various combinations of crosses between knox gene knock-outs including rs1 and kn1 in collaboration with R. Tyers and M. Freeling (U. C. Berkeley) are underway. Those crosses will reveal the redundant functions of these genes.

P131 Characterization of a tissue-specific gene silencing phenomenon involving *B-Bolivia* and CaMV 35S/B chimeric transgenes.

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The *B-Bolivia* allele of the *b* gene directs anthocyanin expression in many vegetative tissues and in the aleurone layer of the seed. To investigate the tissue-specific expression of the *B-Bolivia* allele, we created transgenic maize lines containing 2.1 kbp of the upstream region of *B-Bolivia* and the complete genomic coding sequence. Expression in the transgenic lines reproduces some aspects of the aleurone expression of *B-Bolivia*, but does not reproduce the parent of origin-specific aleurone expression found in the native allele. The plant specific expression in the transgenic lines was quite different from the plant expression pattern of the native allele and only one half of the lines with seed expression displayed any plant color. One of the transgenic lines that initially displayed strong plant color lost this coloration in the T2 and subsequent generations, while maintaining seed expression. To investigate the possibility that the silenced transgene could induce silencing of endogenous *b* allele(s) and other transgene loci, the silenced line and an independent, non-silenced *B-Bolivia* transgene line were crossed with stocks carrying native *B'* and *B-Bolivia* alleles and to a CaMV 35S promoter/B coding sequence (35S/B) transgenic line, which has stable, intense pigmentation of virtually all tissues. Although neither transgene affected the expression of *B'* or *B-Bolivia*, both *B-Bolivia* transgenes efficiently silenced 35S/B transgene expression. Surprisingly this silencing was tissue-specific, because aleurone expression of the 35S/B transgene was unaffected, and heritable, because no plant expression was seen in the progeny of silenced plants. The silencing phenomenon observed here has characteristics of both post-transcriptional (PTGS) and transcriptional gene silencing and may be a unique example of heritable, tissue-specific silencing.

P132 Ac tagging and characterization of a terpenoid cyclase gene induced by herbivore damage

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See Talk Abstract # T31

11:45 am Saturday, 18 March

P133 Novel type of P1-rr suppresison is caused by transgene carrying full length P1-rr promoter

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The maize p1 gene encodes a Myb-like transcriptional activator that regulates the expression of genes required for red phlobaphene pigment biosynthesis. Alleles of the p1 gene are distinguished based on patterns of pericarp and cob pigmentation, whereby a P1-rr allele has red pericarp and red cob. Previously we reported that the P1.2b::GUS transgene, carrying the long distance P1.2 enhancer fragment from the P1-rr gene promoter, induced paramutation of the endogenous P1-rr allele. The ability of the full length P1-rr upstream regulatory region, including the P1.2 fragment, to induce paramutation of an endogenous P1-rr allele was tested by crossing eight independent P6.2b::GUS transgenic events with the heterozygous P1-rr/P1-wr tester. Examination of the F1 progeny indicated that the full length P6.2b::GUS is capable of causing paramutation, but at lower frequency, 2%, than the P1.2b::GUS transgene, ~21%. Surprisingly, in addition to the expected phenotypic classes, the progeny of a single transgenic event, SC16-5-3, exhibited 46% of ears with novel P1-rr suppression phenotype. The novel suppression phenotype was different from P1-rr' paramutation and resembled the P1-rw, red pericarp and white cob, allele of the p1 gene. Strong correlation of the novel suppression phenotype with resistance to herbicide suggested that this effect was caused by the P6.2b::GUS transgene. Preliminary Southern analysis failed to detect changes in DNA methylation in P1-rw-like plants unlike that observed during P1-rr' paramutation. These results indicate that full length P1-rr regulatory region can cause two different suppression effects, one similar to P1-rr' paramutation, and another resembling P1-rw phenotype. The P1-rw-like suppression is likely to be controlled by different epigenetic mechanism than the P1-rr' paramutation; specific transgene structure might be responsible because only one P6.2b::GUS transgenic caused the novel suppression phenotype of P1-rr.

P134 Characterization of the Zea mays Aldehyde Dehydrogenase Gene Family
Skibbe, David S (1); Liu, Feng (1); Wen, Tsui-Jung (1); Cui, Xiangqin (1); Hsia, An-Ping (1); Meeley, Robert B (2); Schnable, Patrick S (1) (1) Iowa State University, Ames, Iowa 50011, USA; (2) Pioneer Hi-Bred Intl., Johnston, Iowa 50131, USA

Cytoplasmic male sterility (cms) is a maternally inherited trait characterized by the inability to produce functional pollen. In T-cytoplasm, sterility arises from the premature degeneration of the tapetal cell layer during microspore development. This sterility can be overcome by the action of two unlinked restorer genes, rf1 and rf2. Previously, rf2 was cloned (Cui et al., 1996, Science) and has recently been shown to encode a mitochondrial aldehyde dehydrogenase (mtALDH, Liu et al., submitted). In an effort to characterize the molecular mechanism of restoration, additional genes with similarity to rf2 (now termed rf2a) were cloned. The rf2b gene encodes an ALDH that is also thought to accumulate in the mitochondria, while the rf2c and rf2d genes encode putative cytosolic ALDHs (cALDHs). The intron/exon boundaries of these four genes are highly conserved. Despite their overall level of sequence conservation, initial enzymatic characterizations have revealed that the RF2A, RF2B and RF2C proteins have somewhat different enzymatic properties.

