MAIZE GENETICS COOPERATION NEWSLETTER
86

May 22, 2013

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NOTE: The 56th Maize Meeting will be held at Beijing China March 13-16, 2013.
Check MaizeGDB for more details.

I. FOREWORD

The Maize Genetics Cooperation Newsletter exists for the benefit of the maize community as an informal vehicle for communication. Its inception and continuation has been to foster cooperation among those interested in investigating maize. This cooperation has distinguished our field from others and as a consequence has moved it forward at a pace greater than would have occurred otherwise. Your submissions are encouraged to disseminate knowledge about our field that might otherwise go unrecorded. We encourage the community to carry studies of general scientific interest to the formal literature. However, there is a great need to share technical tips, protocols, mutant descriptions, map information, ideas and other isolated information useful in the lab and field.

Because maize is both a commercial species and a genetic model system, the danger exists that the sharing of research materials might be diminished. It is imperative for us to work together to prevent this from occurring. Certainly, basic findings should be transferred to the industrial sector and basic advances in industry should be shared with the academic community for the benefit of both. Published materials must be shared for research purposes with the only restriction being against commercial use.

We remind the readers that contributions to the Newsletter do not constitute formal publications. Citations to them should be accompanied by permission from the authors if at all possible. Notes may be submitted at any time and are posted without editing at the staging site. When the print copy is finalized, the staging site copies are updated and a copy provided to the archival site at MaizeGDB, mnl.mgdb.org. **We set an arbitrary cutoff of May 1, 2013 for print copy of volume 87, per call sent by email earlier this year.** Electronic submission is encouraged and is done by sending your contributions as attachments, or as text of an email, to MaizeNewsletter@missouri.edu. Submissions must require minimal editing to be accepted.

Owing to use of electronic address scanning by the postal service, and its requirement for evolving address standards, a number of persons may not have received copies in recent years. If this is a concern, please email the editors: MaizeNewsletter@missouri.edu, after first checking the staging site, http://www.agron.missouri.edu, about the printing status of the issue not yet received. For USA subscribers, please provide your zip + 4 postal code.

We thank Paula McSteen for contributing a maize gene review for *bif2 barren inflorescence2*. Historical notes from David Fisher and Lee Cass were provided this year about John R Laughnan, Marcus M.Rhoades and David H Timothy. We are pleased to report that a note from the Stock Center about the potential utility of *Yg*-N1582* for selecting haplotypes, resulted in nearly instant inquiries to Gerry Neuffer shortly after the note was posted to the staging site, and many months before this print copy. The Maize Genetics Executive Committee report this year was redacted from material posted at MaizeGDB (http://www.maizegdb.org/mgec-activities2012.php), where the results of a MGEC community survey are posted about research directions, bioinformatics needs, education and examples of how maize genetic research has or will impact crop improvement. We include a new section this year, the MaizeGDB Editorial Board, and their literature selections for 2011 and 2012. Virginia Walbot convened the first Board in 2005. Board membership turns over each year. In 2012 the membership included: David Braun, Liza Conrad, Owen Hoekenga, Addie Thompson and Beth Thompson. In 2011, membership included: Aaron Lorenz, Michael Muszynski, Paul Scott, R. Keith Slotkin and Clinton Whipple.

This year, Megan Clark, candidate for 2 concurrent Masters degrees, one in Journalism and one in Public Health at the University of Missouri-Columbia, was responsible for redaction, layout and indexing of the Newsletter Cooperators Notes. She has performed this task with precision, considerable good humor and patience, and much communication with authors. The maize community owes her much gratitude for her service.

<div align="right">

Mary Schaeffer
James A. Birchler
Co-editors
Ed Coe
Distinguished editor

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ALMORA (UTTARAKHAND), INDIA

Vivekananda Parvatiya Krishi Anusandhan Sansthan (ICAR)

Resistance against *turcicum* leaf blight in the north-western Himalayan region of India

— Chandrashekara, C; Jha, SK; Agrawal, PK; Singh, NK; Bhatt, JC

The Indian Himalayan states have long been a center of maize cultivation. *Turcicum* leaf blight (TLB) and Maydis leaf blight (MLB) are the two major diseases that are problematic to high-land maize farmers in this region. *Turcicum* leaf blight of maize (syn. Northern leaf blight) is caused by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs (synonyms *Drechslera turcica* [Pass.] Shoemaker, *Helminthosporium turcicum* [Pass.]) and is also an important foliar disease of maize worldwide. TLB can be severe in mid-altitude tropical regions where high humidity, low temperatures, and cloudy weather prevail during the maize growing season (Singh et al., SABRAO J Breed Genet 36(1):45-47, 2004). In India, this disease is prevalent in the states of Karnataka, Himachal Pradesh, Uttarakhand, Orissa, Andhra Pradesh, and North Eastern Hill states. It also affects the *Rabi* maize in the plains of India. Yield losses easily can exceed 50% if the disease appears before flowering (Raymundo et al., Plant Dis 65:327-330, 1981; Tefferi et al., African J Plant Prot 6:75-82, 1996). However, losses are reduced if the infection takes place at a later stage. Resistance in most maize lines is partially dominant and controlled by many genes (Caldwell, Proc 3rd Int Wheat Genetic Symp pp. 263-272, 1968; Nelson, Breeding Plants for Disease Resistance, State University Press, p. 401, 1973; Sangit Kumar et al., Arch of Phytopath and Pl Protect 44:528-536, 2011; Van der Plank, Disease Resistance in Plants, Academic Press, 1968).

To identify new sources of resistance against TLB, 35 maize inbred lines developed at VPKAS, Almora, along with resistant and susceptible checks were screened for TLB at the Hawalbagh Research farm (29° 38' 3" N, 79° 37' 49" E), Almora, Uttarakhand, which is one of the hot spots for TLB in India. Evaluations were made during *Kharif* 2010 (wet season) in randomized block design with two replications under artificial epiphytotic condition. Each test line was sown in three rows of 3 m, and rows were spaced at 60 cm. Spreader rows of the highly susceptible local inbred V351 were planted at regular intervals as a source of secondary inoculum for the disease development.

The inoculum of the *Exserohilum turcicum* prevalent in north-western Himalayas was prepared by growing the fungal mycelium on sorghum grains. After seven days, the grains were dried under the shade at room temperature. A fine powder of these grains was prepared using a mixer-grinder and a pinch applied to the leaf whorl of each plant, beginning at the 4-5 leaf stage, followed by three more inoculations at 7- to 8-day intervals. During intermittent short dry spells, the plots were irrigated by knapsack sprayer to maintain the relative humidity of more than 80%. The disease first manifests 8 to 12 days following inoculation and becomes severe by the time of silking. Two weeks after inoculation, genotypes were

Table 1. Classification of 35 maize inbred lines based on disease reaction.

Response*	Inbred line
Resistant	V336, V346, V373, V398, V400, V401, V407, V418, VQL2, CM145
Moderately resistant	V152, V334, V335, V338, V340, V341, V345, V372, V383, V390, V403, V404, V405, V409, V410, VQL17, CM141, CM153
Susceptible	V25, V402, V406
Highly susceptible	V351, V414, CM212, VQL1

*Resistant: score 1-2; moderately resistant: 2.5-3.5; susceptible: 4-4.5; highly susceptible > 4.5.

scored on a scale of 0-5 as per CIMMYT protocol (Table 1; CIMMYT, Managing trials and reporting data for CIMMYT's international maize testing program, 1985; Singh et al., 2004).

The present study resulted in identification of 10 resistant inbred lines. Among them V373, V398, V407, V418, VQL2, and CM145 also were resistant to other diseases, including maydis leaf blight. Eighteen inbred lines were observed to be moderately resistant to TLB. The new sources of TLB resistance identified in the present study will be helpful for breeding programs and for basic and applied research.

ALLEGANY, NEW YORK

The maximum height of the maize subspecies: data

— Karl, JR

This is to report a maize plant standing 34 feet from the ground (Fig. 1), furthering the literature on the diversity of natural maize (compendium on the history of maximum height of the subspecies: Karl, Tallest Corn, independent publisher, Allegany, NY, 2010). The 34-foot plant is a sib increase from accession 234 (Figs. 8 & 9) of Colonia (Jesus Sanchez, personal communication, courtesy Major Goodman), Alvarado, Chiapas, adjacent to the state of Huehuetenango, Guatemala. The author appropriated it in 2001 from CIMMYT after finding the work published by Stevenson in the 1972 edition of Crop Science (p. 864). It seems that the collection was made in the initial sweep commencing in 1943 (e.g., GRIN has accession 241 as being collected in 1944). It was cultivated (about 212 days in a tall greenhouse; Figs. 2-4) by the author in Allegany, New York, in 2010. It is a mere Tehua plant (cf. monograph by Wellhausen, Races of Maize in Mexico, Bussey Institute of Harvard University, Cambridge, MA, 1952) that was grown out. The plant has no visible tassel, though indication of tasseling (irregular appearance of the upper plant, entailing the whorl irregularities of being tightly funneled, with upright leaves, irregular leaf spacing, creases, and forked tips, as well as nodal protrusion from leaf sheaths) has been evident since probably 28 feet. The newest leaf is at 33 feet, and the highest visible leaf collar is the 48th at 31 feet. There are four visible leaves above the 48th.

As it is a short-internode strain, the longest internode is 11.5 inches Roots on (shorter) neighboring plants issue from nodes at 20 feet, at which height the 34th internode lies on short-internode strains and the 24th internode on long-internode strains (Table 1, Fig. 5). When a short-internode strain and a long-internode strain

Figure 1. Guide used to measure plant. Relative position of the plant when the standing height of the whorl approximated 31 feet.

October 15, 2010.

Highest Leaf

Newest Leaf

Whorl

Highest Leaf Collar

Key Points of Reference: Standing-Height, Newest Leaf, Highest Leaf-Collar, Whorl, Ground, Highest Leaf, Below Ground, Tassel, Free-Standing Neighboring Maize Plants

to ground
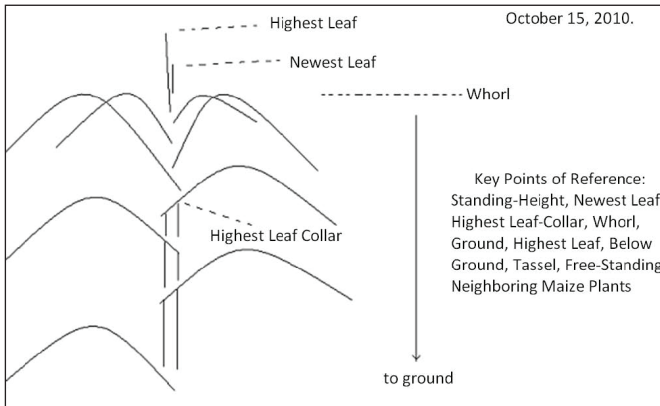
Table 1. Maize internode length in inches. Tallest strain of the subspecies, Chiapas 234, and Ecuador 689, Jala x Hueuetenango Teosinte F2.

Internode	234 plant A, short int.	234 x 689, long int.	234 plant B, short int.	Jala x Hue F2, long int.
5	1	1.5	.5	1
6	1.3	2.7	1	4
7	1.7	5	1.2	5.7
8	2.3	7.5	1.7	8.5
9	3.2	10.3	5.2	10.7
10	4.5	13.5	4.6	13
11	5	15	5.6	14
12	6	15.5	6	10.7
13	7.7	14	8	11.2
14	9	15.5	7.5	14
15	10	15	10.5	14.5
16	10.3	12.5	11.5	15.5
17	8.5	11.5	8.5	15.5
18	7.5	13	7.2	15
19	7	17.5	8.5	15
20	7.5	15.5	10.7	16
21	8.5	14.5	12.3	13.2
22	9.5	16.5	11.5	12
23	9.5	13.5	13.5	13.5
24	10.2	14.5	13.5	12.7
25	10.5	13.2	13.5	10
26	11	11.5	10.3	8.5
27	10.5	9.7	10.7	8.5
28	11	7.7	13	
29	11		11.6	
30	11.7		11.5	
31	11.2		11.5	
32	11		10.7	
33	11		11	
34	10.5		11	
35	10.5		10	
36	9.5		9.6	
37	8			
38	7.5			

(17.5" longest on plants: Chiapas 234 x Montaña race accession 689 of Ecuador; F1; Fig. 6) are at, e.g., 23-27 feet, both showing no signs of tasseling, the short-internode plant will have 13 more leaves (Fig. 7).

Figures 2-9 are available online. Thanks to Frank Kutka and Barb Every for editing counsel.

Heterosis and the night-length reaction: Effect of the night-length reaction on the plant height of the tallest strains of the maize subspecies
— Karl, JR

Heterosis is known to be negative for the trait of short-night reaction in maize. This may mean an F1 will be shorter than the parents in relation to the degree of heterosis when crossing extremely reactive strains; the height difference is due to a change in leaf quantity. This height heterosis evidently does not have a substantial effect in crosses with or within the Tehua race, though inbreeding Tehua may reduce plant height significantly (by perhaps 4.5 m). The heterosis is, however, evident within the Montaña race.

Two tall strains of Tehua (Chiapas 234 and NSL 2825, under short night) were crossed, and the F1 had the same relative height and quantity of leaves (roughly 48). When crossed with a shorter strain, the F1 was shorter (~10.5 m, mature height). This possible absence of heterosis also occurs when the Tehua is crossed with other tallest strains. For example, a cross was made of the populations Chiapas 234 with Ecuador 689, which is of the Montaña race. A plant from the cross was permitted to attain the height of 8 m, and at that height the whorl exhibited no indication of tasseling, similar to the parent strains. (Indication of tasseling entails the whorl irregularities of being tightly funneled, with upright leaves, irregular leaf spacing, creases, and forked tips, as well as nodal protrusion from leaf sheaths.) A shorter Montaña likewise makes a shorter F1 (~10.5 m). 234 crosses (with other tallest strains, e.g., Veracruz 406, from the race Coscomatepec) grow 5.25 m on uncultivated grassy ground in Costa Rica under natural night length (where 234 is still reactive). Two tall strains of Montaña (Ecuador 573, 689) were crossed. The F1 matured at an 8 m plant height, whereas the F2 exhibited no indication of flowering at 7.7 m. The F2 thus matures at a height similar to that of the parent populations (~10.7-12 m).

As maize plant height comprises not only tassel size and internode quantity, but also internode length, aside from the effect of night length in these maizes, it is interesting to note that the peak internode length on some hybrids with or within the Montaña race is 44.5 cm (Fig. 1 online)(cf. 3 consecutive internodes of >43.3 cm with the Jala race), and is 38.5 cm for the Tehua 234 (Fig. 2 online), which is ostensibly not party to such heterosis (perchance 2825 is 234) (Fig. 3 online, Table 1 next page). It seems that Montaña offers many tallest strains of maize; however, it has particularly long internodes, even in the field (.36 m); Tehua seems to be the opposite. Heterosis increases the appearance of ears in these extremely short-night-reactive backgrounds, as is usual for the subspecies in general. This is also a case study in salvaging repository accessions (with genetic diversity inadequate for a population) by hybridizing two of them.

