

ABSTRACTS - TALKS AND POSTER PRESENTATIONS

TALKS:

Session 1. Thursday 7:15 pm March 13

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

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

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

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

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

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

Session 5. Friday 7:30 pm

Steve Henikoff Traditional Genetics Meets Functional Genomics

Susan McCouch (Abstract not available).

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

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

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

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

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

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

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

Session 9. 7:30 pm Saturday March 15

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

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

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

Posters

Bioinformatics

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

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

Genome Resources

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

PHYSICAL MAPPING OF THE MAIZE GENOME

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

Biochemical Genetics

- 42 Bierwagen, Tracie Correlation between SU1 isoamylase expression level and amylopectin structure
- 43 Colleoni, Christophe Identification of a novel starch hydrolytic enzyme in maize
- 44 Dierking, Ryan Comparison of cell wall proteins in drought tolerant and susceptible maize lines
- 45 Dinges, Jason A Comprehensive Real-time PCR Expression Analysis of the Maize Starch Debranching Enzyme Gene Family
- 46 Heine, George F Functional Significance of the Evolutionary Steps that Shaped the *R2R3 Myb* Gene Family
- 47 Herschberger, Nicholas Photosynthetic Mutant Library (PML): A Reverse Genetics Resource for Chloroplast Biogenesis Genes
- 48 Houston, Norma The Protein Disulfide Isomerase Multigene Family in Maize
- 49 James, Martha Evidence for physical interactions among specific starch metabolizing enzymes

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

Cytogenetics

- 62 Bass, Hank Single-locus cytogenetic mapping in maize with marker-selected sorghum BACs as FISH probes on pachytene spreads from maize-chromosome-addition lines of oat.
- 63 Golubovskaya, Inna absence of the first division1 (*afd1*) is a maize *rec8*-cohesin
- 64 Hiatt, Evelyn Genetic and Cytogenetic Analysis of Abnormal Chromosome 10 Indicates that at Least Four Loci are Required for Meiotic Drive
- 65 Kato, Akio Chromosome doubling in maize
- 66 Lamb, Jonathan The maize B chromosome contains multiple centromeric elements located away from the functional centromere
- 67 Lee, Michael Increased Meiotic Recombination in Maize Genotypes After Chronic Water-Deficit Stress
- 68 Pawlowski, Wojtek 'Master switches' of meiosis

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

Developmental Genetics

- 70 Barrell, Philippa A screen for non-reduction mutants in maize
- 71 Barret, Pierre Differential expression of a new HSP70-like gene and of the transposable elements Pong and PREM during in vitro androgenesis in maize NILs underlines new aspects of induction mechanisms
- 72 Bomblies, Kirsten Quantitative analyses and transgenic overexpression support the maize FLORICAULA/LEAFY homologs as QTL candidates.
- 73 Braun, David tie-dyed1 promotes the sink/source transition in developing leaves
- 74 Brooks, Lee An analysis of CNS-Ig3-i2 in non-ligule forming plants and plants with altered ligules
- 75 Buryak, Alla Maize Sprouts Polarity as Factor Defining Exogenetic Phytohormones Influence
- 76 Cartwright, Heather Pangloss1 and Pangloss2 are Required for Polarization of Subsidiary Mother Cells in the Formation of Maize Stomata.
- 77 Christophe, Reuzeau TRAITMILL TM :AN APPLIED GENOMICS PLATFORM FOR THE IMPROVEMENT OF CEREALS.
- 78 Consonni, Gabriella Maize mutants arrested in early embryogenesis disclose an irregular pattern of cell divisions and altered programmed cell death
- 79 Danilevskaya, Olga Functional analysis of the maize FT/TFL homologs reveals potential players in the floral transition.
- 80 Deleu, Wim Biochemical characterization of the FEA2 protein
- 81 Fajardo, Diego Molecular Genetic Analysis of rgh Endosperm Mutants
- 82 Frank, Mary Three Brick Genes Are Required for Polarized Growth and Division in Maize Leaf Epidermal Cells
- 83 Fu, Suneng Functional analyses of EMPTY PERICARP2, an essential regulator of the heat shock response that is required for maize embryogenesis.
- 84 Gibbon, Bryan The Protein Secretory Pathway is Upregulated in Several *opaque* Mutants
- 85 Grimanelli, Daniel GENOMIC CLONING THROUGH APOMIXIS RESULTS IN EXTENSIVE EPIGENETIC VARIATION
- 86 Hake, Sarah Clonal analysis of Wavy auricle in blade (Wab)
- 87 Henderson, David ragged seedling2 fails to maintain the dorsoventral axes of leaf tissues in maize
- 88 Hollick, Jay PARAMUTATION AND PLANT DEVELOPMENT REQUIRE *rnr12* FUNCTIONS
- 89 Inada, Noriko The characterization of pleiotropic shoot phenotype in *leafy coleoptile(lco)1-R*