P135 Expression of the DIMBOA biosynthesis genes
Stahl, Karolin(1); v. Rad, Uta(1); Frey, Monika(1); Gierl, Alfons(1) (1) Technical University, Dept. of Genetics, Garching, Germany

Benzoxazinones are secondary metabolites that play an important role in the defense mechanisms of maize against insects and microbial pathogens. In maize, DIMBOA- and DIBOA-glucosides are the dominant benzoxazinones. The genes (Bx1-Bx9) involved in DIMBOA synthesis have been elucidated recently. The toxic aglucon is glucosylated prior to transport into the vacuole. Biosynthesis requires the coordinate expression and activity of enzymes located in chloroplasts, endoplasmatic reticulum and cytosol. The regulation of the genes have been investigated on RNA level by quantitative RT-PCR. The tissue specific expression is displayed by in situ hybridisation.

P136 The involvement of long distance communication in a natural case of gene silencing in plants, paramutation at the b locus in maize

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Paramutation is a naturally occurring gene silencing phenomenon, whereby one allele causes a directed, mitotically and meiotically heritable change in the expression of another allele of the same gene. With paramutation at b, a regulatory gene of the anthocyanin pigmentation pathway in maize, an allele conferring dark purple pigment, B-I, is altered into an allele conferring light purple pigment, B', in B'/B-I heterozygous plants. The new B' allele is indistinguishable from B' and paramutates naive B-I alleles. Characteristics of paramutation at b are: 1) B' is transcribed at a 10-20 fold lower rate than B-I. 2) The B protein is not required for paramutation; 3) There is no evidence for rearrangements, changes in sequence or methylation within 25 kbp of the paramutated allele (Patterson et al.,1993, Genetics 135:881); 4) Paramutation requires sequences upstream of the coding region (Patterson et al.,1995, Genetics 140:1389). To identify and characterize sequences at the b locus required for paramutation, we are currently mapping sequence polymorphisms between B', B-I and neutral and paramutagenic recombinant alleles. To be able to map 5' sequences that participate in paramutation: 1) crosses were performed to isolate recombinants in the upstream region between B' and an allele insensitive (neutral) to paramutation, B-P. Several recombinant alleles have been isolated, both neutral and paramutagenic; 2) 100 kb of upstream B' sequences have been cloned and upstream single copy probes are being identified. The analysis of three neutral B' alleles, one neutral and one paramutagenic recombinant B-P allele suggest that sequences participating in paramutation are located in between 50 and 90 kbp upstream. To identify the 5' and 3' boundary of the sequences involved in paramutation more precisely, more recombinant alleles are being isolated. We will use the cloned upstream B' sequences to characterize all recombinant alleles in detail.

P137 Wax helps: the glossy1 mutant is more sensitive to ultraviolet radiation by some physiological measures

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Some corn-growing areas are already experiencing increased levels of ultraviolet (UV) radiation as a result of stratospheric ozone depletion. Like all living organisms, corn responds to UV radiation. This wavelength of radiation is known to cause damage to DNA, RNA and proteins, and this damage may be a signal that induces adaptive responses. The waxy layer on the outside

of leaves is a first line of defense—putting a sunscreen in this layer, on the outside of the plant, would protect all of the plant's tissues from damaging UV. We have used a variety of morphological and physiological measurements to compare the UV responses of a mutant with very little wax (glossy1) to a near-isogenic line with normal juvenile wax bloom. We find that glossy1 is significantly more sensitive to UV when leaf rolling and biomass are measured. Thus, under some irradiation regimes, wax acts as a sunscreen for juvenile maize leaves.

P138 Identification of genes transcribed from a QTL

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The MARS recombinant inbred population comprises 976 lines derived from two Pioneer proprietary inbreds. These lines were crossed to an appropriate tester and yield tested at 19 locations over 2 years. In addition these lines were genotyped at 172 RFLP marker loci. A QTL analysis for yield detected many QTLs, but the genes responsible for the QTL effects remain unknown. The objective of this project is to identify genes that are transcribed from a specific QTL. To do this we pooled RNA from MARS lines according to the identity of the parental marker alleles at the QTL. Using Curagen RNA profiling technology to compare the RNA pools, we detected 83 band differences. These band differences, which could arise from either differential gene expression or allelic sequence polymorphisms, will be confirmed by mapping. The value of this approach is that it targets genes that are transcribed from a specific genetic locus and that differ in expression or sequence between the two parents. Presumably, these are the genes mostly likely to be responsible for the QTL affect, yet we have undoubtedly detected only a fraction of the genes that reside at this locus. To identify additional genes, we plan to prepare RNA pools from different tissues, developmental stages, environments, and also from hybrid genotypes.

P139 Conservation of maize VP1 function in the dicot, Arabidopsis.

