

Combined analysis of genomic, transcriptomic and epigenomic variation among maize inbreds

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Most breeding efforts rely upon selection of natural variation present within a species. We have been actively cataloguing variation in the genome structure, epigenome and transcriptome of maize genotypes to further understand the variation that could be targets of selection. There is evidence for wide-spread genomic differences among maize lines including copy number variation (CNV) and presence-absence variation (PAV). A detailed CGH characterization of CNV/PAV performed on a subset of maize genotypes revealed wide-spread differences in genome content affecting ~20Mb of the low-copy portions of the maize genome. A subsequent analysis used a gene-centric microarray to assess gene content variation among a large number of maize lines and the wild ancestor of maize, teosinte. We found that ~10% of maize genes present in the reference genome sequence are either missing or altered in copy number in at least one other genotype studied. These genome content differences among lines can have implications for variation among lines and the content of hybrid genomes. The same array platform was used to assess how the transcriptome was reshaped by domestication. Co-expression network analyses identified many networks that have been affected by domestication. Current studies are focused on assessing epigenomic variation. There are many differences in DNA methylation patterns among different maize genotypes. The analysis of epigenomic and genomic variation highlights examples where PAV/CNV show epigenetic modifications as well as examples in which the two genomes do not have structural variation but do have epigenetic variation.

Maize–Teosinte Introgression Libraries Reveal Greater Allelic Effects for Flowering Time QTL Than Maize Inbreds

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Genetic analysis and improvement of crops relies on variation in genes controlling agronomic traits. In maize (*Zea mays* ssp. *mays*), artificial selection during domestication starting 7500 years ago and/or modern plant breeding over the last century has diminished this critical genetic variation. This is especially true for key genes responsible for traits that define differences between maize and its wild ancestor teosinte (*Zea mays* ssp. *parviglumis*). We have developed ten teosinte introgression libraries, each derived by backcrossing a different parviglumis accession into B73. The ten libraries, comprising 887 BC4-derived introgression lines, were characterized with 768 SNP markers to define the introgressed teosinte chromosomal regions. Each line contains an average of 3 chromosomal segments encompassing ~4% of the teosinte genome. These libraries were evaluated for flowering in five to ten environments. Many of the genomic regions affecting flowering time in the teosinte libraries were in common with flowering QTL identified by Nested Association Mapping (NAM), but larger allelic effects were observed. The development and evaluation of these maize-teosinte insertion libraries will enable

us to evaluate allele series, test the impact of domestication on trait variation, and reintroduce valuable genetic variation into maize germplasm.

Changes in Plant Morphology in Recurrent Selection Programs in the Iowa Stiff Stalk Synthetic Population

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The maize plant phenotype has changed a great deal through the era of hybrid maize production. Some of the observed changes such as upright leaf angle, silking-anthesis interval, and tassel branch number, have well understood contributions to improved grain yield in modern hybrids. However, less is known formally about indirect selection responses for these phenotypes in the context of recurrent selection programs. The objective of this study was to determine if recurrent selection for agronomic performance in Iowa Stiff Stalk Synthetic (BSSS) population has changed important plant phenotypes. Thirty synthetic populations representing a total of 29 cycles of recurrent selection in three recurrent selection programs in BSSS were evaluated in four Iowa locations in 2008 and 2009. The most consistent changes observed across selection programs were for phenotypes that increase light penetration into the canopy, including flag leaf angle, flag leaf size, and tassel branch number. Light-interception phenotypes had more consistent responses in the populations *per se* evaluated here than agronomic traits selected for on a testcross basis in the recurrent selection programs. Selection responses for morphological phenotypes in populations *per se* suggested these phenotypes may have much simpler inheritance than typically assumed for grain yield.

Genetic Analysis of Maize Visual Kernel Color - Segregation, Bulk Segregant and Nested Association Mapping Analyses

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Carotenoids are very important nutritionally, as sources of pro-vitamin A for the developing world, and as antioxidants important for eye health for both developing and developed country populations. Simple visual selection for darker orange color has been associated with an increase in total carotenoids. Visual scoring is much less expensive and time-consuming compared to other quantitative carotenoid measurements such as HPLC and NIR.

Segregation analysis using visual scoring of S1 kernels from a four-parent synthetic revealed that colors segregate in a relatively simple mendelian manner and that very few genes are involved in the conversion from yellow to orange kernel color. Bulk Segregant Analysis (BSA) was performed on bulks of light orange and dark orange kernels from an F2:3 mapping population.

The kernels making up the bulks were scored visually and the genotyping was done using an Illumina MaizeSNP50 chip. Paired t-tests were performed to detect genetic differences between the bulks. Several loci were statistically different, some mapped to regions of the genome near known carotenoid biosynthesis genes.

