Meeting report

Genomic, chromosomal and allelic assessment of the amazing diversity of maize
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Teosinte thrived in the highlands and valleys of central Mexico 8,000 years ago. Human selection for increased seed number, cob size, poor seed dispersal, and nutritional value domesticated this wild plant into what we recognize today as maize. The 2004 Maize Genetics Conference was the first to be held near the site of the origin of maize and the present-day center of species diversity, and questions about the origin, types and consequences of maize diversity were central to the 42 talks and nearly 200 poster presentations. A starlight tour of the Museo Nacional de Antropología [http://www.mna.inah.gob.mx/] allowed delegates to examine the depiction of corn by successive pre-colonial Mexican civilizations for further inspiration.

Modern maize captured the genetic diversity of teosinte
Ed Buckler (USDA-ARS at Cornell University, Ithaca, USA) has analyzed maize diversity by sequencing 18 genes, *in toto* or in part, from more than 100 inbred lines. As a benchmark consider that humans have about 0.09% base substitution in pair-wise comparisons and that as a species we are 1.34% different from chimpanzees. Evaluating pairs of modern inbred lines of maize, previous work has shown that there is 1.42% silent diversity in coding regions! In a typical gene there are between 20 and 25 amino-acid polymorphisms among alleles: 30% are radical changes and a further 22% are 'indel' mutations of missing or added amino acids. This tremendous diversity in maize reflects the maintenance of genetic differences from teosinte: domestication did not involve a bottleneck with a handful of representative alleles; rather, present-day corn has alleles that have been filtered by selection over millions of years. Buckler estimated that a single family gathering teosinte seed to supply 10% of their calories would have required 300,000 plants. The several million people of ancient Mexico at the onset of maize domestication probably used seed from teosinte populations of several billions of plants at all stages of domestication. In contrast, only a few tomato or pepper plants suffice in a kitchen, and the domesticated types exhibit correspondingly low genetic diversity.

Using diverse alleles, association genetics can pinpoint which polymorphisms confer specific phenotypes. To avoid false assignments between genotype and phenotype, a robust knowledge of population structure in maize lines allows line history to be separated from independent genetic changes that confer plant properties. Buckler's group and others have further established that linkage disequilibrium (LD), a measure of the recombinational history of chromosomal regions, decays within 1 kilobase (kb) for landraces (traditional varieties grown by subsistence farmers), within 2 kb for modern maize inbred lines used by geneticists, and in roughly 2-20 kb in the elite commercial inbred lines developed in the past decades for the hybrid corn seed industry. For loci with a major impact on productivity and plant architecture, ancient and modern plant breeders have applied stringent selection, and in these cases LD expands to cover a larger region and the drop in allele diversity can be used to link quantitative trait loci (QTLs) to genic regions likely to be important in domestication and yield. For example, four of six genes in the starch biosynthesis pathway show a significant decrease in allele diversity compared to only 5% of randomly selected loci. Recently published work from Buckler and collaborators describes an analysis of ancient maize specimens and showed that particular alleles of *Teosinte branched1*, which encodes a modulator of stem and floral architecture, and *Pbf*, encoding a regulator of seed storage protein, were fixed about 4,000 years ago in domesticated maize, whereas favorable alleles of *Sugary1*, key to producing sweet corn,

Figure 1
Variable composition of maize chromosomes 2 and 6 in ten modern inbred lines of maize. Multiple FISH probes directed to repetitive elements in the genome were used to visualize the location of these elements on each chromosome. Photograph courtesy of Akio Kato.

were not selected in the corn grown in the southwestern USA until approximately 1,000 years ago.

And what has been the fate of teosinte? Jerry Kermicle (University of Wisconsin, Madison, USA) illustrated that it grows robustly in uncultivated areas, and as a weed in Mexican cornfields, often mimicking the morphology of modern maize so closely that farmers cannot recognize it. How does teosinte persist if it is interfertile with domesticated maize? Kermicle explained that haploid maize pollen performs poorly on teosinte silks, where many centimeters separate pollen attachment and the individual ovules on the ear. Teosinte carries dominant alleles of the *Gametophyte factor1* (*Ga1*) locus that confer preferential growth on a *Ga1* silk; in contrast, modern corn is *ga1* and this pollen is only 1% as successful on teosinte *Ga1* silks. This 'trick' is employed commercially to permit selective pollination within small blocks of sweet corn or popcorn despite the billions of wind-borne pollen grains from nearby standard corn. *Ga1* alone cannot explain the crossing barrier between teosinte and corn, however, because Mexican landraces of corn carry the *Ga1-male acting* allele that is compatible with *Ga1* teosinte silks. Kermicle reported a second gene, *Teosinte crossing barrier1* (*Tcb1*), that reduces inter-crossing many-fold by restricting pollen with the recessive *tcb1* allele from growing on *Tcb1* teosinte silks. Interestingly, the dominant *Tcb1* allele is found primarily in the weedy teosinte in corn fields, where it effectively blocks pollen flow from maize and may thus contribute to an incipient speciation process.