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The maize Vp1 gene is believed to be orthologous to the abi3 gene of Arabidopsis because of similarities of the mutant phenotypes and sequence homology of proteins encoded by each gene (Giraudat et. al., 1992, McCarty et. al., 1991). Here we show that expression of VP1 driven by the 35S promoter can complement abi3-6 , a functional null mutant allele of abi3 (Nambara et. al., 1994). The visible phenotype of seed produced from VP1 expression in the abi3 mutant background is barely distinguishable from wild type. VP1 restored ABA sensitivity of abi3 during seed germination. Ectopic expression of VP1 in vegetative tissue enhances ABA inhibition of root growth. In addition, 35S-VP1 conferred ABA inducible expression of the normally seed-specific cruciferin C gene in leaves. The ectopic expression phenotype of VP1 in vegetative tissues is consistent with the phenotype derived from ectopic expression of ABI3 in Arabidopsis as previously reported (Parcy et. al., 1994). Furthermore, the expression pattern of C1-GUS and cab3-GUS reporter genes are regulated in 35S-VP1 lines in a similar manner to ABI3 wild type lines. One difference is that the level of C1-GUS expression was significantly lower in the seeds of 35S-VP1 lines than in wild type. These results suggest that VP1 is functionally interchangable with ABI3 at least qualitatively. Interestingly, we observed new phenotypes by the ectopic expression of VP1 in the root. Lateral root formation and root tip swelling induced by auxin were suppressed by ABA in 35S-VP1 lines but not in untransformed control.

Moreover, the ectopic expression of VP1 causes ABA induction of C1-GUS in a localized region of the root elongation zone in which auxin is known to be localized. These results imply the possible interaction between auxin, ABA, and VP1 to control development during seed maturation as well. Since C1 expression is dependent on B3 domain of VP1 which is a DNA binding domain conserved in several classes of proteins in plant including Auxin Response Factors (ARFs), we have addressed on the function of the domain by site-directed mutagenesis on the conserved amino acids among all the B3 proteins. The possible interaction between auxin, ABA, and VP1 and involvement of B3 function will be discussed.

P140 rgf, a mutation reducing grain filling in maize through effects on basal endosperm and pedicel development

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The maize cob presents an excellent opportunity to screen visually for mutations affecting assimilate partitioning into the developing kernel. We have identified a defective kernel mutant termed rgf, reduced grain filling, with a final grain weight 30% of wild-type. In contrast to most defective endosperm mutants, rgf shows gene dosage-dependent expression in the endosperm. rgf kernels possess a small endosperm incompletely filling the papery pericarp, but embryo development is unaffected. The mutation conditions defective pedicel development, and greatly reduces expression of endosperm transfer layer-specific markers. rgf exhibits striking morphological similarities to the mn1 mutant, but maps to a locus ca. 4 cM away from mn1 on chromosome 2 of maize. Despite reduced starch accumulation in the mutant, no obvious lesion in starch biosynthesis has been detected. Free sugar levels and sugar flux, measured in cultured kernels, are not markedly altered. However, rgf and WT kernels excised at 5 DAP and cultured in vitro, develop differently in response to variations in sugar regime: glucose concentrations above 1% disrupt placentochalazal development in rgf kernels, but have no effect on cultured WT kernels. These findings lead to the proposal that either uptake or perception of sugar(s) in endosperm cells at 5 to 10 DAP may determine the rgf kernel phenotype.

P141 High resolution AFLP® genetic maps of Maize

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High-resolution AFLP genetic maps were produced of two well-characterized Zea mays L. populations: 1. An Intermated (IM) population developed by the Iowa State University (ISU)/Pioneer Hi-Bred/Dupont cooperative with the parents B73, a central corn belt line derived directly from Iowa Stiff Stalk Synthetic (BSSS) and Mo17, a central corn belt line derived from Lancaster and Krug germplasm. 2. An recombinant inbred (RI) population developed at the INRA France with the parents F252, an American early dent line, and F2, a French early flint line. The generation of high resolution AFLP genetic maps 1) allows to map a large portion of the AFLP alleles in the maize germplasm, 2) provides a framework for the construction of physical maps and 3) will function as a strong tool in maize marker assisted breeding. The high multiplex ratio of the AFLP technique, combined with the high level of polymorphism of maize facilitated to map a large number of AFLP markers in the maize germplasm with relative ease. The two maps produced of 90 individuals consist of 1543 markers (3057 cM) and 1787 markers (1510 cM) respectively. Although the two populations have no parent in common and the four parental lines are not highly related to each other, 442 common AFLP markers were generated that allow the alignment of both maps. AFLP is a registered trademark of Keygene N.V., the Netherlands.

P142 Linkage Map Integration: An integrated genetic map of Zea mays L.

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Multiple genetic maps are created within many crop species as part of molecular breeding projects. We have developed a computer program to facilitate the integration of multiple genetic maps into a single consensus map, which combines the genetic information from all maps in a common framework. This program has been used to create an integrated map of Zea mays L. The integrated map, based on 23 genetic linkage maps, consists of 5650 molecular markers representing the 10 maize chromosomes. The map will continuously be refined through replacing and addition of novel maps. The integrated map is valuable for comparative genome analysis and as a reference collection for marker assisted breeding applications.