QTL analysis was performed on 10 Nested Association Mapping families segregating for yellow and orange kernel color. The results of both individual family and joint family analysis further indicated that only a few genes are largely involved in the conversion from yellow to orange kernel color. Markers near two logical candidate genes, *phytoene synthase 1 (psy1)* and *lycopene ϵ -cyclase (lcy ϵ)*, were significantly associated with the trait in a number of families as well as in joint analyses. Other QTL having smaller effects on kernel color are located near carotenoid biosynthesis genes including *whitecap 1 (wcl)*, *β -carotene hydroxylase 1 (crtRB1)*, *ζ -carotene desaturase 1 (zds1)*, & *yellow 8 (y8)*.

The combined information from these studies provides confirmation that few genes are involved in the conversion from yellow to orange kernel color, and that phenotypes associated with genetic differences at these loci can be detected visually. This proven ability to select for higher total carotenoids visually will be especially useful in the developing world as local breeders and farmers can make selections for darker orange maize kernels which are much higher in provitamin A than the traditional white maize presently consumed. In the developed world, lines containing favorable alleles of the loci detected in these studies will be used in Marker-Assisted Selection (MAS) breeding programs to biofortify maize for higher antioxidant levels. Selection for darker orange kernels can be paired with MAS for alleles of genes associated with increased provitamin A to increase flux into the carotenoid biosynthetic pathway and to improve provitamin A content.

Techniques, technologies and approaches to improve maize aflatoxin resistance

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Contamination of aflatoxin, a carcinogen produced by the fungus *Aspergillus flavus*, in maize is a serious problem in Texas, the southern United States and many developing countries worldwide. Limited germplasm with improved resistance has been bred and QTL that reduce aflatoxins have been identified. New high throughput techniques for inoculation have also been developed. However, a major challenge of identifying resistant maize germplasm, QTL and eventual fine mapping to genes/alleles is the cost and difficulty associated with quantifying contamination by the toxin or the fungus. One approach we are using is near infrared

spectroscopy (NIRS) a rapid and cost effective technique to quantify the composition of a sample using the molecular absorbance/ reflectance of near infrared light. Using 300+ inoculated samples from an association panel, NIRS calibrations were developed for whole and ground samples based on Vicam aflatoxin levels, quantitative PCR fungus levels, and ear disease severity ratings. All calibrations were successful but the calibrations are most likely detecting the fungus and not the toxin *per se*. An additional ~800 inoculated samples from the Texas A&M corn breeding program were also calibrated using the Vicam Aflatest. It was determined that *A. flavus* contamination might be predicted well enough by NIRS for initial screening in breeding and genetic mapping, but not well enough to replace official measurements. These calibrations become more robust with additional samples in the future and further integrated into a maize breeding program. An additional technology being used in breeding is a mutant allele of the maize lipoxygenase (LOX5) gene, shown to decrease levels of aflatoxin. Novel native alleles have also now been identified and will be tested for function in an association mapping experiment this summer.

The Effect of Intermating on Hybrid Trait Variation and QTL Mapping in Maize (*Zea mays* L.)

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The effect of intermating on hybrid trait variation and QTL mapping was studied within a population developed from two phenotypically similar parents. Two populations, a selfed recombinant inbred line (SRIL) and an intermated recombinant inbred line (IRIL), were developed from the same parental inbred lines, the short season Iodent inbred line CG60 and the short season Stiff Stalk inbred line CG102. Plants within these populations were crossed to an inbred line from a different heterotic group, LH295 of the Lancaster Sure Crop heterotic group, and resulting testcross individuals were evaluated for grain yield and a number of agronomic traits in six environments. Genetic variance for these traits did not significantly differ between the IRIL and SRIL populations. In addition, heritability was similar in the SRIL and IRIL populations, and correlations between traits did not differ significantly. Thus, our results indicate that alleles that contribute to phenotypic variation are not in repulsion phase linkage. We also identified 12 QTL in the SRIL population and 6 QTL in the IRIL population. The QTL confidence intervals within the IRIL population were reduced by a factor of 1.65 compared to SRIL. However, few QTL were shared between the IRIL and SRIL populations, suggesting that a number of small effect loci segregate within these populations.

Combining Ability and Acceptability of Temperate Sweet Corn Inbreds Derived from Exotic Germplasm