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Table 1. Maize internode length. Race Tehua and Montaña. Two Montaña accessions crossed, Ecuador 573 x 689. Tehua Chiapas 234, and Tehua 234 x NSL 2825 F1.

Internode	Mont. F1 Plant 1	Mont. F1 Plant 2	Tehua F1	Tehua 234
1				
2				
3				
4				
5			1.5	1
6			4.5	2.2
7	6		6.5	4.7
8	10	9	6.5	9
9	12	11	9	9.7
10	12.5	12	12	10
11	14	14	11	9
12	12.5	14	11	9
13	10	11	11	9.7
14	10.5	11	13.7	12
15	11	8.5	14.3	11.5
16	14	5.5	12	11.2
17	16.5	12	12	12.5
18	16	13.5	12	13.7
19	17.5	16	13.2	14.5
20	15.5	17	14	15.2
21	16.5	16	13	13.2
22	14	15.5	12	12.5
23	13.5	12.5	11.7	9.7
24	11	10.5	10	10.2
25	10	13	10	12.2
26	12	13.5	10.5	12
27	9	12.5	10	11.2
28	8	11	9.2	11
29	10	10	9.5	9.5
30	12	10	10	7.7
31	11	9.5	9	7
32		7.5	9.5	9
33			8.6	10.2
34			8.7	11.2
35			8.7	11.2
36			9.2	11.5
37			8.6	12
38			9.2	10.2
39			7	
40			7.7	

The maximum leaf quantity of the maize subspecies
— Karl, JR

Roughly 65 leaves occurred on a 9.5 m maize plant (height of newest visible leaf, 41st leaf) that had a tassel on the scale of .5 cm (Fig. 1 online). A tassel of that size on a 9.5 m plant is unprecedented; it is 3 m higher than on previously known tallest strains. This means the new strain may accordingly be 3 m taller (15 m). The genetic background of the plant was primarily accession 234 of Chiapas, Mexico, with the Leafy mutation (chromosome 3, H.Cai, personal communication, 2007) inserted (Figs. 2 & 3 online). The plant was cultivated in Allegany, New York, under short nights, inside a wooden frame (Fig. 4) wrapped with plastic. The genotype was derived by backing the mutation once into the Wellhausen Guatemalan accession 863 and then twice into that of Chi-

apas 234 when the allele become public in 2003 (one leaf has been lost with each backcross; 17 leaves above-ear originally, then 16, now 15). With a wild-type sib of the 65-leaf plant maturing at 8.5 m (Fig. 5 online), this Leafy strain could stand more back-crossing. The derived genotypes are not available from a public repository.

A second dissected meristem of the material (eared under long night but likely permitting short-night leafy) indicated the possibility that 65 leaves is a low expression level for a segregant with the leafy phenotype. Short-night leafy has not been confirmed yet in the natural materials in a short-night regime. As leafy involves an increased quantity of leaves above the ear (20+ counted in this work, from Leafy and also short-night leafy), it is pertinent to note the ear height in natural strains is more than 9.5 m (Fig. 6 online). The (approximately) 7 wild-type leaves above the ear bring the height to 12 m. To developmentally contextualize the tall maizes of the subspecies, it is relevant to note there was a peak growth rate of 3.7 m in a month. This was in month 3, when the plant height goes from 2.5 to 6.1 m.

Figure 4. A 14 m high wooden frame wrapped with greenhouse plastic was the environment for the plant. This environment lengthens the internodes compared with the internode length in field cultivation. The predominant hypotheses for the cause of elongation are the preponderance of far-red wavelength because of the filtration by the plastic before the light reaches the plants (on this point, there is the counterpart phenomenon in which the absence of UV wavelength, due to its obstruction by the plastic, dictates expression of the indeterminate allele [Shaver, MNL 41:33, 1967; J Hered 58:273, 1967], which was assumed, in past times, to have wild-type prevalence in the tallest maizes and in teosinte and an allelism with these materials' gross height), and the difference between daytime and nighttime temperature (cf. Irwin et al., HortScience 30:940-949, 1995) being exaggerated due to the greenhouse effect. A more remote possible affector would be the neutralization of air movement near the plants.

The idea of growing maize in a wooden frame wrapped with plastic was introduced to the author via personal demonstration by N. Craven, Stouffville, Ontario, Canada. Via heat units and internode extension, plants in the enclosure can attain a height range of ≥ 8 m at this New York location, compared with ≥ 5 m in the field (frost May 20/Sept. 20).

BERGAMO, ITALY
Unità di Ricerca per la Maiscoltura (CRA-MAC)

Analysis of genetic variability and identification of genes involved in rootworm damage tolerance in maize
— Lanzanova, C; Berardo, N; Hartings, H; Torri, A; Valoti, P; Mazzinelli, G; Balconi, C

Western corn rootworm (WCR, *Diabrotica virgifera virgifera*) is a devastating maize pest in North America and recently in Europe. The major damage to maize plants is caused by larval feeding on roots; the adult stage can cause silk clipping with low fertility of the ear and reduced production. Resistance to insects leads to a reduction in production losses, a decrease of the costs of insecticide treatments, and improved food safety for animals and humans. Among the prevention and containment measures appear effective the use of some agricultural practices such as hybrid selection, crop rotation, sowing early, good availability irrigation, earthing up, and insecticide treatments (Eubanks, Proc NSF Design, Service and Manufacturing Research Conference 2544-2550, 2002; Hibbard et al., Maydica 44:133-139, 1999; Kiss et al., Proc XXI IWGO Conference 29-37, 2001). However, these strategies of control and prevention appear not only poorly effective in containing the pest damage, but also incur high costs and negative effects on the environment, and do not address the ability of the parasite to develop tolerance to different active ingredients.

Therefore, in addition to these strategies, developing resistant maize varieties by classical plant breeding or transgenic approaches have been the most important methods to control this pest (Punja, Can J Plant Pathol 23:216-235, 2001). Maize expressing *Bacillus thuringiensis* (bt) toxins or the *Caryophyllene synthase* gene (Degenhardt et al., Proc Natl Acad Sci 106:13213-13218, 2009), responsible for (E)-β-Caryophyllene production in maize, was used as protection from pests. The identification of genes and molecules underlying the defensive plant response against the corn rootworm is of primary importance for the establishment of plants tolerant to the damage caused by rootworm larvae.

The main topics of our research involve: i) analysis of genetic variability and identification of hybrids with reduced radical damage; ii) identification of genes underlying the plant response to damage by corn rootworm; and iii) validation of candidate genes and polymorphisms mapping.

In our laboratory, WCR eggs infestation tests on maize B73 x Mo17 hybrid roots were set up to obtain root samples for differential gene expression analysis in comparison with uninfested controls. The method involved hatching of diabrotica eggs in contact with corn seeds germinated in Magenta boxes with soil so that the newborn larvae could feed on corn roots. Growth of corn plantlets (hybrid B73 x Mo17) infested (or not as control) was performed in a containment greenhouse. A significant difference in the structure of the root system of infested plants was observed 30 days after infestation (DAI), and associated with smaller plants. At this stage, samples of roots (control and infestation) were collected for microarray analysis to identify differential gene expression. The damage inflicted by larvae on the roots was even more evident when

the plantlets were transferred from Magenta boxes into pots for an additional 15 days. At this stage (45 DAI) the root system was severely damaged and the leaves suffered severe stress.

A preliminary experiment was set up comparing, in addition to the experimental hybrid B73 x Mo17, the response of two different commercial hybrids (Eleonora, PR32G44) to diabrotica eggs infestation. The test (30 replicates for each hybrid and treatment) was conducted under infestation conditions previously described. At 21 DAI, the root system of Eleonora hybrid appeared to be less damaged by infestation; plant height of both genotypes was significantly affected by the infestation. Similarly, data about biomass of leaves and roots indicated for all hybrids a significant effect of reduction by treatment with diabrotica eggs, confirming the reliability and reproducibility of the method of infestation previously set up. The comparison between the uninfested hybrids showed that root weight of B73xMo17 was significantly different from PR32G44 and Eleonora. These controlled WCR eggs infestation tests will be used for the analysis of a broad spectrum of genotypes for the identification of 50 hybrids (commercial and experimental) with reduced radical damage. The data will be compared with the response of the same commercial hybrids tested in agronomical trials performed in two years (2010-2011) at 20 different locations, representative of the maize Italian areas, in the frame of WCR monitoring program (by Pherocon AM traps). Preliminary data indicated that, in all monitored areas, the total mean WCR adults capture was higher in 2011 than in 2010.

Screening of favorable alleles for β-carotene content in maize inbred lines
— Alfieri, M; Berardo, N; Redaelli, R

Carotenoids are natural compounds that play an important role for human nutrition and health; among them β-carotene is quite relevant, being a precursor of vitamin A. Deficiency of this compound is a global health problem that affects many people, especially children, in the South of the world. The biosynthetic pathway of carotenoids has been extensively studied and described by many authors (DellaPenna and Pogson, Annu Rev Plant Biol 57:711-738, 2006). The precursor of both monoxygenated carotenoids, i.e., carotenes, and their oxygenated derivatives (xanthophylls), is lycopene. From it two biosynthetic branches derive, distinguished by a different cyclic-end group. Two beta rings lead to the β, β branch (β-carotene and its derivatives: β-cryptoxanthin, zeaxanthin) whereas one beta and one epsilon ring define the β, ε branch (α-carotene and its derivatives: zeinoxanthin and lutein). The gene *hydroxylase3* (*HDY3*) controls the synthesis of one of the hydroxylases involved in the conversion of provitamin A carotenes to non-provitamin A xanthophylls.

Recently, a simple and fast PCR assay was developed to identify the alleles of *HDY3* potentially associated with an enhanced or reduced provitamin A content (Vallabhaneni et al., Plant Physiol

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151:1635-1645, 2009). Allele A was found to be correlated with a low-medium content of provitamin A, whereas alleles B and C appeared to improve provitamin A content, therefore more interesting from the nutritional point of view. This PCR assay was used to screen a group of genotypes (100 Italian inbred lines and nine public lines) to identify the lines carrying the optimal alleles. Among the Italian germplasm, 91 lines presented allele A, three lines showed allele B, one line had alleles AC, and five lines carried alleles AB. Among the public lines, five presented allele B; the others had allele A. To verify the introgression of optimal alleles in hybrid seeds, 20 crosses among the lines were carried out in 2011. As expected, hybrid grains contained the alleles of both parents.

Some of the lines tested with molecular assay had been previously selected for a breeding program focused on maize nutritional quality; they were therefore used for the extraction and quantification of total carotenoids. The extraction procedure was based on the protocol described by Schaub et al. (Maize quick carotenoid extraction protocol, 2004); the content of total carotenoids was quantified by spectrophotometer using the Lambert-Beer equation and expressed on a dry matter basis. The quantification of total carotenoids in the 13 inbred lines showed a range of variation from 23.16 (Lo1189) to 50.10 (Lo59) µg/g d.m. with a mean value of 33.21 ± 8.9 µg/g d.m. Both the mean value and the range of variation were quite high compared to the data reported for inbred lines in recent works (Chander et al., J Agric Food Chem 56:6506-6511, 2008; Ibrahim and Juvik, J Agric Food Chem 57:4636-4644, 2009; Kuhnen et al., J Sci Food Agric, 91:1548-1553, 2011; Vallabhaneni et al., Plant Physiol 151:1635-1645, 2009).

The results confirmed those obtained in previous papers about the richness in total carotenoids of traditional Italian maize germplasm (Berardo et al., Innov Food Sci Emerg Technol, 5:393-394, 2004; Berardo et al., J Agric Food Chem, 57:2378-2384, 2009). On the other hand, the five public lines analyzed showed a narrower range of variation, from 14.67 (F2) to 36.66 (Oh43) µg/g d.m., and a lower mean value (23.93 ± 8.7 µg/g d.m.). The carotenoids content was quantified also in hybrid grains; the results were quite different, depending on the genetic composition of the hybrid, and the amount of carotenoids ranged from 31.88 to 64.97 µg/g d.m. Quantification of the carotenoid components by HPLC will help to verify if the amount of β-carotene in these genotypes is related to the content predicted on the basis of *HYD3* alleles.

Screening of Italian maize inbred lines for nutritional quality, resistance to *Fusarium verticillioides*, and differential gene expression in resistant and susceptible genotypes

— Balconi, C; Hartings, H; Locatelli, S; Lanzanova, C; Panza, L; Torri, A; Alfieri, M; Berardo, N; Redaelli, R

The introduction of hybrid maize (*Zea mays* L.) has significantly increased grain yield and resistance to pathogens. Recent focus in breeding has been on nutritional quality of food plants, so as to promote good health through a convenient diet. The international maize germplasm possesses a large genetic variability for the main components of the grain. In particular, in Italy the availability of a large number of populations and ecotypes represents an interesting starting point for the identification of genotypes with good nutritional value and safety characteristics (Berardo et al., J Agric Food Chem 57:2378-2384, 2009). Mycotoxin contamination in maize grain is a global threat to both safety of human food and animal feed (Balconi et al., World Mycotoxin Journal 3:239-250, 2010; Berardo et al., Food Additives and Contaminants: Part B., 4:116-124, 2011).

Table 1. Italian maize genotypes.

Italian inbred lines	Source
Lo 002	Nostrano dell'Isola
Lo 003	Nostrano dell'Isola
Lo 005	Nostrano dell'Isola
Lo 017	Nostrano dell'Isola
Lo 018	Nostrano dell'Isola
Lo 020	Nostrano dell'Isola
Lo 021	Nostrano dell'Isola
Lo 033	Isola basso
Lo 043	Scagliolo
Lo 051	Bianco Oderzo
Lo 058cmsC	Marano
Lo 067	Scagliolino G.V
Lo 093	Scagliolino G.V. precoce
Lo 186	Marano x Isola basso
Lo 249	Scagliolo Marne
Lo 309	King Ko (Foggia)
Lo 387	Cinquantino San Fermo
Lo 404	Sacra Famiglia 43
Lo 434	Cinquantino Bianchi
Lo 435	Cinquantino Bianchi
Lo 441	Scagliolo Marne
Lo 465	Nostrano dell'Isola Finardi
Lo 491	Nostrano dell'Isola Finardi
Lo 514	Dente di cavallo
Lo 520	ICAR 54
Lo 589	Nostrano dell'Isola
Lo 241	Lo3 x Lo38
Lo 295	70 x 110
Lo 352	Lo32xLo18
Lo 446	Lo80 x Lo71
Lo 452	Lo5^2 x Lo19
Lo 457	Lo43 x Lo58
Lo 1264	P3394 (Cecilia)
Lo 577	N.I. maranizzato
Lo 578	N.I. maranizzato

With the aim to find new sources of genetic variability to improve the nutritional quality of maize hybrids and their resistance to pathogens, a set of 35 traditional Italian inbred lines (Table 1) and six public inbred lines (A632, B73, DSP1771, F2, MBS847, and W117) was evaluated in 2009 and 2010 in Bergamo. A preliminary survey was undertaken to test their response to the fungal pathogen *Fusarium verticillioides*, the causal agent of ear rot in most maize-growing areas of Southern Europe. This test employed: i) artificial inoculation of the ears in field trials at 15 DAP (days after pollination) through the KIA (Kernel Inoculation Assay) with a spore suspension obtained from a mix of two toxigenic *F. verticillioides* strains isolated in Northern Italy; ii) visual rating of the infected ears; iii) evaluation of the internal infection of the kernels; iv) quantification (ELISA) of the mycotoxins (fumonisins) present in the grain. Controls were not inoculated or inoculated with sterile H2O ears. Most of the inbred lines (around 60%) both in 2009 and in 2010 showed, after artificial inoculation, a low susceptibility to *F. verticillioides*, as deduced by visual rating evaluation. Both during 2009 and 2010, artificial inoculation induced an evident increase in fumonisin content. Large variability was observed between the genotypes. During both years, around 15% of the tested inbred lines showed fumonisin content ≥ 105 μg/kg after artificial inoculation; the other genotypes were almost equally distributed in two groups depending on fumonisin content, respectively: ≤ 104 μg/kg and 104 ≥ 105 μg/kg.