P143 Characterization of the 140-kb Multigenic a1-sh2 Interval

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Rates of recombination per kb vary across the genome. Due to its multigenic nature, the 140-kb a1-sh2 interval is being used as a model to test the hypothesis that all recombination hot spots are genes. Sequence analyses conducted to date on a 50-kb portion of the a1-sh2 interval have revealed the presence of at least two novel genes (genes x1 and yz1). A sequence from the rice a1-sh2 interval was predicted by Chen and Bennetzen (1996) to be a gene (which they termed "gene x"). Our sequence analyses of the maize a1-sh2 interval identified a similar sequence. RT-PCR and cDNA cloning have established that this conserved sequence (gene x1) is indeed genic. Similarly, the yz1 gene was identified by gene finding algorithms including SplicePredictor, NetGene2, Grail and Genie during sequence analysis of the maize a1-sh2 interval. The validity of these predictions was established via RT-PCR and by the subsequent characterizations of cDNA and genomic clones. Comparisons between the predicted and actual gene structures of the x1 and yz1 genes as well as the a1 and sh2 genes indicate that SplicePredictor and NetGene2 are the most suitable prediction algorithms for gene discovery in maize and rice.

P144 Phosphate Transporters in Maize

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PHOSPHATE TRANSPORTERS IN MAIZE Mining the Pioneer/DuPont EST database, we have identified to date six full-length Zea mays inorganic phosphate transporter genes designated ZmPT1, ZmPTII, ZmPTIII, ZmPTIV, ZmPTV, and ZmPTVI. These genes were cloned, re-sequenced, mapped and analyzed showing strong homology to previously identified phosphate transporters. We conducted the re-sequencing (2 strand plus) using the Locus Pocus method (Novagen). Our study indicates that the phosphate transporters in maize comprise a multigene family arising from 12 of our cDNA libraries. All six genes were mapped to the maize chromosomes with three that were binned together on chromosome 1. Currently, we have a Mu insertion line in ZmPT1. These phosphate transporters will undoubtedly prove to be useful for agronomic improvements.

P145 Relationships between yield, stability, and density tolerance

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The MARS recombinant inbred population comprises 976 lines derived from two Pioneer proprietary inbreds. These lines were crossed to an appropriate tester and yield tested at 19 locations over 2 years. In addition, these lines were genotyped at 172 RFLP marker loci. To identify lines that differ in their response to different environmental conditions or stresses, we applied stability analysis to the existing yield data. The yield of each individual line was regressed against the location average yield over the 19 yield test locations, and the slope of the straight line fit to the data was interpreted as the stability of the line. The correlation between yield and stability was weak. We performed a QTL analysis for stability and detected many QTLs, only some of which coincided with the yield QTLs previously detected in this population. Because plant density is a form of environmental stress, we used stability to predict the density tolerance of the MARS lines. To test these predictions, we yield tested two contrasting subpopulations of MARS lines based on stability. Preliminary results are consistent with our predictions. These results demonstrate that stability analysis can usefully be applied to a recombinant inbred population, that stability can be associated with genetic loci, and that stability can be used to predict density tolerance. These results also suggest that we may usefully analyze changes in gene expression that occur in response to environmental conditions by using plant density to impose stress, and they define MARS lines that would be appropriate for this experimental approach.

P146 Genetics of Chlorogenic Acid and Maysin Synthesis in Maize Silks

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Chlorogenic acid and maysin are products of the phenylpropanoid and flavonoid pathways, respectively. Our laboratory is studying the genetic basis of synthesis of these compounds as a model system for understanding the genetic control of biochemical pathways. In this poster we will summarize results for QTL analyses of three F_2 populations; A619 x Mp708, W23*c2/whp1/in1* x Mp708, and W23*c2/whp1/in1* x NC7A. Mp708 was chosen as a parent of two of the populations based on the very high levels of chlorogenic acid present in silk of this inbred line. Our analyses indicate the high level of chlorogenic acid in Mp708 is primarily due to positive alleles of two major QTLs, one from the p1 region of chromosome one and one from chromosome two. W23*c2/whp1/in1* was chosen as a parent to study the role of the chalcone synthase genes in maysin synthesis and to study the interconnectedness of the phenylpropanoid and flavonoid pathways. NC7A was chosen a parent because it accumulates apimaysin, a mono-hydroxylated form of maysin. The population with NC7A will allow us to examine differential synthesis of maysin and apimaysin. Together, the three populations address many questions on regulation of these biochemical pathways.

P147 Effect of p1 locus on synthesis of silk maysin, apimaysin, 3'-methoximaysin and chlorogenic acid in maize

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Maysin, predominantly, and related compounds such as apimaysin, 3'-methoxymaysin, and chlorogenic acid have been pointed out as important antibiotic compounds against corn earworm (Helicover zea Boddie). The objective of this work was to obtain molecular markers associated with synthesis of maysin and related compounds in a mapping population for resistance to Aspergillus flavus infection and aflatoxin contamination. Total 112 probes were used to screen polymorphisms and detected 46 codominant RFLP markers that were used to score 205 F2 lines derived from the cross of lines GT-A1 and GT119. F2:3 families were used to evaluate silk flavonoid compound content. Locus p1, on the short arm of chromosome 1, explained 54.0%, 42.1%, and 28.3% of phenotypic variance for maysin, 3'-methoximaysin plus apimaysin, and chlorogenic acid concentrations. The presence of functional allele in p1 is necessary to activate

the branch of the flavonoid pathway that lead to maysin, 3'-methoxymaysin and apimaysin, but could also affect chlorogenic acid content which branched out at early stage of the flavonoid pathway. Locus p1 alone could be used as molecular marker to select progenies with high silk maysin (above 2%) and related compounds in this population. The usefulness of p1 as marker should be further tested in wide-range germplasm and efforts should be done to convert the RFLP-marker into a PCR-based marker for use friendly.