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

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

Genome Structure/Syteny

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

Molecular Genetics

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

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

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

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

Quantitative Traits

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

Heterosis

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

Transposable Elements

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

Talk Abstracts

Session 1. Thursday evening.

Vicki Vance

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

Allison Mallory, Lewis Bowman and Vicki Vance.

Department of Biological Sciences, University of South Carolina, Columbia, SC 29212 USA

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

Neelima Sinha
The Development and Evolution of Leaves

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

Section of Plant Biology, University of California, Davis, CA 95616 *Department of Ecology and Evolution, State University of New York, Stony Brook, NY - 11794-5245

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

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

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

Friday Morning

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

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

Paramutation is an epigenetic phenomenon in which one allele heritably reduces expression of the other allele. We are using the well defined maize p1 gene as an experimental system to address questions crucial for elucidation of the mechanism of paramutation: 1) what regulatory sequences are required for paramutation and 2) what trans-acting factors are involved in maintenance and establishment of paramutation. The p1 gene is a myb-like transcriptional activator that regulates the expression of structural genes for flavonoid pigment biosynthesis and leads to accumulation of the red phlobaphene pigment in floral organs of a mature plant. The active state of the paramutable P1-rr allele results in uniform red pigmentation of pericarp and cob, while paramutated state, named P1-rrf, has streaky pericarp and pink cob. Major progress has been made towards identification of the sequences involved in p1 paramutation when we showed that a 1.2 kb enhancer fragment from P1-rr induces P1-rrf paramutation when introduced as a transgene. In the absence of the inducing transgene, P1-rrf was heritable and paramutagenic to a naïve P1-rr allele (Sidorenko and Peterson, 2001, Plant Cell:13, 319-335). Subsequent experiments established that the bulk of paramutagenic activity is localized within a 400 bp sub-fragment of the 1.2 kb enhancer. Here we will present results of ongoing transgenic experiments that aim to define paramutagenic sequences to an even smaller fragment of approximately 200 bp. As a step toward identification of trans-acting factors involved in p1 paramutation, we have begun to test whether transacting mutants that affect paramutations of other genes also affect maintenance of the P1-rrf state. Intriguingly, the results indicated that the maintenance of p1 paramutation was not disrupted in homozygous mop1 (mediator of paramutation 1) background; this is in contrast to the observation that mop1 disrupts b1 and p11 paramutation (Dorweiler et al., 2000, Plant Cell:11, 2101-18) and similar to r1 paramutation which persisted in the presence of mop1 (Chandler and Kermicle, unpublished data). Importantly, mop1 is required to establish paramutation at b1, p11 and r1. Experiments to test whether mop1 is required for establishment of p1 paramutation are in progress. The other two mutations tested, rmr1 and rmr2 (required for maintenance of repression 1 and 2), also had no significant affect on the maintenance of p1 paramutation and this result is different from that for b1 (Chandler, unpublished data) and p11 (Hollick and Chandler, 2000, Genetics:157, 369-378). Based on obtained results we hypothesize that maintenance of paramutation at the b1 and p11 loci and r1 and p1 loci differ in their requirements for transacting factors encoded by mop1, rmr1, and rmr2 genes.