The inbred lines were also characterized by NIRS in terms of grain chemical composition (protein, lipid, and starch content). Crude protein and lipid contents, expressed on a dry matter basis, were determined during both years on ears harvested after different treatments. Inoculation with *F. verticillioides* in 2009 appeared to slightly reduce the nutritional compounds as compared to the control inoculations, whereas in 2010 the lowest content of protein and lipid was found in the ears not inoculated.

In order to address the study of differential gene expression in resistant and susceptible genotypes, two of the inbred lines under study were selected: i) Lo 186, exhibiting abundant mycelium growth and high level of fumonisins, and ii) Lo 435 with a far more resistant phenotype and low fumonisin content. Material was collected at two time points after inoculation; total RNA was then isolated from each of the collected samples in order to prepare hybridization probes, which were subsequently used to hybridize an Affymetrix maize array. Gene expression data analyses were conducted in R language using the Limma package from Bioconductor, comparing expression profiles of sterile H2O-inoculated (control) and *F. verticillioides*-inoculated samples. Upon comparison, about 500 genes (P-value ≤ 0.05) identified in Lo186 kernels after fungal inoculation were differentially expressed. The classification in GO (Gene Ontology) functional categories, showed their relative involvement mainly in i) the response to biotic stimuli (e.g., mechanisms related to response to fungus); ii) anatomical development processes.

In summary, results from the research indicated that i) most of the inbred lines both in 2009 and in 2010 showed a low-medium susceptibility to *F. verticillioides*, as deduced by visual rating evaluation; ii) during both years, around 15% of the tested inbred lines showed a fumonisin content ≥ 105 μg/kg after artificial inoculation; the other genotypes could be almost equally partitioned into two groups depending on fumonisin content; and iii) plant defense against *F. verticillioides* infection involved the expression change of several genes; the knowledge of their association with main functional pathways might disclose important information regarding those molecular processes active during fungal infection.

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Argentinean high-lysine and modified starch corn hybrids: Days and growing-degree days to silking

— Corcuera, VR[1-3]; Kandus, M[1-2]; Salerno, JC[1-2]

The duration of the different phenology phases expressed in days varies among environments due to changes in relative humidity, air and soil temperature, sun radiation, and photoperiod. Because of the poor correlation between the number of days and the growing-development of the plants it is not possible to obtain acceptable results when the genotypes are classified according to the number of days to flowering, though its utility as guidance must be admitted. Most of the variation in days to flowering and maturity can be explained by differences in temperatures amongst locations and years, so it is evident that this is the main factor affecting the development rate of maize. The effect of the temperature over the development rate can be described using the concept of thermal time (e.g., growing-degree days or GDD). Consequently, the duration of the vegetative cycle of maize may also be defined by the sum of temperatures or thermal time (TT) required to reach silking (R1).

In our work we have used diverse foundation lines and also new crosses between adapted genotypes and exotics from other research centers, so as to count on enough genetic variability. The hybrids tested may be grouped according to their grains' characteristics within four types or categories: i) materials with modified starch (MS) by action of the single mutant genes *waxy* and/or *amylose-extender*; ii) double mutant genotypes with starch and protein modified by the action of the mutant genes *waxy* and *opaque-2* and/or its variant *opaque-5* (DR); iii) materials with high-lysine content or high-quality proteins by action of the genes *opaque-2* or its allele *opaque-5* (HL); and iv) the flint hybrids ACA 929 and ACA 2000 used as testers.

The cycle to flowering of 30 single- and double-crosses was field evaluated in the Instituto de Genética Dr. E.A. Favfret (IGEAF-INTA Castelar). The results are shown in Table 1, and the basic statistics estimated for the different groups of hybrids are shown in Table 2. The single-crosses reach silking on average at 54.3 ± 3 days from emergence and after accumulating 693.6 ± 43.7°C (ranges: 50 to 59 days, 631.2°C to 759.9°C; see Table 1). Some very pre-

Table 1. Evolutive cycle to flowering of the single- and double-crosses tested during the 2010/11 growing season.

Hybrid	Cross	Type	Days to R_1	GDD (°C) R_1	FAO class
HC49	single	MS (*wx ae1*)	54	689.7	300-400
HC50	single	MS (*wx*)	59	759.9	500
HC52	single	MS (*wx*)	50	631.2	200
HC55	single	DR (*wx o2 o5*)	52	659.5	200
HC57	single	HL (*o2 o5*)	54	689.7	300-400
HC58	single	MS (*wx*)	57	737.6	500
HC59	single	DR (*wx o2*)	56	721.8	300-400
HC66	double	DR (*wx o2/ Oh43*)	59	759.9	500
HC67	double	DR (*wx o2*)	51	645.2	200
HC69	double	DR (*wx o2*)	59	759.9	500
HC70	double	DR (*wx o2 o5*)	57	737.6	500
HC72	double	DR (*wx o2/ Oh43*)	56	721.8	300-400
HC73	double	DR (*wx o2*)	56	721.8	300-400
HC74	double	DR (*wx o2 o5*)	56	721.8	300-400
HC75	double	DR (*wx o2*)	58	748.1	500
HC77	double	DR (*wx o2/ Oh43*)	58	748.1	500
HC78	double	DR (*wx o2*)	59	759.9	500
HC80	double	DR (*wx o2*)	52	659.5	200
HC82	double	DR (*wx o2*)	53	674.0	200
HC83	double	DR (*wx o2/ Oh43*)	59	759.9	500
HC85	double	DR (*wx o2 o5*)	57	737.6	500
HC90	double	MS (*wx ae1*)	54	689.7	300-400
HC91	double	DR (*wx o2*)	58	748.1	500
HC92	double	MS (*wx*)	55	706.0	300-400
HC93	double	DR (*wx o2 o5*)	56	721.8	300-400
HC94	double	MS (*wx*)	56	721.8	300-400
HC95	double	DR (*wx ae1*)	53	674.0	200
HC96	double	DR (*wx o2 o5*)	56	721.8	300-400
HC97	double	DR (*wx o5*)	56	721.8	300-400
HC98	single	MS (*wx*)	52	659.5	200
ACA2000	single	Flint or vitreous	59	759.9	500
ACA929	three way	Flint or vitreous	62	794.1	600

Table 2. Basic statistics calculated for the hybrids evaluated in the IGEAF-CNIA Castelar (2010/11 growing season).

Days to R1			
	Average ± s.d.	Variance	Variance/n
MS	54.4 ± 2.7	7.3	0.8
Vítreous (Testers)	60.5 ± 2.1	4.5	2.2
DR	56.2 ± 2.5	6.1	0.3

Thermal time (°C) to R1			
	Average ± d.s.	Variance	Variance/n
MS	696.6 ± 39.8	1583.9	176.0
Vítreous (Testers)	777.0 ± 24.2	584.8	292.4
DR	722.5 ± 35.8	1278.2	63.9

gree of precocity (class FAO 200). Some other double-crosses (e.g., HC72, HC73, HC90, HC92, HC93, HC94, HC96, and HC97) belong to class FAO 300-400 because they reach silking after piling up 689.7°C to 721.8°C from emergence. The single-crosses HC50 and HC58, the same as the double-crosses HC69, HC70, HC75, HC77, HC78, HC83, HC85, and HC91, must be included within the classes FAO 500 and FAO 600. Consequently, these latter hybrids are considered as non-precocious and have an intermediate or complete cycle to flowering like the testers ACA 929 and ACA 2000.

The mean contrasts ($t_{Student}$), revealed the MS hybrids do not differ statistically from double mutants in the number of days or thermal time necessary to reach silking (± t = 1.7 n.s. for both physiological traits). Nevertheless, highly significant differences were observed between the MS materials and both testers ACA 929 and ACA 2000 (± t = 3.6 and 3.7 for days and thermal time, respectively; p ≤ 0.01). Likewise, the double mutant hybrids (DR) differ in a highly significant way from the testers ACA 929 and ACA 2000 both in the necessary number of days and thermal time to reach R1 (± t = 2.7 and 2.9, respectively; p ≤ 0.01). A very strong correlation between the number of days and the thermal time necessary for silking was found in the hybrids evaluated in Castelar (r = 0.99; p ≤ 0.01).

The results obtained show a great degree of precocity in the materials studied. Due to their degree of precocity, 7/30 hybrids could be recommended for late sowings in the Northern Pampa zone and in the Argentinean Corn Region VI. Likewise, due to the length of their evolutive cycle, measured in number of days or thermal requirements, the precocious genotypes could be used indifferently in the Western or Southern Pampa areas, characterized by having a shorter period free of freeze than other areas of the Argentine corn region. A higher degree of precocity helps adaptation to areas with short summers and humid falls, making possible a further increase of the culture surface of this species but in this case making use of specialty germplasm suitable for diverse industrial and feeding purposes.

cocious genotypes to R1 were detected among these materials, as for example the *waxy* hybrids HC52 and HC98 and the double mutant HC55. These single-crosses must be included within the class FAO 200 because they reach R1 as soon as they accumulate 630°C to 660°C. On average, double-crosses reached silking after 56.1 ± 2.3 days from emergence (range: 51 to 59 days), and their mean heat requirement to the same phase was 713.1 ± 22.8°C (range: 645.2°C to 759.9°C). The double-mutants HC67, HC80, HC82, and HC95 stand out amongst them due to their high de-

Argentinean high-lysine and modified starch corn hybrids: Determination of chemical composition
— Corcuera, VR; Kandus, M; Salerno, JC

The gross chemical composition of grains from single- and double-crosses grown at the Instituto de Genética Dr. E.A. Favret (IGEAF-INTA Castelar) during the 2010/11 growing season was determined using an infrared spectrophotometer model Foss Infratec 1241 Grain Analyzer to quantify through a non-destructive assay protein content (%), starch content (%), and oil content (%). Analyses were performed on two 60 g grain samples of each genotype obtained by hand pollination to prevent contamination and *xenia* effect particularly on oil content. These results were averaged to obtain the final values and to complete others published previously (see MNL 83:12-13, 2009).

Maize is the best energy source for animal dietary rations. On average, corn kernel oil content is relatively low (3%-5%) and is mostly found in the germ. According to data published by ILSI and based on samples taken worldwide (ILSI Crop Composition Database version 2.0, www.cropcomposition.org) corn oil content varies from 1.74% to 5.56%. Nevertheless, MAIZAR (Argentine Maize Association) eventually reported that the oil content measured by NIRT technology on 48 commercial hybrids sampled within the limits of the ZMT and the southeastern area of the province of Buenos Aires during the 2004/05 growing season NIR ranged from 3.9% to 6.5%. High-oil content (HOC) corn shows up to twice the content of this component and a higher protein quality than dent maize, so it also has a greater energy value and may replace other high-cost fat and protein sources. According to the U.S. Grain Council any genotype yielding grains with an oil content of ≥ 6% should be considered HOC.

Kernel protein content is highly variable and depends mainly on the variety, sampling, and production environment, as well as calculation factors used to convert N into protein. In accordance with ILSI Argentina, kernels' average protein content is about 9.5% (from a sample of 109 commercial hybrids grown in the provinces of Cordoba and Buenos Aires between 1999 and 2001).

The gross chemical composition of the grains produced by the single- and double-crosses tested in the experimental field of the IGEAF-INTA Castelar is shown in Table 1. Average oil content was 5.3% (range: 4.1% to 7.3%). Four crosses could be classified HOC. The *waxy* single-cross HC52 shows the highest oil concentration (7.3%), followed in decreasing order by the double mutant HC69 (6.2%), the high-lysine single-cross HC57 (6.1%), and the double mutant HC59 (6%).

The average protein content of the 30 hybrids tested was high (avg. = 12.2%; range = 10.8% to 13.6%). The double mutant HC59 yielded the highest protein content (13.6%), followed by the double mutants HC55 and HC95, which produced kernels with 13.3% protein. A high level of protein content (≥ 12%) was detected in the grains of the genotypes HC49, HC50, HC57, HC58, HC67, HC70, HC73, HC74, HC75, HC80, HC85, and HC92. In brief, 18 out of 30 hybrids yielded grains with more than 12% protein. Finally, the average content estimated for the 30 hybrids was 68.4%, ranging from 66.7% to 69.9% (Table 1).

Table 1. Gross chemical composition (via NIRT) of 30 crosses tested in the experimental field of the IGEAF-INTA Castelar during the 2010/11 growing season.