P148 Genetic variation for phenotypically invariant traits detected in teosinte: implications for the evolution of novel forms

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See Talk Abstract #22

12:05 pm Saturday, 18 March

P149 Heterotic Patterns Among Elite Flint Maize Populations from Argentina

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The development of new heterotic patterns based on the evaluation of exotic germplasm is a concern for many maize breeders interested in broadening commercial germplasm base. A study was carried out to evaluate the combining ability effects and heterotic patterns among flint elite populations from Argentina. Six open-pollinated populations with different geographic origins (13-035, 01-102, 14-057, 17-006, 16-035, and 16-053) were crossed in a complete diallel mating scheme. The parental populations, their crosses, and checks (Dekalb 4F37, a commercial hybrid, and Pitagua, an open pollinated variety) were evaluated at Pergamino, Argentina in a randomized complete block design with four replications in two environments during 1990. Significant differences were observed among entries ($p<0.01$). No interaction was detected between entries and environments. Diallel analysis of variance following the Gardner and Eberhart (1966) model II showed significance for variety effects ($p<0.01$), heterosis ($p<0.05$), and variety heterosis effects ($p<0.01$) but no significant average heterosis effects, and specific heterosis effects. Average heterosis for yield was 154.13 kg/ha (2.83 %). The highest yield among crosses was observed for the crosses 17-006 x 16-035 (6110 kg/ha) and 13-035 x 16-035 (6061 kg/ha) which also showed the highest mid-parent heterosis with 14.1 % and 12.6 %, respectively (significant at $p<0.01$). The superiority of these crosses was due to the variety heterosis effects of the three populations and to the variety effect of 16-035. Compared to the checks, 17-006 x 16-035 yielded 98 % of Pitagua (6244 kg/ha) and 79 % of Dekalb 4F37 (7716 kg/ha), and 13-035 x 16-053 yielded 97 % of Pitagua and 78 % of Dekalb 4F37. Root and stalk lodging were 17 % and 26%, respectively, for 13-035, 20 % and 21 % for 17-006, and 22 % and 16 % for 16-035. Results indicate that the crosses 17-006 x 16-035 and 13-035 x 16-035 could potentially represent an exotic heterotic group for flint maize germplasm. A possible strategy is to form a composite between populations 17-006 and 13-035 and exploit the heterosis with population 16-035. However, it is necessary to improve populations root and stalk lodging prior to the selection of inbred lines.

P150 Associating Phenotypic Traits With Sequence Variation in Maize id1

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This work focuses on associating important agronomic traits with sequence variation. Once the genotype-phenotype connection is made for a trait, useful alleles can be identified that may improve agronomic performance. id1 is a candidate gene for the flowering time phenotype in maize. QTL studies have suggested that id1's genomic region has significant phenotypic effects. 100 inbred lines (both tropical and temperate) are used in PCR reactions that represent the range of phenotypic variation. Sequencing of PCR reactions gives us a sample of allelic variations and will identify putative functional polymorphisms in maize lines. Currently, sections of id1 have been amplified, cloned and sequenced. Linkage disequilibrium will be discussed, along with

id1's association with ear height and flowering time.

P151 New members of RiceMutaor elements by deletion and non-homologous recombination with ectopic DNA segments

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RiceMutator transposable element is a member of super-Mu family distributed from bacterial genomes to higher plant genomes, which harboured in rice genome. It shares structural similarity to maize Mutator and might be regulated by a mudrA like gene. RiceMutator family is composed of elements in RMu1 class which carry a candidate tranposase gene, and those in RMu2 class just carrying homologous TIRs without any genes. We have cloned several RMu1 elements and sequenced to find putative autonomous elements. Some of them are being introduced into Arabidopsis genome to find a new transposition. RMu2 elements have been also cloned and characterized. Some of them were deletion derivatives generating directly from RMu1 elements. The others carried un-related internal sequences to the RMu1 element. One of them, RMu1-A1b carried an insertion in the right terminal inverted repeat, which showed high similarity of the internal sequence of wanderer transposable element. It was not a transposition of wanderer. It was exchanged by ectopic recombination targetted at a short strech, AAG. Additional two elements carried novel sequences. These RMu2 elements will be also examined the ability of the transpositions.