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Excellent table quality is an essential characteristic of commercial sweet corn (*Zea mays* L.) and commonly held paramount as selection criterion. With such emphasis on table quality, breeding for other desirable characteristics such as improved plant vigor or enhanced disease resistance in sweet corn has been considerably restricted in comparison to U.S. dent corn breeding efforts. The narrowness of current sweet corn germplasm suggests potential exists for yield enhancement through new heterotic combinations or reduction in the susceptibility to disease pressure by expanding genetic diversity through introgression of non-sweet germplasm. Our research objective was to examine the potential for incorporation of exotic breeding material in the development of improved temperate sweet corn varieties, analyzing the general combining ability (GCA) and specific combining ability (SCA) of traits of interest. Parents of the experimental entries included six inbred, four of which are classified as Mexican dent, derived from a cross of Mexican lowland tropical maize to *shrunken2* germplasm. The remaining two inbred lines have backgrounds incorporating U.S. dent corn, with the first derived from an A632 x IAsH2 cross and the second derived from a cross of Dairyland ST 1180 x Contender. Those six inbred lines were crossed with three *shrunken2* germplasm testers, all of which are temperate varieties. Experimentation was conducted at four Wisconsin environments: Arlington and West Madison in 2009 and 2010 with three replications in all environments. Twenty-one plots comprised of the eighteen hybrid entries and three commercial checks were arranged as a randomized complete block design with each plot consisting of two rows in 2009 and four rows in 2010. Significant differences between hybrids and checks were identified for several traits of interest, suggesting incorporation of exotic germplasm in sweet corn breeding program holds potential.

Metabolic reprogramming and genetic variation associated with pre-mature senescence in maize

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Leaf senescence is a highly organized process characterized by breakdown of proteins, lipids, and chlorophyll and remobilization of breakdown products to the sink. Since these events lead to loss of photosynthetic productivity, delayed senescence can potentially extend carbon fixation and increase grain and biomass yield. Aim of this study was to better understand the molecular mechanisms regulating leaf senescence. We induced premature leaf senescence in B73 inbred of

maize by preventing pollination and assessed differential metabolic and global transcriptional changes in leaves and internodes at six stages during normal grain filling. Early senescence was associated with increased accumulation of glucose in leaves, and to a lesser extent in internodes during the early and middle period of grain filling. Interestingly, these differences were not observed at later stages, likely due to loss of photosynthetic activity in early-senescent plants. In contrast, pentose sugars showed lower accumulation in leaves and internodes of early senescing plants. Microarray analysis revealed substantial transcriptional reprogramming in leaves at 18 DAP (days after pollination) upon onset of pre-mature senescence that is consistent with major changes in the metabolic profile. Expression patterns of genes involved in sugar transport were consistent with accumulation of these metabolites in leaves and internodes. We also looked at genetic variation for the pre-mature senescence phenotype and the associated hyperaccumulation of free sugars in 39 diverse maize inbred lines and found substantial variation for both traits. Exploration of natural variation for pre-mature senescence and associated metabolic changes along with transcriptional and metabolic profiling provides a framework for detailed investigation of molecular basis of senescence. We are now working on identification of novel genes that will be potential candidates involved in the regulation of senescence in maize.

Index and sampling size for evaluating corn earworm injury to corn ears

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Corn earworm, *Helicoverpa zea* (Boddie), is a major pest of corn worldwide. Widstrom's penetration index is the prevalent method for evaluating corn earworm injury level. However this method does not consider the ear length and may lead to the imperfect understanding of corn resistance and injury level. Frequency distributions of the data derived from Widstrom's method are anormal in some cases. We introduced a penetration ratio index, which is the Widstrom's penetration index divided by the ear length. Penetration ratio index generates normal frequency distributions and represents the relative injury level. Field evaluation of corn earworm resistance in a breeding program is labor intensive because numerous genotypes must be examined. The current prevalent practice is to hand-harvest 10 ears per plot to examine the feeding damage. However, to our knowledge, there is no report regarding the minimum number of ears per plot for reliable results. The coefficients of variation of penetration ratio index fluctuated greatly when the number of ears changed from one to four per plot, and stabilized in most of the hybrids when the number of ears reached five in all experiments. When five ears were examined per plot, the means of penetration ratio index in 98.9% cases in three years were still within the 95% CIs of the means of 10 ears per plot. The results showed that five ears per plot can generate acceptable results and sampling additional plants does not change the results. This can save 50% on labor compared to 10 ears per plot.

Effective Recombination in Plant Breeding and Linkage Mapping Populations: Testing Models and Mating Schemes

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Effective recombination events are recombination events that result in novel genetic combinations which can be directly observed; always less than the actual and expected number of recombination events. Population designs can improve effective recombination which is often a limiting factor in breeding and genetic linkage mapping. Using a simulation approach, this study sought to model and quantify effective recombination under various population designs. The number of markers needed to observe all effective recombination events and the distribution of the expected number of effective recombination events were then estimated. Three recombination models were used including one with recombination rates fit to the large *Zea mays* L. nested association mapping (NAM) dataset. Strong evidence was found in the empirical NAM dataset supporting a two- pathway modified Poisson model of recombination events with separate rate λ for each chromosome, reflecting the significant differences in effective recombination rates found across chromosomes. A positive linear relationship between the mean number of effective recombination events per generation and genomewide heterozygosity was observed. Primarily because of this phenomenon, dihybrid and doubled haploid populations increased the number of effective recombination events per generation when compared to traditional biparental recombinant inbred line populations. This study will be useful for quantitative geneticists and breeders in identifying efficient production of effective recombination events as well as researchers simulating recombination.