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

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

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

3

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

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

4

MaizeGDB: A Next Generation Maize Database

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

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

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

5

**The Maize Genome Sequencing Project at the Donald Danforth Plant Science Center
Barbazuk, Brad Barbazuk {1} Whitelaw, Catherine {2} Quackenbush, John {2} Bennetzen,
Jeff {3} Schubert, Karel {1}**

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

6

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

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

{1} The Institute for Genomic Research {2} The Donald Danforth Plant Science Center {3} Orion Genomics {4} Purdue University
Maize is both a classical genetic model for plant research and an economically important crop; however, the size and complexity of the maize genome deem it recalcitrant to whole genome sequencing. Current estimates indicate that genes constitute a mere 15-20% of the maize genome with the remainder consisting of highly repetitive DNA. The initial objective of the Consortium for Maize Genomics is to evaluate two approaches to sequencing the maize "genespace" (methylation filtration and high Cot selection) in order to provide the most rapid and cost-effective alternative to sequencing the whole genome. At TIGR, we are generating paired end sequence reads from 250,000 methylation filtered clones and 250,000 high Cot clones. The sequences are clustered and assembled, both independently and in combination, at quarterly intervals. The resulting maize genomic assemblies and singletons are annotated based on homology searches, with subsequent development of improved methods for annotation. The results of these analyses, a BLAST-searchable database and Maize Assembly Annotator are presented in the TIGR Maize Database (<http://www.tigr.org/tdb/tgi/maize>). The results of the latest assemblies will be presented and discussed.

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

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

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

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

8 A Role for Maize ROP2 GTPase in the Male Gametophyte

Fowler, John {1} Arthur, Kirstin {1} Vejlupkova, Zuzana {1}

{1} Oregon State University

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

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

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

{1} UMR 5667 INRA-CNRS-ENSL-UCBL {2} Biogemma SA

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

10

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

Bommert, Peter {1} Running, Mark P {2} Vollbrecht, Erik {3} Hake, Sarah {4} Werr, Wolfgang {1}

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

11

Molecular and evolutionary analysis of *ramosa1* in inflorescence architecture

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

{1} Cold Spring Harbor Laboratory

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

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

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

Steve Henikoff {1}

Traditional Genetics Meets Functional Genomics

Till, Bradley J. {1} Greene, Elizabeth A. {1} Ng, Pauline C. {1} Reynolds, Steven H. {1} Young, Kim {1} Codomo, Christine A. {1} Enns, Linda C. {2} Johnson, Jessica E. {2} Burtner, Chris {2} Odden, Anthony R. {1} Taylor, Nicholas E. {1} Henikoff, Jorja G. {1} Colbert, Trenton {1} McCallum, Claire M. {1} Comai, Luca {2} Henikoff, Steven {1}

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Single-nucleotide changes can be induced in practically any organism using traditional chemical mutagens that have been widely used by geneticists for several decades. We have introduced a reverse-genetic strategy we call TILLING (for Targeting Induced Local Lesions IN Genomes) based on deleterious point mutations. TILLING uses chemical mutagenesis of reference individuals and screening for point mutations in a region of interest. Because base substitutions in proteins provide allelic series, and not just knockouts, this strategy can yield refined insights into protein function.

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

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

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

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

12

Population structure and gene diversity of American maize landraces

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

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

{4} North Carolina State University

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

13

The genomic origin of maize revisited

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

{1} Iowa State University {2} Stanford University

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

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

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

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

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

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

The *rp1* rust resistance locus of maize consists of a cluster of NBS-LRR genes. Different *rp1* haplotypes can be very different structurally due to mispairing and recombination in meiosis. Different maize haplotypes may carry as few as one to more than 40 *rp1* genes, most or all of which have no detectable phenotypes. The phenotypically undetectable genes may be functional but simply do not recognize any of the currently prevalent rust biotypes. We are characterizing recombinant haplotypes with novel phenotypes to identify the genes controlling the phenotypes and to characterize the types of recombination events that give rise to them. We have generated and characterized novel haplotypes with nonparental race specificities (Hrp1-D11, DI28 and D2I). Comparisons of these haplotypes to the parental haplotypes has indicated most of the novel race specificities are due to the reassortment of the *Rp1* genes into novel combinations. We have also characterized haplotypes with more unusual phenotypes. Two of these confer defense reactions to any rust isolate and confer lesion mimic phenotypes under normal growing conditions, and a third induces defense responses spontaneously (Hrp1-Kr1N). These phenotypes are controlled by genes derived from recombination events in their LRR-coding regions. We have constructed genes similar to some of these *in vitro* and are now testing them in transgenic plants to examine the structural basis for their novel regulation of defense responses. We are also testing them in transient transformation assays in maize and other cereals to determine the extent to which *Rp1* genes can function in different taxa. The identification and characterization of genes with novel phenotypes will shed light on how complex resistance genes function and evolve.