P152 Tourist traps in the maize genome

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Tourist is a family of miniature inverted-repeat transposable elements (MITEs). Compared to other MITE families, the Tourist family of maize is distinguished by its small size (133 bp in average) and strong potential to form secondary structure. In addition, some Tourist elements are present as tandem repeats, a feature not observed for other MITEs. Tourist elements were isolated from a maize genomic library and by a PCR assay. In addition to identifying Tourist monomers, dimers, trimers and even a tetramer were isolated. These multimers contained a variety of nested and distantly related elements, ranging in size from 126 -138 bp. Interestingly, most of the insertions occur at 10 bp intervals from the TIR (terminal inverted repeat) of the target element. These apparent non-random insertion sites suggest an unusual target site preference, which may or may not be related to the target site preference that distinguishes all MITE families. From an evolutionary standpoint, preexisting Tourist elements would make ideal

targets for the insertion of other MITEs.

P153 *MuDR*-like Sequences are Widespread in the Grasses

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The regulator element for the *Mutator*system is *MuDR*, which contains two genes: *mudrA*(the putative transposase) and *mudrB*(the mystery gene). Database searches using the *mudrA*gene sequence reveals that this element is present in both monocot and dicot plant species. We are interested in comparing the evolution of *MuDR*with the evolution of its hosts. Because the evolutionary relationships among the grasses have been well characterized, and the *Mutator*system is potentially extremely active, such a comparison should provide a model system for exploring the coevolution of host and transposon. Using conserved primers we have amplified and sequenced regions of homologous elements in a wide variety of grasses, including representatives of each of the major subfamilies. Many of these sequences are consistent with continued function despite a great deal divergence, suggesting that *MuDR*has been or is currently active within these species. Phylogenetic analysis based on sequence reveals that although some relationships are consistent with the species tree, some are clearly inconsistent, either due to selection within particular clades, or the presence of multiple paralogous sequences within each species. Southern blot analysis reveals that the *mudrB*gene, which has no significant homology to anything in the database, is present in multiple copies in several members of the subfamily *Panicoidea*. We also have evidence for the presence of both *mudrA*and *mudrB*together on the same element in *Zea luxurians*. These analyses reveal that the evolution of the *MuDR*class of *Mu*family transposons predates the grass family, is extraordinarily complex, and involves "extinctions and blooms" of *MuDR*lineages. How a typical *MuDR*line becomes extraordinarily active, as with maize Robertson's *Mutator*Lines, remains a mystery.

P154 ASSESSING THE UTILITY OF MITES AS MOLECULAR MARKERS

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High resolution genetic maps are prerequisites for gene discovery and QTL identification. Generating such maps can both costly and labor-intensive. Although these techniques are used for a wide range of applications, each marker has its limitations. RFLPs and SSRs do not always show a reasonable amount of polymorphism, RAPDs are difficult to reproduce, while AFLPs have a tendency to cluster in the heterochromatin region. To date, there is no available marker technology that specifically targets genic regions and QTL. It is in this light that the use of miniature inverted repeats transposable elements (MITEs) as markers is being explored. MITEs are short, high copy number elements that preferentially inserts in genic regions of maize, rice and possibly all flowering plants. A few MITE families (Hbr, Hb2 and mPIF) were shown to be stable in several lines of maize and teosinte. Extensive polymorphism was exhibited in several lines of maize. These characteristics make MITEs even more appealing for marker development. The use of MITEs in a modified AFLP technique called MITE display may generate markers that are anchored in genic regions. Hb2 is a family of markers that is approximately 315 bp long with 15-bp terminal inverted repeats (TIR). They have approximately 12,000 copies in the maize genome. The utility of Hb2 to generate molecular markers is being assessed with the use of 100 maize inbred lines generated by crossing B73 and Mo17. A total of 563 polymorphic loci, which represents about 57% of all loci detected, were identified. Mapping of these loci on the maize genome, using Pioneer Hi-Bred's current framework of markers, and assessment of their utility as markers are on-going.

P155 Study of the chilling-induced chlorosis by using the virescent mutants of maize.

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Chlorophyll-deficient tissue is a frequent symptom in plants when they experience low temperature. These symptoms also arise in mutated populations of plants. The virescent character is a genetic variant in pigmentation for which a large number is known in maize. It is generally accepted that expression of this character is influenced by temperature sensitivity. Seedlings of the virescent mutants v1, v2, v3, v4, v13, v16, v18, v19, and v26 of maize exhibit chlorosis when grown at temperature below 18-20°C. Chlorotic leaves, grown at 15°C, contain plastids that appear to have been arrested at an early stage of development. The level of photosynthetic pigments are severely reduced. From an analysis of the fluorescence quenching parameters, it is shown that all mutants possess a functioning, fully reversible, non-photochemical quenching mechanism. This is most developed in the v13, v18 and v19 mutants. These three mutants also have a relatively high primary photochemical yield for photosystem II and a functioning photosystem I. Cloning of the virescent genes may allow the study of nuclear genome control

over chloroplast development. Since the cloning of genes affecting chloroplast development at low temperatures, may help application oriented activities in the field of temperature tolerance, gene tagging experiments were developed for the v1 gene using the Ac/Ds transposable elements. We have used the AFLP procedure to amplify a co-segregating DNA fragment. The cloned sequence shows homology to the GTP-binding protein aG68 of A. thaliana involved in the translocation of soluble proteins. The genetical and molecular mapping of the v4 gene are also in progress by integrating molecular and genetical maps.