Hybrid	Cross	Type	% Oil	% Protein	% Starch
HC49	single	MS (*wx ae1*)	4.8	12.0	69.2
HC50	single	MS (*wx*)	5.0	12.9	68.7
HC52	single	MS (*wx*)	7.3	10.8	66.7
HC55	single	DR (*wx o2 o5*)	5.5	13.3	67.4
HC57	single	HL (*o2 o5*)	6.1	12.1	67.0
HC58	single	MS (*wx*)	5.1	12.0	68.8
HC59	single	DR (*wx o2*)	6.0	13.6	67.4
HC66	double	DR (*wx o2/ Oh43*)	4.7	13.0	68.2
HC67	double	DR (*wx o2*)	5.1	12.3	68.2
HC69	double	DR (*wx o2*)	6.2	11.8	69.1
HC70	double	DR (*wx o2 o5*)	5.1	12.5	68.0
HC72	double	DR (*wx o2/ Oh43*)	5.0	13.0	68.4
HC73	double	DR (*wx o2*)	5.4	12.1	68.9
HC74	double	DR (*wx o2 o5*)	5.4	12.8	67.3
HC75	double	DR (*wx o2*)	4.5	12.9	68.6
HC77	double	DR (*wx o2/ Oh43*)	5.4	11.7	68.9
HC78	double	DR (*wx o2*)	5.4	11.7	68.6
HC80	double	DR (*wx o2*)	5.6	12.5	68.1
HC82	double	DR (*wx o2*)	5.5	13.1	67.2
HC83	double	DR (*wx o2/ Oh43*)	5.4	11.9	68.3
HC85	double	DR (*wx o2 o5*)	5.5	12.6	68.3
HC90	double	MS (*wx ae1*)	4.1	11.8	69.1
HC91	double	DR (*wx o2*)	5.8	11.1	69.3
HC92	double	MS (*wx*)	5.6	12.9	67.3
HC93	double	DR (*wx o2 o5*)	5.0	11.3	69.9
HC94	double	MS (*wx*)	5.0	11.6	69.0
HC95	double	DR (*wx ae1*)	4.4	13.3	68.0
HC96	double	DR (*wx o2 o5*)	5.6	11.7	69.0
HC97	double	DR (*wx o5*)	5.3	11.5	69.2
HC98	single	MS (*wx*)	5.6	11.4	68.7
ACA2000	single	Flint or vitreous	5.2	11.4	70.3
ACA 929	three-way	Flint or vitreous	4.9	11.6	69.8

These values agree with those detected by MAIZAR through an NIR analysis of 48 commercial hybrids grown in the main Argentine corn area (ZMT) and southeast of the province of Buenos Aires during the 2004/05 season. The single-crosses HC57 and HC59 yielded kernels with both high protein and oil content. These results strengthen previous results obtained during the

Table 2. Pearson's correlation coefficients (r) among the chemical components determined through NIRT technology in experimental specialty corn hybrids grown in the 2010/11 growing season.

	r
Oil-protein	-0.32 (nonsignificant)
Oil-starch	-0.45 (significant at p ≤ 0.05)
Protein-starch	-0.48 (significant at p ≤ 0.01)

2009/2010 growing season and are infrequent in improved genotypes of this species.

Pearson's simple correlation coefficients among oil, protein, and starch content were calculated (Table 2). The results suggest negative and significant correlations between starch and oil protein content (r = -0.45; $p \leq 0.05$), negative and highly significant associations between starch and protein content (r = -0.48; $p \leq 0.01$), and, as in previous MNL reports, no significant correlations between oil and protein content were found (r= -0.32; n.s.).

The results of mean contrasts ($t_{Student}$) for the different chemical components of the kernels of the groups of hybrids studied revealed highly significant differences between DR and Flint hybrids for protein and starch content (± t = 4.7 and 5.5, respectively; $p \leq 0.01$) as well as between MS and Flint genotypes for kernels' starch concentration (± t = 4.5; $p \leq 0.01$).

The kernel oil content (6% to 7.3%) of the hybrids HC52, HC57, HC59, and HC69 suggest they could have competitive advantages for animal feeding. Likewise, the oil and protein content found in these experimental hybrids could be of special interest for animal nutrition and/or the corn transformation industry.

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Combinative properties of maize double haploids
— Mikhailov, ME; Maslobrod, SN; Sarmaniuc, M

We describe here tests of the combinative capacity of the double haploid (DH) lines derived from the maize hybrids MK01 x A619 and Rf7 x Ku123. In DH lines, genes from both the parents (P1, P2) are combined at random, so their combinative capacity varies and depends on the number of favorable alleles obtained from each parent.

We determined the combinative capacity of grain yield in DHxP crosses using the equation

$$C(P_1) = \frac{M(DHxP_2) - M(P_2)}{M(F_1) - M(P_2)}$$

where M is grain yield of the genotype indicated in parentheses. The $C(P_1)$ parameter measures the combinative capacity of the DH line relative to the P_1 parent. It shows the increase in grain yield, which gives the DH line compared with increase, which gives the P_1 line (in crosses with the same P_2). The value of $C(P_1)$ depends on the number of favorable alleles inherited by DH line from P_1. The distribution of the DH lines by values of C is shown in Figures 1-4. The general statistics and C values are given in Table 1. Grain yields of parents and F_1 were as follows (gm/plant at the density 4 plants/m^2):

2010 year: MK01 - 100.8 ± 5.6, A619 - 22.4 ± 4.4, MK01xA619 - 181.6 ± 5.4

2011 year: Rf7 - 105.6 ± 5.2, Ku123 - 76.7 ± 3.4, Rf7xKu123 - 191.9 ± 4.2

In the absence of nonallelic interactions (additive-dominant model) C should be distributed symmetrically about the value of 0.5. In fact, only C(Ku123) is distributed symmetrically; otherwise C are less than 0.5, and in two cases significantly (see Table 1). This suggests significant role of nonallelic interactions of complementary type between the favorable alleles of the lines MK01 and Rf7. Nonallelic interactions are not significant in the case of A619 and are absent in the case of Ku123. Apparently, in these cases gene effects are summarized to additive-dominant model.

In Figs. 1-4 the differences in the excess are clearly visible. The distributions of C(MK01) and C(Rf7) have the sharp peaks. In the distributions of C(A619) and C(Ku123) the peaks are less expressive. Sharpness of the peak should depend on the number of genetic factors affecting C: The more genetic factors, the sharper the peak. The number of genetic factors can be estimated from Castel-Wright. In our case, it looks like

$$N = 0.25 / (\sigma^2 - e^2)$$

where N is number of genetic factors, σ^2 is variance of C, and e^2 is mean square error of partial value of C.

The estimated C values shown in Table 1 reflect the number of favorable alleles (or groups of linked alleles) that are responsible in the corresponding line for heterosis of grain yield in crosses. In both of the studied hybrids the same phenomenon is observed. The more productive parent has more alleles influencing heterosis, and significant nonallelic interactions appear between them. In the less productive parent the number of favorable alleles is smaller, and nonallelic interactions between them are less significant or absent.

Table 1. Relative combinative capacity of DH lines.

Hybrid	Parameter	Number of DH lines	Mean C	Standard deviation of C	Mean error of partial C	Estimated number of genetic factors
MK01 x A619	C(MK01)	41	0.387 ± 0.021*	0.135	0.059	16
	C(A619)	42	0.442 ± 0.030	0.192	0.101	9
Rf7 x Ku123	C(Rf7)	26	0.378 ± 0.027*	0.137	0.049	15
	C(Ku123)	25	0.489 ± 0.044	0.220	0.096	6

* Difference from 0.5 is significant at $p < 0.001$.

Figure 1. Distribution of DH lines from combinative capacity relatively MK01 (in crosses with A619).
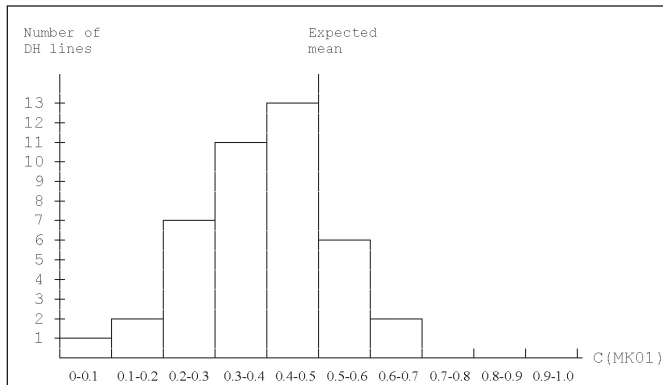

Figure 2. Distribution of DH lines from combinative capacity relatively A619 (in crosses with MK01).
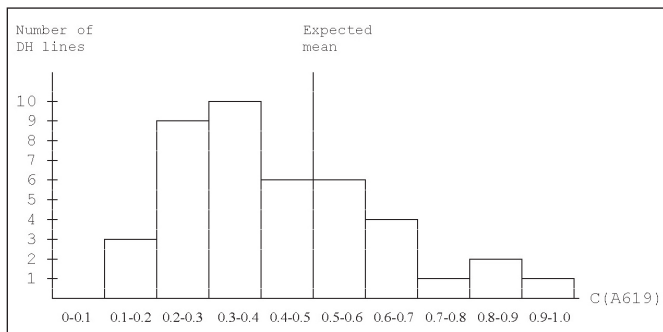
Figure 3. Distribution of DH lines from combinative capacity relative to Rf7 (in crosses with Ku123).
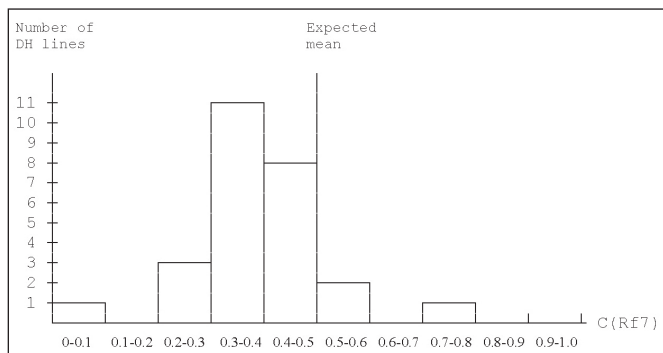
Figure 4. Distribution of DH lines from combinative capacity relative to Ku123 (in crosses with Rf7).
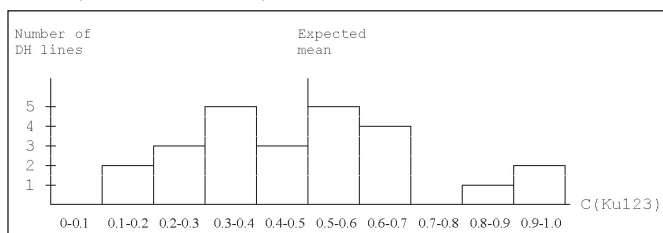
Mottling expression is curious, and so is blotching: What is responsible?

— Coe, E

Mottling and blotching in the aleurone tissue display patterns inconsistent with random on/off switching of pigmentation during cell division. Microscopic images of the "cobblestones" of the tissue showing mottling and blotching are in MaizeGDB (Fig. 1).

Figure 1. Microscopic images of mottling and blotching. Sources (clockwise from top left): http://images.maizegdb.org/db_images/Variation/coe9209-1413/05.jpg; http://images.maizegdb.org/db_images/Variation/coe9209-1413/03.jpg; and http://images.maizegdb.org/db_images/Variation/cd7101-3161-0702/52.jpg.
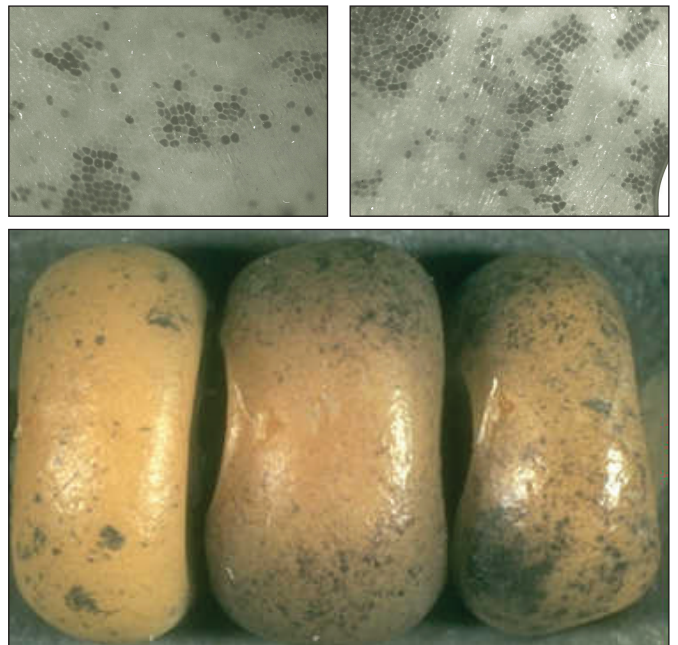
Some cells show pigmentation; some do not. The groupings of colored cells in mottling and blotching contrast dramatically with cellular morphogenesis of the aleurone tissue, which occurs by systematic cell divisions in alternating planes (Coe in Walden (ed), Maize Breeding and Genetics, Wiley & Sons 447-459, 1978). Specifically, when *R1-st* mutates to colored during development the morphogenetic progression is revealed by the contiguous clusters of cells in binary series, from alternating planes of division: "squares to bricks to squares to bricks" (i.e., when *R1-st* mutates just before the last division, a cluster of 2 is formed; if in the preceding division, a cluster of 4 in 2x2; 8 in 2x4; 16 in 4x4, and 32 in 4x8).

What is the pattern in mottling? The three images of mottling in Figure 1 are unlike *R1-st* patterns, and patterns are difficult to discern. More instructive are the expressions in highly paramutant *R1-iv* or *R1-v* (four or five times paramutagenized), where pigmented cells are greatly reduced in frequency. I am unaware of photographs, but there are typically as few as 10-20 colored cells in a whole aleurone tissue. Surprisingly, the colored cells occur not in

11
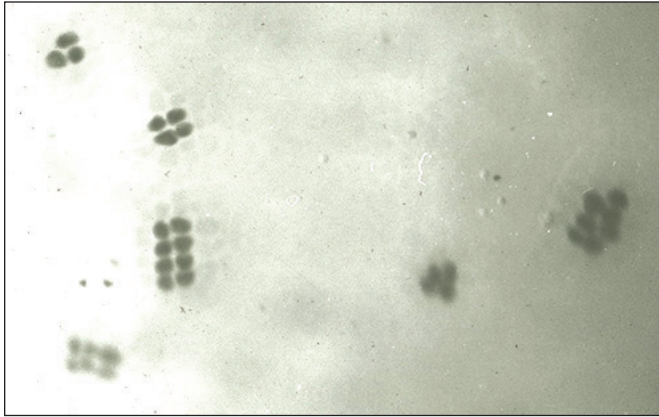
Figure 2. Aleurone cell sectors following mutation to colored in the last few cell divisions. Sources (from top): http://images.maizegdb.org/db_images/Variation/coe9209-1413/06.jpg; http://images.maizegdb.org/db_images/Variation/coe9209-1413/04.jpg.
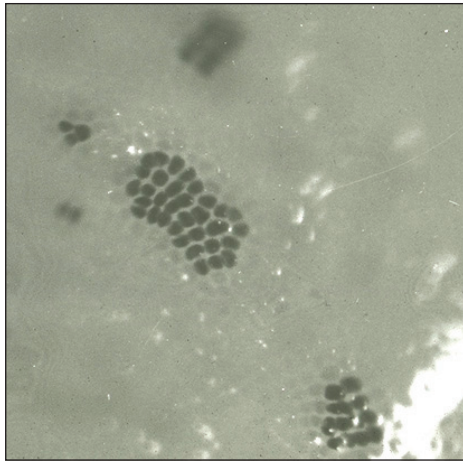
single, scattered, independently pigmented cells, but in irregular, very localized clusters — i.e., neither a Poisson distribution nor a morphogenetic switching to "on," but something in between (Fig. 2). Despite the fact that the clusters are not contiguous, neighboring cells tend to be pigmented (Coe and Mouli, Genetics 68:s12, 1971). In standard highly mottled aleurone tissue the patterns are also not contiguous but are suggestive of clustering, consonant with the irregular clustering in highly paramutant *R1*.

Does mottling expression reflect constancy/inconstancy of epigenetic changes in cell lineages, signal propagation and decay, or the distribution of RNA or protein moieties during cell divisions?