16 Identifying active DNA transposons in the genomic era

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

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

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

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

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

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

18

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

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

19

Distribution of retroelements in centromeres and neocentromeres of maize

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

20

Cytological crossover maps for all maize bivalents using recombination nodules

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

Cytological markers such as chiasmata, MLH1 foci, and recombination nodules (RNs) are useful for defining the frequency and distribution of crossovers along the length of chromosomes. Of these, RNs provide the highest resolution currently available because they are observed by electron microscopy of synaptonemal complexes (SCs) in extended pachytene chromosomes. The most useful cytological crossover maps are those in which each bivalent can be unequivocally identified and related to a specific linkage group. To date, such maps have been generated only for tomato (using RNs) and mouse (using MLH1 foci). To achieve this goal for maize, we have prepared an SC karyotype for the maize inbred line KYS, in which each SC can be identified based on its relative length and arm ratio. Each SC was related to the proper chromosome and linkage group using inversion heterozygotes. Using this karyotype, we mapped RNs on more than 2000 SCs to produce high resolution maps of RN frequency and distribution on each bivalent. The average RN frequency per bivalent is closely correlated with SC length. The crossover frequency using RNs is about 10% higher than estimated using chiasmata. While the total length of the RN map is about two-fold shorter than current linkage maps, the correlations between the RN and linkage maps are good ($r^2 > 0.63$) when the cM lengths of the bivalents are compared. Each bivalent has a unique distribution of crossing over, but all bivalents share certain general characteristics such as a high frequency of distal RNs and a severe reduction of RNs at and immediately adjacent to kinetochores. The frequency of RNs at knobs is either similar to or higher than the average frequency of RNs along the SCs. These RN maps represent an independent measure of crossing over along maize bivalents and provide a means to integrate genetic linkage maps with chromosome structure.

21

The pathway of early meiotic prophase events in maize

Cande, Zac {1}

{1} University of California, Berkeley

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

Session 8. Saturday afternoon QTL workshop Torbert Rocheford.

Torbert Rocheford

Overview of Maize QTL Studies

Department of Crop Sciences, University of Illinois

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

Mike Lee

Map Construction and Use for Mutant Clone & QTL Mapping

Iowa State University

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

Martin Bohn

Methodologies of QTL Analysis and Statistical Considerations

Department of Crop Sciences, University of Illinois

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

Mike McMullen: QTL Approaches to Study of a Pathway

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

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

We investigate the molecular mechanisms that regulate changes in the distributions and densities of specialized epidermal features and cell types during shoot development. To complement our traditional developmental genetic analyses of *glossy15*, *dwarf1*, *macrohairless1* and *macrohairless2*, we have undertaken multiple QTL investigations to identify, map and characterize the functions of other genetic factors affecting these distributions and densities. Here we report, as *users* of the community resource, several results that demonstrate the improved resolution of map positions for QTL detected using the IBMRI resource. For example, QTL analyses of the wax and macrohair markers for vegetative phase change each detected a QTL which corresponds to *glossy15* on chromosome 9L, while analysis of macrohair density on leaf 9 detected a QTL which likely corresponds to *macrohairless1*, which maps 5cM distal to *gll5* on a non-intermated map. The increased length of the IBM genetic map allows the clear resolution of these two separate effects on macrohairs, whereas a non-intermated map would not. This improved map resolution allows more robust inferences concerning function and pleiotropic action of individual QTL when related traits are investigated. Since such inferences require further investigations, we present two strategies that employ the IBMRI resource for further experiments. Both center on the identification of IBMRI lines carrying specific combinations of QTL alleles identified in silico, which are then treated as Nearly Isogenic Lines (NILs) for breeding or used directly for molecular genetic analyses. We believe our success with using this tool to analyze epidermal traits will be generalizable, since B73 and Mo17 are phenotypically quite similar for some of the traits for which we detected numerous robust QTL

Ed Buckler: Principles of Associative Genetic Analysis

USDA-ARS, North Carolina State Univ.

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

Session 9 Saturday evening

Hugo Dooner

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

Waksman Institute, Rutgers University

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

Session 10. Sunday morning.

22 Initial cloning and characterization of vp13 in maize

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

23

A multidrug-resistance associated protein involved in anthocyanin transport in *Zea mays*.

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{1} Stanford University

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

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

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

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

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

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

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