P156 An Overview And Some Observations From Work On Mutator-Based Reverse Genetics

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An overview of Pioneer's Trait Utility System for Corn (TUSC) will be presented. PCR is used to query a linear index of genomic DNA prepared from over 42,000 (F1) maize plants mutagenized by active, high-copy Mutator (Mu) stocks. A matching index of F2 progeny from each individual is archived for the propagation and study of selected target::Mu alleles. A recent addition to our screening service includes a test for target::Mu allele heritability, based on PCR confirmation in a sample pool of F2 gDNA. This has significantly increased our precision by ruling out interference caused by somatic insertion. Over five years of this experience has produced a host of observations that we will attempt to summarize. Some 50 different Mu donor lines were used (exclusively as males) in this construction in our attempt to minimize any starting bias in the population and to maximize a theoretical saturation of the genome. The simple linear array has been an advantage in one way by preserving pedigree relationships in large blocks of sibling plants. Array hybridization results thus instantly reveal conspicuous cases of parental, grand-parental, and "super-parental" insertion types. The super-parental classes are noted by their ubiquitous presence throughout the pedigrees of the collection, and their common occurrence in the 5' regions of a growing list of target genes. This has been supported by sequence analysis of a number of Mu-Mu PCR products that appear to arise by novel transpositions downstream of these historical events. At the other end of the spectrum, some of our chosen targets appear to be immune to Mu insertion; not just on a germinal, but strikingly on a somatic basis. This appears largely independent of target gene size, predicted function or lethality, and map position. This suggests that other features qualify a gene's susceptibility to Mu insertion.

P157 Development of an En/Spm transposon system for barley

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Barley (Hordeum vulgare L.) is the world's forth most important cereal crop with a genome size of 5,5 x 109 bp and probably contains more than 30 000 genes. Assigning specific functions to these genes will be one of the major

challenges over the coming years. One strategy for resolving the biological role of genes is through the use of transposable elements for insertional mutagenesis. Integration of a transposon into the coding sequence of a gene disrupts its function causing a mutation that may lead to a phenotype reflecting the activity of the gene in question. A more powerful alternative to determine the function of a gene for which only sequence data is known is to use transposon based reverse genetics. In maize a "gene machine" consisting of a large population of plants where statistically every gene is "tagged" by transposon insertion can be screened by PCR to identify a transposon-induced mutation in a gene of interest. The mutant phenotype occuring in the selfed progeny can then be correlated with the gene sequence. The well characterized autonomous En/Spm element from maize has been shown to be active in a number of heterologous plants e.g. Nicotiana tabacum, Solanum tuberosum and Arabidopsis thaliana. In our laboratory we are developing a two component En/Spm tagging system for barley where a plant line carrying a dSpm element is crossed with a second plant line carrying a "clipped wing" element that can activate transposition of the dSpm. In contrast to autonomous elements here the induced mutations can be stabilized by outcrossing the activator. Two efficient, reproducible methods for introducing foreign DNA into barley plants have been developed: 1) Particle bombardment using high pressure helium gas to propel gold particles carrying DNA into the target tissue 2) A natural gene transfer system with Agrobacterium tumefaciens carrying a binary vector construct. The advantages and disadvantages of both methods will be dicussed concerning copy number, insert length and introduction of marker genes (bar and gus) in T0 and T1 plants. In addition we will present the current status of the En/Spm two component system for plants produced by particle bombardment.

P158 Transposition Frequency of *Rescue Mu*

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The large 2500 Mbp maize genome contains a high level of non-coding repetitive DNA (~80%) complicating the discovery of maize genes. The Maize Gene Discovery Project collects maize ESTs (**http://www.zmdb.iastate.edu/** and utilizes transposon tagging to find new genes. A tool has been developed based on an endogenous maize transposon family that preferentially transposes into coding sequences to target genes for isolation. The naturally occurring *Mutator* family of maize transposable elements has been engineered to create *Rescue Mu*, a transposon that enables isolation and cloning of genes from maize into *Escherichia coli* (Manish Raizada and Virginia Walbot). The engineered element was introduced into maize using biolistic transformation. The introduced elements within the transgene arrays showed a high frequency of somatic transposition, but a low frequency of germinal transposition (<<10%). We have hypothesized that the low frequency of germinal transposition was due to transgene silencing, thus our strategy was to identify germinal transposition events in which the *Rescue Mu* element transposed to a new genomic location. We then measured the number of new transposition events in plants that contained a single *Rescue Mu* element and no transgene locus. This experiment was carried out at University of Arizona, Berkeley and UC San Diego, and ten independent transposed *Rescue Mu* elements were isolated. Approximately half of these new active elements generated new transposition events in the next generation. For example, one of these plants (M388-86) served as the progenitor for multiple new insertions, all into different maize locations at a frequency of 25%. Plasmid rescue, sequence analysis and database searches of the new insertions show that *Rescue Mu* is in fact transposing into *bona fide* maize genes (Manish Raizada and Gillian Nan). In the subsequent generation, plants with an even higher frequency of transposition (40-60%) were revealed. Several plants containing multiple copies of active *Rescue Mu* were identified and are serving as the progenitors for *Rescue Mu* tagging grids for the Maize Gene Discovery Project.