DICKINSON, NORTH DAKOTA
Seed We Need Project

Using *Ga2s* to limit undesirable fertilization in commercial organic maize
— Kutka, F

There is interest by organic maize growers to have lines that would not cross with transgenic varieties. Traits that prevent unwanted crossing between lines are well known. Gametophytic cross incompatibility was first noticed in popcorn that would set little seed if pollinated by dent corn pollen (Thomas, Agron J 47:440-441, 1955). This trait is controlled by *Ga1s* (Neuffer et al., Mutants of Maize, Cold Spring Harbor Laboratory Press, 1997). (The su-

perscript S [e.g., in *Ga1s*] denotes strong activity.) Most maize cannot pollinate such lines, though there are a few white and yellow dent lines in the U.S. with the trait (Kutka, MNL 84:18, 2009; Poneleit in Hallauer (ed), Specialty Corns, CRC Press, 2000). Zeigler and Ashman (in Hallauer (ed), Specialty Corns, CRC Press, 1994) reported that the trait is used to protect popcorn.

Ga1s works because plants with this trait have silks that do not support normal pollen tube growth for pollen carrying the ga allele. Lausser et al. (J Exp Bot 61(3):673-682, 2010) reported 0-5% of ga pollen tubes growing 8 cm into silks of homozygous *Ga1s* plants with most growing no more than 2 cm. Dent outcrosses are usually low in popcorn fields due to cross incompatibility and the abundance of more competitive *Ga1s* pollen in the popcorn fields (Zeigler and Ashman, 1994).

Tcb1s and *Ga2s* are gametophytic cross incompatibility alleles from teosinte that have been crossed into field corn lines (Evans and Kermicle, Theor Appl Genet 103:259-265, 2001; Kermicle and Evans, J Hered 101(6): 737-749, 2010). These genes work in a similar fashion to *Ga1s*, and normal dent lines in the U.S. are ga2 ga2 and tcb1 tcb1 in genotype. Both genes could reduce undesirable outcrossing in commercial maize.

Lines carrying *Ga2s* from the Maize Genetic Stocks Center were used as donor parents in 2011 in a backcrossing project funded in part by the Organic Farming Research Foundation. Recurrent parents include flint, Oh43, W153R, Mo17, Iodent, B14, and B73 types. *Ga2s* is being crossed into these lines following methods developed by popcorn breeders for *Ga1s* (Thomas, 1955; Zeigler and Ashman, 1994). At the end, plants homozygous for *Ga2s* will be identified by pollinating with pollen from a blue or purple seeded line on one day followed by selfing the next day. Those with strong resistance to outcrossing should have few or no colored kernels at harvest and will be released to the public, though new breeding lines carrying *Ga2s* in recurrent parent cytoplasms should be ready for release to breeders and researchers late in 2013.

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Identification of multiple aleurone in CG Coroico Flour
— Puga, P[1]; Kutka, F[2]

The aleurone layer in maize seeds consists of the single, outermost layer of endosperm cells just below the pericarp. Maize populations with seeds exhibiting aleurone layers of 2 to 4 cell layers or more were found among the Coroico/Piricinco racial complex from Bolivia and Peru (Fig. 1; Welch et al., Plant and Soil 155/156:215-218, 1993; Wolf et al., Crop Sci 12:440-442, 1972). This multiple aleurone trait is controlled by a single, partly dominant gene, and kernels with the trait have been found to be substantially higher in protein and mineral content, making the trait useful for the nutrient enhancement of maize.

We were interested in finding the extent to which existing work with Coroico germplasm has already resulted in northern

Figure 1. Stained section of kernel from Loreto 8 showing 3-4 aleurone cell layers between the normal endosperm cells at the top and the pericarp below.
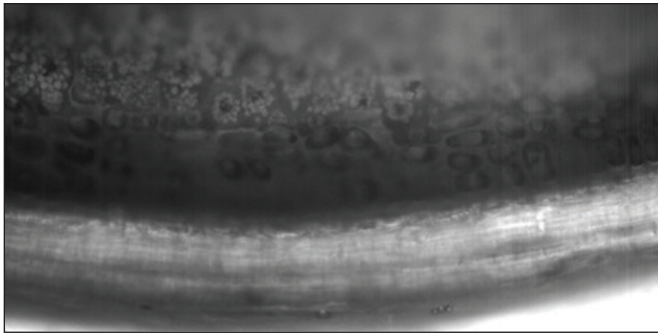
adapted populations that carry the trait. Cargill released to the USDA a temperate adapted population called Cargill North Temperate Zone Coroico. Kannenberg and others at the University of Guelph in Ontario selected this population for earliness and released it as CG Coroico Flour (Lee et al., Crop Sci 46:2728-2733, 2006). We examined these populations, three Coroico/Piricinco populations from Bolivia and Peru, three inbred lines from the Maize Genetics Cooperation Stock Center carrying the multiple aleurone trait, and three adapted inbreds from Minnesota and Manitoba to see how prevalent the trait was among these sources.

Following the methods of Welch et al. (1993), kernels were soaked in water overnight and then sectioned with a razor blade. Sections were stained with amido black in a 7% acetic acid solution to improve visibility of the aleurone cells under light microscopy. As described by Wolf et al. (1972), the North American lines did

Table 1. Results of aleurone cell layer counts for selected North American and Coroico populations of maize.

Variety	Seeds examined (No.)	Single layer	Multiple layers	Multiple layers (%)
A681	10	10	0	0
A682	10	10	0	0
CM7	10	10	0	0
Stock 5708E	10	4	6	60
Stock 5708F	10	0	10	100
Stock 5708G	10	2	8	80
Bolivia 792	100	81	19	19
Loreto 8	60	2	58	97
Coroico Composite	99	54	45	45
Cargill North Temperate Zone Coroico	96	73	23	24
CG Coroico Flour	119	107	12	11

not exhibit the trait, and few sources with the multiple aleurone trait appeared to have the trait fixed (Table 1). Seeds with multiple aleurone layers were found in decreasing proportions from the original Coroico, to the Cargill population, to CG Coroico Flour. Also, when present, the second aleurone cell layer in CG Coroico Flour seeds was largely limited to the crown of the seeds and was usually discontinuous, unlike the original Coroico populations. Whether this was due to a heterozygous state, the loss of modifier genes during selection, or some other cause is unknown. However, having this trait in this early maturing population should help plant breeders interested in using it in early maturing cultivars.

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Callusogenesis in maize inbred DK212 under sodium chloride
— Derkach, KV; Abraimova, OE; Satarova, TM

Resistance to salinity or high sodium chloride is an important agricultural characteristic for maize in some soils. The application of in vitro tissue culture method for creation of genotypes resistant to chloride salinity is a perspective direction of biotechnology investigations (Urechean, Bulg J Plant Physiol Special Issue, 336-352, 2003). Regenerated plants resistant to sodium chloride have been obtained in barley (Ignatova, Cell Biotechnology in Plant Growing, Genetics and Selection, 224, Astroprint, 2011), rice (Priya et al., Afr J Biotechnol 10(36):6947-6953, 2011), beet (Chugunkova, Physiol and Biochemistry of Cultivated Plants 6(242):509-515, 2009), wheat (Koutoua et al., Int J Biosci 1(4):12-25, 2011), potato (Homayoun et al., American-Eurasian J Agric & Environ Sci 11(5):729-732, 2011).

The increase of contents of sodium and calcium ions, the decrease of the concentration of potassium ions, and the significant decrease of ratio K+/Na+ occur in tissues under salinity. Higher activity of superoxide dismutase was discovered in salt-resistant forms, and the decrease of enzyme activity under abiotic stress was revealed both in seedlings and callus tissues (Terletskaja, Biology of Plant Cells in Vitro and Biotechnology, 390, 2008). The salinity of the environment breaks osmotic and ionic homeostasis of cells and provokes a secondary oxidative stress associated with the accumulation of active forms of oxygen and subsequent oxidation of lipids. This leads in turn to the disruption of membrane structure and function (Gurr et al., Lipid Biochemistry: An Introduction, Blackwell Science Ltd, 2002). Tissue culture allows simulation of salinity stress by adding sodium chloride to nutrient medium, and can be used to screen for resistant maize cells and tissues, which may lead to resistant plant on regeneration.

We investigated the callusogenesis under salinity in maize

inbred DK212, which belonged to subplasm Oh43 of Lancaster germplasm. Immature zygotic embryos, 1-1.5 mm in length, were harvested on the 11th day after self-pollination from field donor plants and cultivated scutellum up on modified inductive N6 medium (medium *Ind*) in the darkness. Calli derived in 60 days in culture were transplanted to next modified MS media: control medium (*C*), control medium + 0.1 Mol/l sodium chloride (*6C*) and control medium + 0.5 Mol/l sodium chloride (*30C*) and cultivated at the light. Medium *Ind* as compared to media *C*, *6C*, and *30C* had contained less sucrose for delaying the osmotic load because the osmotic pressure was created later with sodium chloride.

50% and 20.45% of green calli were observed on media *C* and *6C*, respectively, at the 30th day of cultivation at the light. Green coloration was disappearing through 30 days after its appearance. Visual changes in calli sizes depending on sodium chloride contents were noted only to the 60th day of cultivation at the light. To estimate callus cultivation, specific diameters and specific raw weights of calli were measured (Figs. 1 & 2). Specific dry weights and humidity of calli were determined at the end cultivation (Table 1).

Values of specific raw weights and diameters of calli after 60 days of cultivation at the light were appreciably differed from analogical values fixed before transplantation. Maximal value of specific raw weight of calli was observed on the 120th day of cultivation
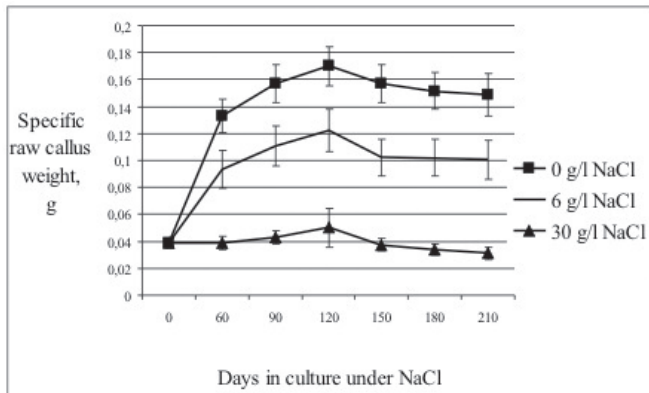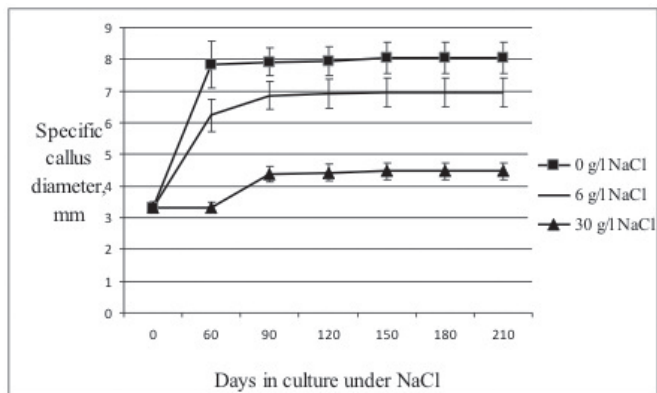
Table 1. Maize callus characteristics on the 210th day of in vitro culture under different concentrations of NaCl.

Concentration of NaCl in the medium (g/l)	Number of calli cultivated	Specific raw callus weight (mg)	Humidity of calli (%)
0	44	7.42 ± 0.98	95.01 ± 6.64
6	44	6.07 ± 1.06	93.96 ± 7.26
30	45	2.60 ± 0.45	91.65 ± 8.34

at the light in all variants of the experiment. The specific diameter values achieved to the 150th day were preserved to the 210th day of cultivation at the light. The tendency of the decrease of specific dry callus weight and humidity under the increase of sodium chloride concentration was observed. Such a tendency indicates the inhibitory effect of sodium chloride on the accumulation of dry weight of calli (Table 1). The reduction of calli humidity related to salt contents in the medium characterizes sodium chloride as an osmotic agent.

Thus, inhibitory effect of sodium chloride on callus growth depends on the age of culture and the sodium chloride concentration. 0.5 M sodium chloride is non-lethal concentration for tissue culture of maize inbred DK212 and can be further examined as a selective one.

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Maize genebank, Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT)

Genetic relations among *Tripsacum* species revealed by genomic variation
— Taba, S; Chavez-Tovar, VH

We studied genomic variation among several *Tripsacum* spp. and teosinte species in collaboration with Floragenex Inc. from Portland, Oregon, USA. Materials were 18 *Tripsacum* clones from Mexico and South America, seven teosinte race populations, and one tropical maize inbred (Tables 1, 3, and S1). A preliminary analysis revealed interesting results for relationships between *Zea* and *Tripsacum*. As part of this work, we curated a *Tripsacum* garden at the Tlaltizapán station and updated the passport data of exiting clones. We note that some clones from the South American highlands suffered from high temperatures at the station.

Sequencing and SNP (polymorphism) discovery. Paired-end Illumina/Solexa sequence reads of the material were anchored to the *Tripsacum* clone sample 12 (Table S1 online) reference assembly using custom short-read software with a Needleman-Wunsch alignment algorithm. Specified alignment thresholds allowed approximately three base pair changes between the ~50 bp Illumina/Solexa read and the reference (>90 percent identity). Small gapped alignments (short indels) spanning one or two base pairs were permissible. A total of 25,878 contigs were constructed, with 5,705,986 base pairs. Contig sequence length ranged from 100 to 625 bp, with an average of 220.5 bp. Using the *Tripsacum* clone 12 as the reference, the number of contigs with at least one polymorphism was 11,558. Within the 11,558 contigs, 7,756 were aligned onto the B73

Figure 1. The dynamics of specific raw callus weight under the influence of NaCl.

Figure 2. The dynamics of specific callus diameter under the influence of NaCl.

genome, where 45,449 polymorphic loci were anchored. In total, 72,413 polymorphic loci were identified. Of these, 1,036 were high-quality polymorphic loci identified across all 26 lines; 10 were InDel genomic variation, and 1,026 were single nucleotide polymorphisms (SNPs).

Clustering analysis of *Tripsacum*, teosinte, and maize. Information on the material analyzed in this study is shown in Tables 1-3. Figure 1 shows that among the *Tripsacum* species studied, those from South America (i.e., *T. peruvianum*, *T. cundinamarc*, and *T.*

dactyloides) were genetically closest to *Zea* (teosinte and maize), though *T. zopilotense* (Mexico) had similar results. The next closest species to *Zea* were *T. latifolium* and *T. andersonii*. The teosinte species were clustered as shown in Figure 1. *T. zopilotense* reportedly crosses well with maize (Savidan et al., Apomixis Newsletter 6:19-20, 1993) and with *T. australe* (Raymundo, MS thesis, Colegio de Postgraduados, Montecillo, Mexico, 1993). *T. dactylodes* is known to cross well with maize. Unfortunately, we did not have *T. australe* and *T. jalapense* in our study.