Chromosome organization: surprises in the 'junk' DNA

Maize genes, like those of rice and *Arabidopsis*, are generally compact with short introns and key promoter motifs located close to the coding region; a typical gene occupies 2-10 kb. But the maize genome is 20 times larger than that of *Arabidopsis* and 6 times larger than that of rice, as a result of the amplification of diverse families of retroelements. Individual or small clusters of maize genes are 'islands' of coding region

in a vast sea of inactive transposons that occupy most of the genome; recombination is at least one or two orders of magnitude higher in the genes. And these genes are on the move: a published study of the 32 kb region around the *bronze1* gene by Fu and Dooner in 2002 established that there are inbred lines with nine additional genes as well as inbred lines in which some of these genes are on other chromosomes or are entirely absent. To ask if the repetitive 'backbone' of the chromosomes was also rapidly changing, Jim Birchler (University of Missouri, Columbia, USA) reported the work of his postdoctoral fellow Akio Kato, who has developed a suite of fluorescent *in situ* hybridization (FISH) probes to detect moderately repetitive sequences that can distinguish each of the ten maize chromosomes in somatic cells. Comparing ten modern inbred lines of maize revealed that each line had a distinctive chromosome pattern as illustrated for chromosomes 2 and 6 in Figure 1. The repetitive component of the genome is, therefore, varying quantitatively (as shown by a range of signal strengths from specific probes) and perhaps qualitatively on an individual chromosome basis (shown by the absence of hybridization of individual probes).

Allele dominance mediated by RNA interference

The robust allelic series available for maize genes also permits the elucidation of the molecular basis for the dominant and recessive nature of particular alleles. Chalcone synthase catalyzes the first committed step in anthocyanin pigmentation; the *C2* allele encodes active enzyme while *c2* lines are deficient in this enzyme. Chris Della Vedova (University of Missouri, Columbia, USA) reported that *C2-Idf* is a dominant, complex, multi-copy allele found in Peruvian maize. *C2-Idf* suppresses anthocyanin pigmentation in leaf tissues of *C2/C2-Idf* heterozygotes (Figure 2). Full-length *C2* transcripts are virtually absent from *C2/C2-Idf* lines, but transcription, as measured by run-on transcription assays, is nearly at wild-type levels. Abundant small RNAs of 21-23 nucleotides in length are derived from throughout the transcribed region whenever the *C2-Idf* allele is present, and
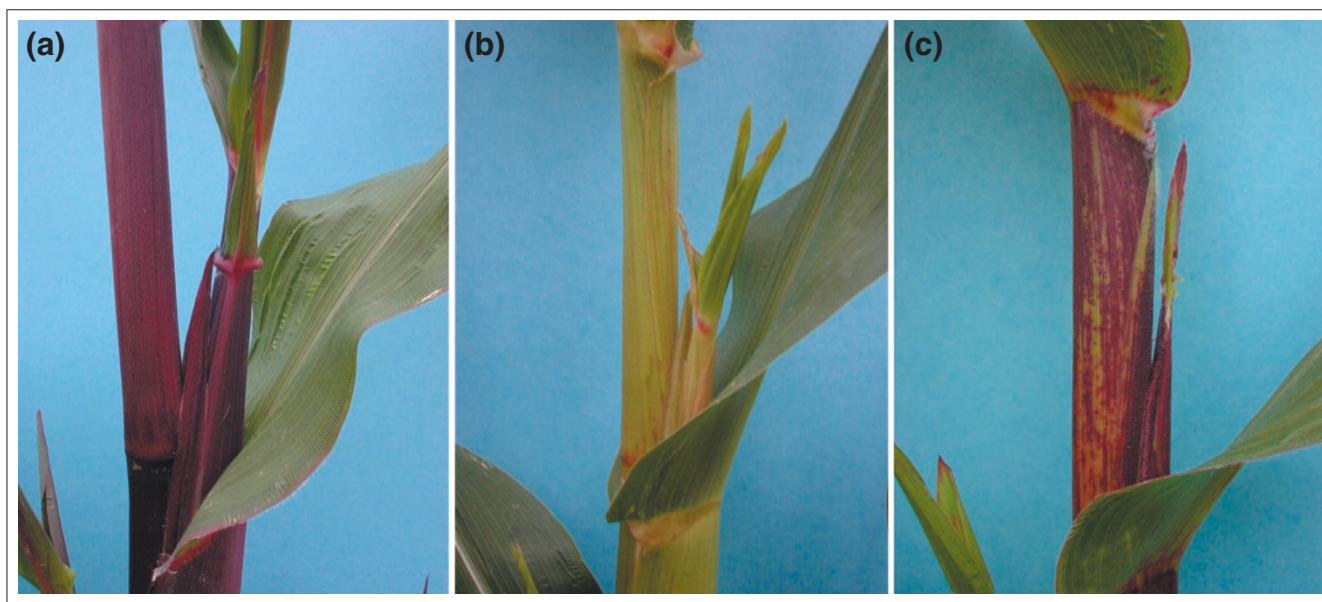
Figure 2
The effect of the *C2-Idf* allele on anthocyanin pigmentation. **(a)** *C2* confers intense pigmentation in a plant with strong activators of transcription of the *B, Pl* anthocyanin pathway, but **(b)** there is a significant reduction of the pigment in a *C2/C2-Idf* heterozygote. **(c)** Infection with maize dwarf mosaic virus, a potyvirus that encodes the P1/Hc-Pro suppressor of gene silencing, restores pigmentation in a pattern similar to that of viral spread. Maize necrotic streak virus infection also restores pigmentation (not shown), presumably through the action of P19, which binds small interfering RNAs (siRNAs). Photograph courtesy of Chris Della Vedova.

their presence mirrors the decrease in pigmentation. Transiently infecting *C2/C2-Idf* leaves with plant viruses that encode suppressors of gene silencing restores pigment production in a pattern similar to that of viral spread without altering the intrinsic transcriptional rate. These results indicate that *C2-Idf* is inducing the post-transcriptional degradation of transcripts from the *C2* allele.

This talk and many others illustrated that the diversity of maize can be exploited by both molecular and population geneticists to answer fundamental questions about genetic interactions at the allele or karyotypic level within a plant and over short and long evolutionary time scales. The next harvest of maize results will be the 47th Annual Meeting to be held 10-13 March 2005 in Wisconsin, USA [http://www.maizegdb.org/].