P159 Jittery, a low-copy, Mu-related transposon apparently mobilized by BSMV infection

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Stocks infected with BSMV (barley stripe mosaic virus) are known as AR (aberrant ratio) stocks for historical reasons (Sprague and McKinney, 1966, Genetics 54: 1287-1296; Nelson, 1981, Maydica 26: 119-131). In these AR stocks, new mutations at various loci arise with frequencies of 10^{-4} to 10^{-5} and chromosome breakage events occur with high frequency (Mottinger et al. 1984). The *Bs1* retrotransposon and an insertion designated *Tz86* were found to be inserted respectively, in an *Adh* mutant (Johns et al., 1985, EMBO J. 4: 1093-1102) and in a *sh* mutant (Dellaporta et al. 1985, CSHSQB. 49: 321-328) that arose in AR stocks, suggesting a relationship between viral infection, chromosome breakage, and transposition in maize. Interestingly, *bz-m039*, a *bz* mutation also isolated from Sprague's AR stocks, produces a spotted (i.e., unstable) phenotype and reverts frequently in the germline, indicating that the transposon in this mutant is not a retrotransposon. Based on preliminary genetic data, the transposable element at the locus appears t be autonomous. We have isolated and sequenced the insertion present in *bz-m039*. The element, which we have called *Jittery*, is 3.9 kb long, has inverted terminal repeats of 177 bp, and causes a 9-bp direct duplication of the target site. Curiously, the inverted repeats are not perfect: 4 extra bases occur 1 bp away from the 5' end. Sequences homologous to the *Jittery* ends are present in low-copy number in all maize inbred lines examined, but are absent in other grasses. Only a few inbreds appear to have an intact copy of a *Jittery* element, of as yet unknown activity. The putative transposase encoded by *Jittery* is homologous to a *Mutator*-like truncated transposase encoded by a 1.3-kb tomato insertion (Young et al., 1994, Genetics 137: 581-588) and to several *Mutator*-like transposases detected recently in the *Arabidopsis* genome. Thus, *Jittery* resembles *Mutator* in the length of the element's inverted repeats, the size of the target site duplication, and, distantly, in the make-up of its transposase, but differs from it in its low copy-number and in the high frequency with which it excises to produce germinal revertants.

P160 mPIF Elements: Possible Non-autonomous Members of the PIF Transposable Elements

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A novel miniature inverted-repeat transposable element (MITE), named miniature PIF (mPIF), was isolated in a PCR reaction using primers derived from the terminal inverted repeats (TIRs) of the PIF DNA transposon family previously discovered at the maize r locus. Southern blots have shown that mPIF is present at high copy number in maize, teosinte, Tripsacum and coix, but absent from rice and sorghum. We estimated the copy number of mPIF to be 10,000 in maize by genomic library screening. Sequence analysis of 32 randomly chosen mPIF-containing genomic clones showed that members of this family are highly conserved in both nucleotide sequences (90%) and length (358 bp on average), have 14 bp TIRs, flanked by a 3-bp direct repeat, and show strong target site preference. Like most previously described MITEs, mPIF elements are preferentially inserted into single or low copy regions in the maize genome. With the transposon display method we found that the insertion sites of mPIF are highly polymorphic among different maize strains. Interestingly, mPIF elements share identical TIRs and the 3-bp target site duplication, as well as some homology in the subterminal regions, with members of the PIF family. Taken together, our data suggest that mPIF family was active in the recent past and may still be active in some maize strains. Furthermore, mPIF and PIF elements may share a common transposition mechanism, with mPIF being non-autonomous and activated in trans by the transposase encoded by the autonomous PIF elements. We are currently looking for evidence of the mobility of mPIF and its association with the activity of PIF elements.

P161 Non-linear Ac/Ds transposition and maize genome reorganization

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Transposable elements have long been considered as potential agents of large-scale genome reorganization. In maize, particular configurations of transposon termini can induce chromosome rearrangements at high frequencies. We analyzed several genomic rearrangements derived from an unstable allele of the maize P1 (pericarp color) gene carrying both a full-length Ac (Activator) transposable element and a Ac terminal fragment termed fAc (fractured Ac). In one case, a classical maize ear twinned sector yielded two rearranged chromosomes; one contained a large inverted duplication, and the other contained a corresponding deficiency. The rearrangement breakpoints have target site duplications and a transposon footprint, thereby proving that the duplication and deletion chromosomes were generated by a single transposition event involving Ac and fAc termini located on sister chromatids. Because the transposition process we describe involves transposon ends located on different DNA molecules, it is termed non-linear transposition (NLT). Non-linear transposition can rapidly break and rejoin chromosomes, and thus could have played an important role in generating structural heterogeneity during genome evolution. Nine additional NLT-induced large deletions were analyzed. The deletions are all anchored at the Ac/fAc insertion, and extend to various endpoints up to 4.6 cM proximal of the P locus. We conclude that NLT events can efficiently generate interstitial deletions, and that the resulting nested deletions are potentially useful for dissection of local intergenic regions, and for rapid correlation of genetic and physical maps. Finally, a modified NLT model can explain the origin of several complex maize chromosome rearrangements isolated by McClintock.

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