Table 1. *Tripsacum* clones sampled for this study at CIMMYT Tlaltizapán station, 2009.

| Clonal sample ID	Collection No.	Population No.	Species classification	Chromosome number	Possible ploidy	Country of origin	Collection site and province or state	Collection site altitude (m), latitude, longitude
01	T.1069	95	TMN (*T. manisuroides* de Wet and Harlan)	36	diploid	Mexico	Mirador, Los Chiapas, Chiapas	1200, 16:37; -91:85
02	T. 5243	613	TCD (*T. cundinamarce* de Wet and Timothy)	36	diploid	Colombia	Viota-Tamarindo, Cundinamarca	1095, 4:42; -74:52
03	T. 5222	603	TMR (*T. dactiloydes var.meridionale* de Wet and Timothy)	36	diploid	Venezuela	El Caimito-5km from Libertad, Tachira	1400, 7:58; -72:32
04	T.7003	1	TZP (*T. zopilotense* Hernandez and Randolph)	36	diploid	Mexico	Cañon de Zopilote, Guerrero	354, 18:35; -101:70
05	T. 5087	554	TMR (*T. dactyloydes var.meridionale* de Wet and Timothy)	36	diploid	Colombia	Sota, 7km Carr. a Sota, Boyaca	1000, 6:32; -72:70
06	T.69	18	TLT (*T. latifolium* Hitchc.)	54	triploid	Mexico	Pluma Hidalgo, Oaxaca	1100, 15:92; -96:42
07	T.7146	4	TMZ (*T. maizar* Hernandes and Randolph)	54	triploid	Mexico	San Jose II, Nayarit	30, 22.47; -105:30
08	T.552	6	TBV (*T. bravum* Gray)	72	tetraploid	Mexico	Rancho Nuevo, 87.7 km from Iguala, Guerrero	780, 18:17; -101:40
09	T.569	9	TDH (*T. dactyloydes var. hispidum [Hitchc.]* de Wet and Harlan)	72	tetraploid	Mexico	Zacazonapan, 2 km N. of Zacazonapan, Mexico	1450, 19:07; -100:20
10	T.139	24	TDM (*T. dactyloides var.mexicanum* de Wet and Harlan)	72	tetraploid	Mexico	Aguascaliente, 13km S. of Acatlán de Juarez, Jalisco	1420, 19:98; -104:00
11	T.210	31	TIT (*T. intermedium* de Wet and Harlan)	72	tetraploid	Mexico	Ojo de Agua, 1km W. of Ojo de Agua, Colima	170, 19:32; -103:80
12	T.1070	96	TJL (*T. Jalapense* de Wet et Brink)	72	tetraploid	Mexico	La Coyota, km 14.9 Carr. Sumidero, Chiapas	1150,
13	T.7221	1	TPL (*T. pilosum* Scribner and Merrill)	72	tetraploid	Mexico	Jilotlán, Jalisco	740, 19:22; -103:01
14	T.1610	39	TDM (*T. dactyloydes var.mexicanum* de Wet and Harlan)	72	tetraploid	Mexico	La Toma, 3 km W. of Tequila, Jalisco	1200, 20:90; -103.80
15	T.2131	142	LC (*T. lanceolatum* Ruprecht ex Fournir)	72	tetraploid	Mexico	Rio Chico, Carr. Mazatlán, Durango	2100, 23:93; -104.80
16	T.2368	153	TDD (*T. dactyloides* (L.) L.)	72	tetraploid	Mexico	Lamasinta, Carr. Chilapa-Tlapa, Guerrero	1500, 17:60; -99:17
17	T.5081	550	TPR (*T. peruvianum* de Wet and Timothy)	90	pentaploid	Peru	Tingode Saposoa, San Martin	299, -7:12; -76:62
18	T. 5023	522	TAD (T. andersonii Gray)	64	hybrid (64)	Brazil	Campinas Institute of Agronomy, Sao Paulo	661, -22:88; -46:07

Table 2. Plant traits measured for the *Tripsacum* clones analyzed in this study and for some additional clones at CIMMYT Tlaltizapán station.

Clonal Sample ID	Collection No.	Population No.	Species classification	Plant height (m)	Leaf length (cm)	Leaf width (cm)	Culm width (cm)	Culm thickness (cm)
Tripsacum clones used for phylogenetic study in A1 plot at Tlaltizapán, Morelos, Mexico.								
01	T.1069	95	TMN (*T. manisuroides* de Wet and Harlan)	3.28	152	3.3	1.5	1.35
02	T. 5243	613	TCD (*T. cundinamarce* de Wet and Timothy)	3.15	158	4.2	2.15	1.65
03	T. 5222	603	TMR (*T. dactiloydes var.meridionale* de Wet and Timothy)					
04	T.7003	1	TZP (*T. zopilotense* Hernandez and Randolph)	2.39	144	1.6	0.6	0.4
05	T. 5087	554	TMR (*T. dactyloydes var.meridionale* de Wet and Timothy)	2.5	127	2	1.2	1
06	T.69	18	TLT (*T. latifolium Hitchc.*)	1.8	135	4.1	2.3	2
07	T.7146	4	TMZ (*T. maizar* Hernandes and Randolph)	2.07	128	6	2.3	2.15
08	T.552	6	TBV (*T. bravum* Gray)	1.68	114	1.9	1.4	1.15
09	T.569	9	TDH (*T. dactyloydes var. hispidum [Hitchc.] de Wet and Harlan*)	1.15	122	1.43	0.7	0.55
10	T.139	24	TDM (*T. dactyloides var.mexicanum* de Wet and Harlan)	3.2	177	3.13	1.7	1.25
11	T.210	31	TIT (*T. intermedium* de Wet and Harlan)	3.28	179	3.66	0.95	0.8
12	T.1070	96	TJL (*T. Jalapense* de Wet et Brink)	3.24	178	3.6	1.25	1.1
13	T.7221	1	TPL (*T. pilosum* Scribner and Merrill)	3.53	223	4.1	2.2	1.7
14	T.1610	39	TDM (*T. dactyloydes var.mexicanum* de Wet and Harlan)	2.77	155	3.67	1.8	1.5
15	T.2131	142	LC (*T. lanceolatum* Ruprecht ex Fournir)	1.64	129	1.65	0.7	0.5
16	T.2368	153	TDD (*T. dactyloides* (L.) L.)	2.5	150	0.76	0.65	0.45
17	T.5081	550	TPR (*T. peruvianum* de Wet and Timothy)	2.73	140	4.1	1.5	1.1
18	T. 5023	522	TAD (*T. andersonii* Gray)	2.08	163	6.73	2.5	2.1
Additional *Tripsacum* clones measured in A0 plot at Tlaltizapán, Morelos, Mexico.								
A01	TZP-DEC- 0506		TZP (*T. zopilotense* Hernandez and Randolph)	2.18	142	0.9	0.35	0.3
A02	T.5085	553	TPR (*T. peruvianum* de Wet and Timothy)	2.14	129	2.96	0.7	0.55
A03	TLX-DEC-05-72		TLX(*T. laxum* Nash)	4.4	144	5.1	2.15	1.65
A04	TLT-DEC-05-70		TLT (*T. latifolium Hitchc.*)	2.95	203	3.46	1.85	1.05

Table 3. Maize and teosinte samples included in this study.

Sample ID	CIMMYT Bank ID	Race name	Population	Collection site or seed origin
Maize line	16417	Tuxpeño pool	CML 247	G24F119*G24F54-6-4-1-1-BB,CIMMYT
Teosinte 01	8765	Balsas	K67-22	Tinganbato, Michoacán, Mexico
Teosinte 02	8768	Chalco	K68-1	Milpa Alta, México, Mexico
Teosinte 03	9478	Guatemala		Chiquimula, Guatemala
Teosinte 04	9479	Huehuetenango		Jacaltanango, Huehuetenango, Guatemala
Teosinte 05	10003	Diploperennis	San Miguel	Cautitlán, Jalisco, Mexico
Teosinte 06	11367	Central Plateau	K69-5	Chacandiro, Michoacán, Mexico
Teosinte 07	11403	Balsas	W.S.92-12	Ixcapzalco, Guerrero, Mexico

Figure 1. Neighbor-joining tree of *Tripsacum*, teosinte, and maize, which was constructed based on the Rogers distance, calculated using 1036 SNPs.
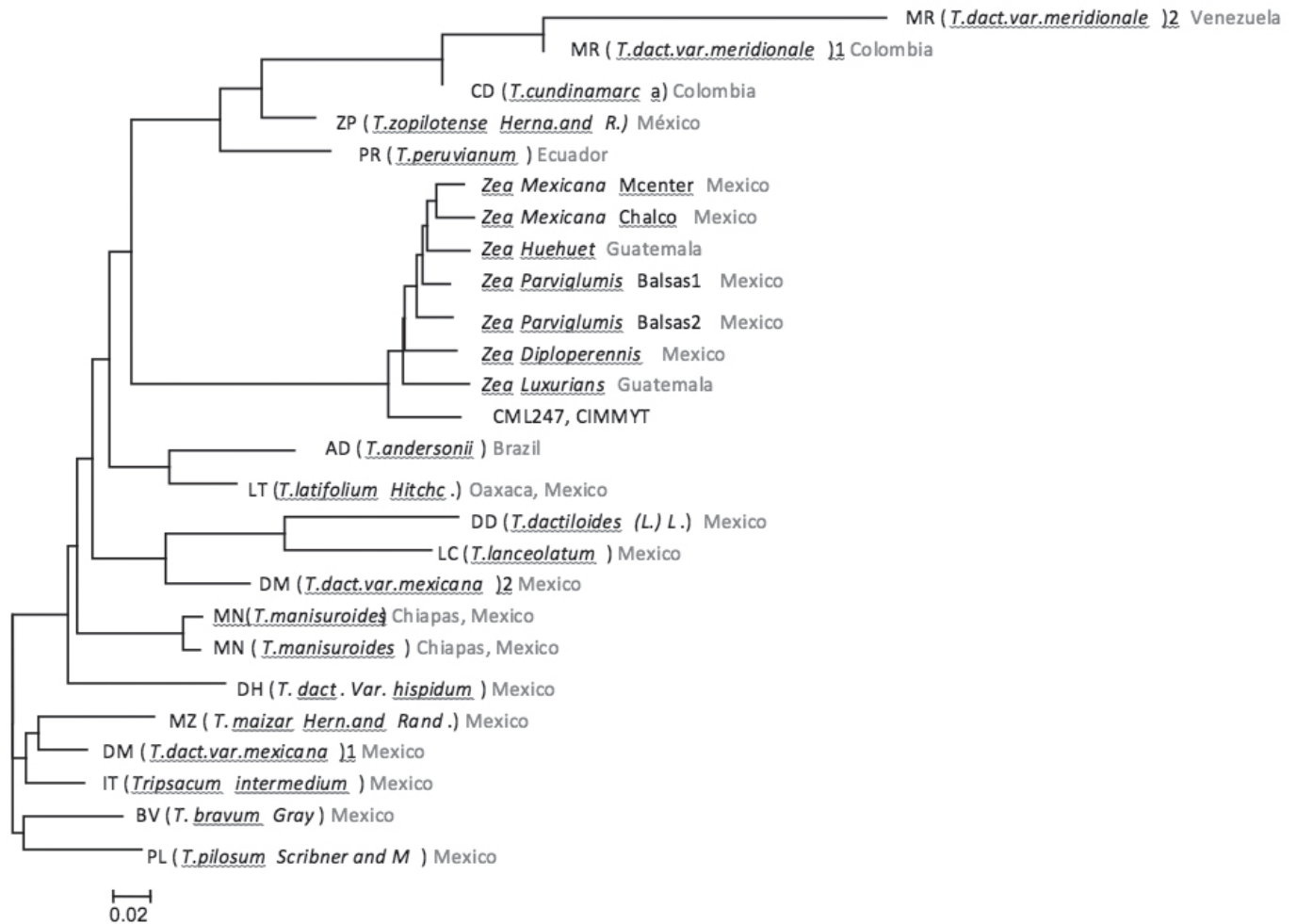
From the current relationship among the taxa of *Tripsacum*, teosinte, and maize, we recommend developing inter-specific gene pools of *Tripsacum* species and *Tripsacum* X maize gene pools to exploit useful genes and allelic diversity (Dewald and Kindiger, Am J Bot 85:1237-1242, 1998; Li et al., Ann Bot 84:695-702, 1999) that may exist in *Tripsacum*.

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Aberrant doubled haploid lines in maize
— Rotarenco, V; Dicu, G

Doubled haploid (DH) maize lines generally are created from *in vivo* induced haploid plants by artificial chromosome doubling using mitosis-inhibiting compounds such as colchicine (Gayen et al., MNL 68:65, 1994; Wan et al., Theor Appl Genet 77:889-892, 1989). DH lines therefore should be homozygous; however, we have observed a significant phenotypic variation in the progeny of some DH lines.

Possible reasons for this phenomenon include:

1. Mutagenesis

a) Colchicine does not act only as an inhibitor of mitosis but may also induce mutations. If this happens in the diploid sporophytic tissue of a treated haploid plant, selfed progenies will be heterozygous leading to segregation in subsequent generations.

b) Since the diploidy is the natural ploidy level of maize, the mutation rate of haploids, due to the influence of environmental conditions, might be higher than that of diploids.

2. Paternal gene transfer

Haploid inducers acting as pollinator generally do not transmit any genes to the resulting haploids, i.e., the induced haploids carry genes from the maternal genotype only. Yet occasionally limited male gene transfer (DNA introgression) was reported in the literature (Fisher, 2004; Liang Li et al., 2009). This leads to a transformation of genetic material; no heterozygousity will occur at the DH level. However, if a male chromosome segment is added to the female genome leading to aneuploidy, segregation may occur in subsequent selfing generations.

Our studies showed a significant influence of inducers on the manifestation of quantitative traits in haploid plants, and that, most likely, was associated with the DNA introgression (Rotarenco et al., 2009). Our objective was to reveal the most likely reason for the instability of DH lines. A well-known inbred line, A619, and a DH line, 134, were used in the study. The 134 line is one of the DH lines derived from our breeding synthetic population, SP, and after three generations, we noticed a significant phenotypic variation within this line. In contrast, the inbred line A619 is characterized as a rather stable genotype. Both lines were crossed with a haploid-inducer line, MHI (Chalyk, MNL 73:53-54, 1999).

Haploids produced from each line were divided into two groups. The first group was planted in the field; the second group was subjected to a chromosome-doubling treatment (Deimling and Geiger, Vortr Pflanzenzuchtg 38:203-204, 1997). There were about 150 haploid kernels in each group. Haploids planted in the field were randomly pollinated with a bulk of pollen from their diploid lines; doubled haploids were self-pollinated. By the pollination of haploids with their diploid analogues, eight new lines, called reconstituted lines, have been obtained from each initial line. Six DH lines have been produced from both A619 and 134 lines by chromosome doubling.

Reconstituted and DH lines have been compared with the initial genotypes in S2 and S3. Plant height, ear length, and coefficients of variation of these traits were estimated. The experiments were carried out in three replications on two-row plots. No significant differences were revealed between the line A619 and its derivatives, whereas among the lines produced from the line 134, a significant variability for the estimated traits was detected. We did not reveal any significant differences between the S2 and S3 generations. Results comparing the initial lines with their DHs in S3 are presented in Table 1.

Lines 134DH4 and 134DH5 were significantly inferior to the initial line, 134, for plant height; 134DH1 and 134DH3 significantly exceeded the initial one for ear length. Four reconstituted lines, either for plant height or for ear length, differ significantly

Table 1. Means of plant height and ear length and coefficients of variation of these traits in the lines A619 and 134 and their DHs (S3).

Initial lines and DH progenies (S3)	Plant height		Ear length	
	Mean	Coef. var.	Mean	Coef. var.
A619	202.2 ± 2.2	6.6	15.7 ± 0.7	20.7
A619DH1	198.2 ± 2.8	6.4	14.2 ± 0.5	13.5
A619DH2	200.5 ± 2.6	5.8	16.1 ± 0.7	17.0
A619DH3	200.3 ± 1.9	4.7	16.7 ± 0.7	15.9
A619DH4	201.8 ± 1.4	3.8	15.5 ± 0.3	8.2
A619DH5	207.1 ± 2.6	5.4	16.0 ± 0.7	17.3
A619DH6	197.4 ± 2.4	6.1	17.7 ± 0.9	13.5
134	269.5 ± 5.6	10.6	15.1 ± 0.19	10.7
134DH1	275.2 ± 2.9	4.9	16.9 ± 0.21***	7.6
134DH2	267.8 ± 2.0	3.5	14.5 ± 0.16	7.6
134DH3	270.2 ± 3.9	6.6	16.8 ± 0.26***	9.1
134DH4	227.3 ± 2.2***	5.4	15.1 ± 0.13	5.8
134DH5	223.4 ± 2.5***	6.4	14.7 ± 0.16	7.1
134DH6	268.0 ± 2.1	3.7	15.1 ± 0.17	7.1
*** Significantly (P < 0.001) different from initial line				

from the initial genotype 134 (data is not presented). Additionally, among those 14 lines derived from the line 134, we have noticed a variation for the beginning of flowering — up to 10 days.

In all DH lines, the coefficients of variation have reduced in comparison with the initial genotypes (Table 1). Thus, most likely, the influence of colchicine is not the main reason for the instability of DH lines; the same can be said about the influence of environmental conditions since we did not obtain any significant differences among the reconstituted lines derived from the line A619 (data not presented). Differences among the lines produced from the line 134 may be connected with the fact that the initial genotype represents a heterogeneous material. At the moment, aneuploidy is the preferred version of the segregation in the progeny of some DH lines.

Every year, we notice a high frequency of unstable DH lines, named *aberrant doubled haploid* lines, among so-called spontaneous doubled haploids. There might be something in common between these phenomena, and we are assuming that is aneuploidy. We expect further work will bring us more answers on this topic.

Improvements of *in vivo* haploid induction in maize
— Rotarenco, V; Dicu, G

Currently, doubled haploid technology is the main way homozygous inbred lines are developed in maize breeding. It be-

came possible after the creation of haploid inducers — specific genotypes used as pollinators to produce maternal haploids *in vivo*. Modern inducers have relatively high rates of haploid induction and a system of marker genes allowing haploids to be identified at different stages: dry kernels, seedlings and mature plants (Rober et al., Maydica 50:275-283, 2005; Rotarenco et al., MNL 84:21-22, 2010). However, there are some limiting factors in the haploid induction technique, namely: the frequency of haploids has a significant variation in different donors (Prigge et al., Crop Sci 51:1498-1506, 2011); the identification of haploids among dry kernels might be very complicated or even impossible in cases when the donors are homozygous for certain genes (*R1*, *C1-I*) (Belicuas et al., Euphytica 156(1-2):95-102, 2007; Geiger in Bennetzen and Hake (eds), Maize Handbook Vol II: Genetics and Genomics 641-659, Springer Verlag, 2009); the marker genes *B1* and *Pl1* are frequently affected by paramutations both in crosses with donors and in inducers themselves (Chandler et al., Plant Mol Biol 43(2-3):121-145, 2000). Additionally, in some inducers, we have noticed partial male sterility and reduced pollen production. Poor seed set in inducers complicates their maintenance and reduces the yield of haploids in crosses with donors.

Chalyk et al. (MNL 77:29-30, 2003) found a high frequency of aneuploid cells in haploid inducers. The authors concluded that aneuploidy could be the reason of the haploid-inducing ability. Based on this hypothesis, it is possible to assume that there should be a positive correlation between the haploid-inducing rate and the frequency of aneuploid cells in inducers. On the other hand, aneuploidy can lead to irregularities during meiosis and be the reason for both partially male sterility and poor seed set.

In 2010, using the new haploid inducers, PHI (Rotarenco et al., 2010), genotypes were found with high rates of haploid induction (more than 10%), and also good pollen production and seed set. In 2011, the main objectives were to estimate the connection between the haploid-inducing ability and partially male sterility in the PHI inducers and to determine the correlation between the frequency of haploids and the yield of haploids per ear in different donors.

Besides inducer lines PHI-1, PHI-2, PHI-3, and PHI-4, two hybrid inducers, PHI-3 × PHI-2 and PHI-3 × PHI-4, have been tested. Five donors were used in the experiment: two hybrids, A619 × A464 and B73 × Mo17; a synthetic population, SA; and two inbred lines, B73 and A619. To synchronize the flowering time, the inducers and donors were planted at different times. The donors were pollinated on the third day after the emergence of silks, which is the best time for haploid induction (Rotarenco et al., MNL 81:9-10, 2007). In each donor, 5 to 10 ears were pollinated with the pollen of each inducer. Partially male sterility (segmental shedding) was revealed in the inducers PHI-3 and PHI-4, whereas the inducers PHI-1 and PHI-2 had a good shedding. As checks, we used the initial inducers, MHI (Chalyk, MNL 73:53-54, 1999) and Stock6 (Coe, Am Naturalist 93:381-382, 1959) (Figs. 1 & 2).

Figure 1. MHI: segmental shedding.

Figure 2. Stock 6: good shedding.

In three donors, A619 × A464, B73 × Mo17, and the SA population, the highest rate of haploid induction had an inducer with a good shedding: PHI-1. The same inducer line had the highest yield of haploid kernel per ear in those donors (Table 1, Fig. 3).

Remarkably, the hybrid inducer PHI-3 × PHI-4 did not have any signs of male sterility in contrast with the parent lines. More-over, its haploid induction rate, 14.1%, was higher than in the parents and the number of haploids per ear was the highest, 19, in the donor A619 × A464. The inducer PHI-3 had the highest haploid induction rate, 24.6%, in B73; however, the average yield of haploids was the lowest, 6.6. The highest yield of haploids, 36.8, in B73 was obtained by the PHI-1 inducer with the haploid induction rate of 21.6%. The same inducer showed the best result in the second donor line, A619 (Table 2, Fig. 4).

All coefficients of correlation had positive values. In two donors, A619 × A464 and B73 × Mo17, they were statistically sig-

Table 1. Haploid induction rates, average numbers of haploid kernels per ear, and the coefficients of correlation between these traits in three donors obtained by crosses with six inducers (2011).

	Donor					
	A619 × A464		B73 × Mo17		Population SA	
Inducer	Haploids (%)	Haploids per ear	Haploids (%)	Haploids per ear	Haploids (%)	Haploids per ear
PHI-1	15.3	17.0	14.7	18.1	17.1	28.0
PHI-2	10.7	7.2	10.2	11.3	11.7	9.6
PHI-3	12.2	8.1	10.1	8.0	15.2	6.7
PHI-4	12.0	8.8	8.4	10.0	14.3	14.0
PHI-3 × PHI-2	9.1	9.2	-	-	-	-
PHI-3 × PHI-4	14.1	19.0	-	-	-	-
Coefficient of correlation	0.77*		0.89*		0.72	

*Significant at p < 0.05.

Table 2. Haploid induction rates, average numbers of haploid kernels per ear, and coefficients of correlation between these traits in two donors obtained by crosses with four inducers (2011).

	Donor			
	B73		A619	
Inducer	Haploids (%)	Haploids per ear	Haploids (%)	Haploids per ear
PHI-1	21.6	36.8	15.2	12.2
PHI-2	14.0	10.0	7.4	1.6
PHI-3	24.6	6.6	14.3	3.4
PHI-4	18.5	18.3	10.3	4.6
Coefficient of correlation	0.13		0.71	

Figure 3. Female: synthetic population SA.

Figure 4. Female: B73.

nificant ($p < 0.05$).

Our results revealed that the haploid-inducing ability, partially male sterility, and seed set are not such strong connected inducers' characteristics. Thus, we are able to create inducers with the high rates of haploid induction good both shedding and seed set. Using hybrid inducers could be a way to improve these inducers' properties as well. However, the hybridization of different inducer lines, based on our experience, might have a negative effect on the haploid induction rate and the marker genes. Therefore, first, the most successful hybrid combinations have to be identified.

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Activation of silent *Ac* sequences
— Schwartz, D

In a comparison of active and silent *Ac* sequences I have found that a single nucleotide polymorphism always appears at position 20, counting from the *Ac9* 5' end. All active *Ac* sequences have a T at that position. The B73 sequenced genome does not contain an active *Ac*, only a silent 4554 base *Ac* as well as 55 *Ac* fractionals that begin at the 5' end. All of these have a G at that position. The portions of *Ac* critical to the formation of the transposase, promoter and translated segment extend from the 5' end to base 2451 of *Ac9*. The first 3100 bases of the active *Ac9* and the 4554 base *Ac* are identical except for seven single nucleotide polymorphisms. Of these, only the T to G change at base 20 is seen in all the 56 *Ac* sequences in the B73 genome. A T to G change is a rare mutation, but it is always seen at base 20 in silent *Ac*. By comparison, of the transposons with strong similarity to *Ac* that is limited to the first 110 bases, only one out of 175 have G in place of T at position 20. Also, of the 17 transposons in which the similarity to *Ac* is limited to the first 206 bases, only one has a G in place of T at position 20.

Studies are testing the hypothesis that stress-induced activation of the silent *Ac* involves a G to T nucleotide change at position 20. The transposase activity of *Ac9* causes much damage to maize plants as a result of chromosome breakage with the subsequent breakage-fusion-bridge cycle, as well as gene inactivation from *Ac*-induced insertions. Thus, in a stable environment, plants with an active *Ac* would be selected against. However, under conditions of stress caused by drastic changes in the environment, an active *Ac* would play a major role in producing the mega-changes required for survival of the species. What would be better than activation induced by the simple change of only a single nucleotide?

On *Ac*-induced chromosome dissociation
— Schwartz, D

Barbara McClintock was able to identify the presence of the activator, *Ac*, by its action of chromosome dissociation, *Ds*. Although the events involved in the mobility of the *Ds* transposon are clear, I know of no studies on *Ac* breakage of the chromosome. Three possible events come to mind. Breakage can result from *Ds* mobility if the two broken ends do not join to produce an eight-base direct repeat

(1). It may result from a single break at the 5' end that contains the nearby AAACGG binding site of the transposase (2). The last event (3) is breakage at the 3' end. The fusion of the two complementary strands of DNA at a break point that must occur to give rise to McClintock's breakage-fusion-bridge cycle affords a simple way to distinguish between these three possible events.

Ds1 terminal sequences of about 40 bases were duplicated and pasted end to end, either at the 5' or at the 3' termini, and used as query in BLASTs of the sequenced genome of B73. The BLASTs were searched for hits that extended across the paste point. According to (1), no such hits would be expected when either the duplicate 5'-5' or 3'-3' terminal sequences were used as query, since the fusion would join the flank sequences and not *Ds1*. According to (2), hits that extend across the paste point should be detected only with the duplicate 5-5' used as query and not with the duplicate 3'-3' query. The opposite should result according to event (3) with the extended hits detected only when the query is the duplicated 3'3' sequence.

Tests with a number of *Ds* transposons clearly showed that event (2) is correct. In BLASTs with duplicate 5'-5' pasted termini as query, multiple cases were detected with hits that extended for as long as 20 bases on both sides of the paste point. No such cases were detected with the duplicated 3'-3' pasted queries.

The AAACGG transposase binding site is very close to the terminus, starting at position 9. Proximity is not required for the cleavage that occurs at both the termini in the case of *Ac*/*Ds* transposon mobility where the 5' and 3' termini are separated by as many as 4,500 bases, but it may be a factor in breakage at only one end that gives rise to a breakage-fusion-bridge cycle. In order to check if a nearby transposase binding site at the 3' end would give a positive result, a test was performed using *Ds* transposons with terminal sequences that had an AAAGGG sequence close to both the 5' and 3' ends. The test results were clearly positive, showing that the breakage could occur at either the 5' or 3' termini.

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Farmers in Sub-Saharan Africa cry for tightly closed ear drooping maize phenotypes
— Muoma, JO[1]; Ochieno, DMW[1]; Alakonya, AE[2]

Maize is a very important staple food crop in Sub-Sahara Africa (SSA), but the production has been below optimum. One important constraint to maize production is grain rot/ear rot, which has been reported to cause yield losses ranging between 13% and 70%. Of concern is that most of the ear-rot-causing fungi like *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp. produce mycotoxins, which are fungal metabolites that are hazardous to human and animal health. Some of the worst aflatoxin outbreaks were reported in Kenya between 2004 and 2007, during which hundreds of people died of mycotoxin-related sicknesses. This was considered one of the worst epidemics in human history.

Figure 1. Maize ears showing stalk borer damage and ear rot symptoms on a commercial variety in western Kenya.

Commercial seed producers, particularly the large multinational companies, have been supplying high-yielding maize germplasm to farmers in SSA. Unfortunately, such commercial varieties seem to be susceptible to endemic pests and diseases, especially birds, stemborers, and ear rots. For instance, the upright position of the ears and their opening at maturity makes it easy for weaver birds to remove the husks (shucks), allowing secondary pest infestations and entry of rain water, which predispose maize to ear rot fungal infestations. Despite breeding being the main avenue in the development of superior maize germplasms, there seems to be unrealized efforts by commercial seed companies in producing ear rot resistant maize. Farmers planting the commercial cultivars have been challenged with protecting their apparently high maize yields from losses caused by ear and grain rots. SSA farmers, noting these issues, look at such "foreign germplasm" with open reservations. The farmers have been reverting to their traditional seeds, which exhibit closed and drooping ears at maturity. Farmers claim less ear rot occurs in these traditional cultivars, though they have been admitting their yields are quantitatively lower than those from commercial hybrids. This move is unfortunate because some of the high-yielding commercial hybrids could help solve the food security problem, but only if the closed-tip traits and the ear-dropping traits could have been incorporated during breeding. Regardless of our views as researchers, the farmer remains the "boss" and therefore evaluates our performance.

As scientists, we are aware many genes and even loci could be responsible for the novel traits our "bosses" require. Because the incorporation of such traits offers a major opportunity in minimizing field and post-harvest maize pest and disease infestations, efficient conventional breeding approaches need to be adopted to enhance the high-yielding commercial cultivars with closed and ear-drooping genes. The challenge is that ear drooping is polygenic, and the success of transferring the phenotype by conventional breeding methods may not be predictable. This calls for maize breeders to employ molecular tools like SSR and ISSR markers in their breeding programs if a solution to this problem is to be developed in the near future.

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Kashmir landraces: Present status, future prospects
— Najeeb, S; Rather, AG; Zarger, MA; Ahangar, MA; Sheikh, FA; Parray, GA; Bhat, ZA; Sofi, PA; Subash; Kashap, C; Ishfaq, A; Dar, ZA; Mehfoza, H; Bardi, ZA

In the state of Jammu and Kashmir (India), maize is the second most important crop after rice and is a staple food of some tribal areas such as Gujar and Bakarwall (nomadic race). The main maize crop generally is grown as rainfed and on marginal lands, particularly the hilly terrains of the Kashmir Valley at an altitude range of 1,850-2,300 m above mean sea level. Its latitude and longitude are 32.50-36° N and 73-76.2° E, respectively. The Kashmir Division is agroclimatically a typical temperate region. In the Kashmir Valley maize is grown as a sole crop and in some cases intercropped with pulse crops such as beans, green gram, etc., to increase the cropping intensity. The farmers go for intercropping because through traditional knowledge systems they know the positive impact on compatibility of two crops and soil health aspect. Maize is also grown in a few pockets of the plain belts of the valley (1,450-1,650 m AMSL), without irrigation and most often as a backyard/kitchen garden crop. The plants are not allowed to go to seed development. The crop is usually consumed just before dough stage as roasted or boiled cob.

A number of landrace populations of maize have been documented from the Kashmir Valley. Table 1 lists important landraces with their salient features. These are grown as sole crops. The main reasons for their popularity, even when there are available high-yielding varieties:

• Good adaptation. They adapt to specific agroecologies and have assumed a niche status. They have very good population buffering.

• Wide resistance to natural factors. These landrace populations have a wide range of genetic variability and adaptability. They possess the genes for tolerance to various biotic and abiotic stresses. Most important to mention are drought, cold, and pest resistance.

• Early maturing. They escape the cold injury risk at later stages of crop growth. Snowfall is likely in September, particularly in the hilly terrains when the crop is under dough stage.

• Very good culm quality. The unusually thin and succulent stems are highly relished by the cattle in the lean season when there is no other fodder crop available.

• Ability to grow on marginal lands. This includes a low input environment and the areas where irrigation is absent. Since these landraces have much specific adaptability, they are often a "safer" crop and thrive even under low input conditions (Farr, The benefits of traditional agriculture in Mexico, e-Mexico Journal 1, 2001; Ortega, Causes of genetic erosion in maize, presented at "Gene Flow: What Does It Mean for Biodiversity and Centers of Origin," 2003).

• Good grain quality. Because of grain quality they are mostly preferred over hybrids and synthetics bred by Mountain Crop Research Station (SKUAST-K) for different agroecologies of Kashmir

and from outside sources of equivalent ecologies.

 • Food and fodder value. These landraces usually serve as a staple food of 15% of the population of Kashmir Valley. The maize kernels are ground to fine flour (called makai atta*). The unleavened bread prepared with this flour is much liked by the local people, even the rice consumers. The bread is usually supplemented with locally prepared ghee, which adds taste and aroma to the unleavened bread. The byproduct of the grinding process is rough maize flour (called satoo) and is consumed with salt tea. It is used throughout Kashmir Valley as a breakfast food and is a cheaper alternative to wheat bread for low-income families. In some cases maize is consumed just like rice. After boiling maize grains a sticky dish is prepared and is supplemented with curd. Highly relished by elderly people in the hilly areas, this is called makai wart. In the local system of medicine it is recommended to diabetic patients and those having urinary problems. Figure 1 (next page) provides information about the broad-spectrum variability in cob characters, color variations, cob sizes, and textures. Figure 2 shows the products of maize directly consumed by local population: satoo (rough flour) used as breakfast food, atta (fine flour) for bread making, and makai wart (boiled kernels of maize just like rice).

The roasted and boiled cobs of local landraces fetch a very good market rate because of their taste and sugary content. Where fodder values of these landraces are concerned, their thin and succulent stems (culm) are highly relished by cattle. Because the valley remains cut off from outer parts of the world during winter months, these hilly terrains have no alternative except to use maize stover as cattle fodder.

The maize farmer growing for home consumption often has little reason to choose hybrid maize over locally adapted open-pollinated maize. When maize is a main food source, texture, flavor, and even appearance may be more highly valued than absolute productivity under rarely achieved, optimal conditions (Anderson, Plants, Man and Life, Little Brown, 1952; Hernández in Ramamoorthy et al. (eds.), Biological Diversity in Mexico, Origins and Distribution, Oxford University Press, pp. 733-753, 1993; Ortega, 2003). Prices for local maize can be several times higher than for common, yellow imported maize (Barkin in Esteva and

Table 1. Important landrace populations of maize of Kashmir.

Landrace	Color	Salient features	Usage/market value
Anantnag safed	White	Semi dent, medium maturity, good straw quality	Bread, satoo*, poultry ration; low market value
Anantnag lader	Yellow	Semi dent, medium maturity, good straw quality	Bread, poultry ration; medium market value
Pahalgam wuzg	Deep orange	Semi dent, medium maturity, good straw quality	Satoo, cattle feed; high market value
Pahalgam safed	White	Semi dent, early maturity, good straw quality	Bread, satoo, poultry ration; low market value
Vailoo local	Yellow	Semi dent, early maturity, good straw quality	Bread, poultry ration; medium market value
Gowran wuzg	Deep orange	Semi dent, extra early, good straw quality	Satoo, cattle feed; high market value
Matihundi safed	White	Semi dent, small cob, early, good straw quality	Bread, satoo, poultry ration; low market value
Matihundi wuzg	Deep orange	Semi dent, plant and ear characteristics poor, early maturity, good straw quality	Satoo, cattle feed; high market value
Gurdamn wuzg	Deep orange	Semi dent, plant and ear characteristics poor, extra early, good straw quality	Satoo, cattle feed; high market value
Gurdamn lader	Yellow	Semi dent, plant and ear characteristics poor, early maturity, good straw quality	Bread, poultry ration; medium market value
Tangwin safed	White	Semi dent, plant and ear characteristics poor, early maturity, good straw quality	Bread, satoo, poultry ration; low market value
Khreti wuzg	Deep orange	Semi dent, plant and ear characteristics poor, early maturity, good straw quality, low yielding	Satoo, cattle feed; high market value
Aru wangan	Purple	Semi dent, plant and ear characteristics poor, early maturity, good straw quality, very low yielding	Bread, poultry ration; medium market value
Aru wuzg	Deep orange	Semi dent, plant and ear characteristics poor, early maturity, good straw quality, low yielding	Satoo, cattle feed; high market value
Satura safed	White	Semi dent, plant and ear characteristics poor, early maturity, good straw quality, yield potential medium	Bread, satoo, poultry ration; low market value
Paner lader	Yellow	Semi dent, plant and ear characteristics poor, early maturity, good straw quality	Bread, poultry ration; medium market value
Nagbal wuzg	Deep orange	Semi dent, plant and ear characteristics poor, early maturity, good straw quality	Satoo, cattle feed; high market value
Ganderbal safed	White	Semi dent, plant and ear characteristics poor, early maturity, good straw quality, good yielding	Bread, satoo, poultry ration; low market value
Gund wuzg	Deep orange	Semi dent, plant and ear characteristics poor, early maturity, good straw quality	Satoo, cattle feed; high market value

*Satoo: rough flour of maize kernels used as breakfast food

Figure 1. Maize populations collected from Kashmir Valley.

Figure 2. Products made from maize and consumed by local population.

Marielle (eds.), Sin maíz no hay país, Consejo Nac Cultura y Artes, pp. 155-176, 2003), but at the national level, with current governmental policies, there is pricing discrimination against native, open-pollinated maize (Ortega, 2003).

However, these landraces are losing their popularity and gradually are becoming extinct. They face tough competition from newly developed hybrids and synthetic varieties. The reasons for the decline of these landraces include their low yielding potential, leading them to be replaced by high-yielding hybrids and synthetic varieties; low resistance to biotic stresses such as *turcicum* leaf blight and common rust, which are taking a heavy toll; lower sensitivity to inputs, as they respond at a very slow rate to favorable environments and to costlier inputs such as inorganic and bio-fertilizers; and the socioeconomic plight and poor resources of the farmers, who are not growing their landraces on modern scientific lines.

Ways to protect maize landrace populations

• Population improvement program. Few simple recurrent selection cycles can genetically improve the base population of these landraces because they possess broad-spectrum genetic variability within the population for various economic traits. This not only improves the population per se, but improved inbreds can be derived from the populations for hybrid development.

• Initiating the hybrid development program. Development of elite inbreds from improved populations and using one of the parents from exotic sources can exploit the heterosis. The two lines would be genetically dissimilar, and this wide diversity between the lines is the raw material for heterosis.

• Biotechnological interventions. Using the latest biotechnology tools, such as molecular selections, can rectify some economic traits of these landraces.

• Innovative approaches for crop improvement. New scientific management technologies, such as proper crop husbandry

practices for better production, in turn could improve farmers' socioeconomic plight.

• Participating plant breeding (PPB) approaches. PPB will work with farmers to develop the varieties based on the priorities of clients. This is because in formal breeding programs varieties generally are developed in favorable environments but proposed for different environments. The expensive cost and long lag phase from development to actual availability also plays a role in slow adoption of good varieties. The resource-poor maize farmers generally are located in marginal environments, and such conditions are not being given due consideration. It is here these landraces can be popularized after genetic enhancement and genetic purification right in the farmers fields. The community seed production units can be established for informal seed multiplication chain. Thus a participatory role directly involving farmers and designing agroecology-specific varieties is necessary.

Landraces are a reservoir of important allelic resources of a crop. Nature has bestowed them with wide resilience against many kinds of stresses such as cold, drought spells, and some diseases and pests. They have the genes for adaptability, besides grain and straw quality. These populations are reservoirs of useful genes that could combat coming challenges and other socioeconomic issues.

Improvement of maize: Emerging trends in the state of Jammu and Kashmir
— Najeeb, S; Wani, SA

Maize occupies an area of 0.32 M ha in Jammu and Kashmir state (Government of Jammu & Kashmir Directorate of Economics and Statistics, 2009). The maize cultivation assumes much more significance in the state's hilly tracts, where it is the chief source of livelihood and staple food for more than half a million families. Eco-

nomic growth has led to increased demand for mutton and poultry and has given further importance to maize. Additionally, maize is the only alternative to rice in the circumstances of drought. Over the past decade the demand for maize grain has grown because of two key factors: i) a 38% increase in the population of state of Jammu and Kashmir; and ii) a change in food habits to include bread made of maize flour, in addition to common staples rice and wheat.

Production in the state is very low when compared to national and international averages. It is estimated that per hectare productivity in the state of Jammu and Kashmir is just 1.8 tons. The low production and productivity come from various factors and include the predominate use of low-yielding open pollinated varieties (OPVs)/ landraces. The OPVS developed by CIMMYT for resource-poor farmers lose their genetic advantage or their phenotypic identity during successive cycles of seed sowing. Commercial and small holding maize growers do not meet demand for seed of improved varieties on time. As a result farmers plant the land with their own saved seed.

In addition, there is no stable market and farmers cannot be assured of a reasonable price for their crop, so they do not take any risk in the investment to grow it. Transportation is inadequate to bring in supplies in a timely fashion and to transport products to the market. Farmers are resource-poor, and do not use many chemical fertilizers, and soil health has deteriorated. There is no access to new technologies developed at the research stations of agricultural universities and are located far from actual growers. Extension services and links are not adequate to make maize growers aware of urgent needs during different cropping operations and new challenges brought on by demographic changes and urbanization.

The maize sector must overcome all these bottlenecks and meet the demands of other sectors of the state that directly or indirectly depend on the success of maize crops. Due to urbanization, industrialization, and demographic pressures, we are losing a major portion of arable land. Thus to satisfy future demand for maize production, increasing emphasis must be placed on making improvements in breeding and cultural practices as there seems little opportunity for any increase in area. Cultural and breeding programs must go hand in hand if the full potential of emerging production technologies is to be realized. Maize productivity has reached a plateau in developed countries as the hybrid and production technologies have been exploited fully (USDA, Agricultural Statistics 1930-1998, Washington, DC, 1999; World Resources Institute, Washington, D.C., 1999). In 1998, 43% of maize area in developing countries (CIMMYT, CIMMYT 1993/94 World Maize Facts and Trends. Maize seed industries, revisited: Emerging Roles of the Public and Private sectors, Mexico, 1994) was planted to hybrids, though when Argentina, Brazil, Chile, and China are excluded, only 15% of maize planting is hybrid.

It appears that use of hybrids and development of hybrid seed industries in the state will continue to grow. To many farmers the perceived advantage of hybrids over local landraces/OPVs is not only their added yield, but also the fact that hybrids provide derived genetic improvements with repeatable precision and do so in a clearly distinguishable package. A number of HYVs and hybrids have been bred locally by the state agricultural university (Table 1). As Jammu and Kashmir is a physio-geographically diverse state, uni-

Table 1. Varieties of maize bred locally and released for different agro-ecologies of the Jammu and Kashmir state.

Varieties	Yield potential (q ha-1)
Composite-6 (C6)	45-50
Composite-8 (C8)	40-50
Composite-15 (C15)	45-50
Composite-4	50-55
Super-I	50-60
Shalimar-KG-I	45-50
Shalimar-KG-II	40-45
Shalimar Maize Hybrid-1	60-65

form breeding strategies and production technologies cannot fulfill the demand and aspirations of maize growers of the state. It is estimated that only 15-20% of farmer demand for seed is satisfied by the seed industry. Farmers need a revitalized maize seed industry that supplies improved varieties on time and, more importantly, updates the offering annually to meet evolving needs for new kinds of pest resistance, adoption to new cultural methods, and, in the circumstances of climate change, ever-increasing levels of stress resistance. These industries need to breed different cultures intended to raise yields at higher plant densities in spite of heat and drought (Edmeades et al., Developing drought- and low N-tolerant maize: Proceedings of a symposium, Mexico, 1997). The collaborative methodology of development and invention of new varieties and innovative technologies will go beyond the usual concept of extension services, which were conceived as acting primarily to deliver useful breeding products to farmers. The extension services need to be reviewed in a broader perspective and updated so transfer of technology from research units to maize growers is more efficient and effective. It would prove one of the most productive investments to improve the economic and social well being of rural poor in the state, though it would require investment in time and people that may exceed financial capabilities or political will.

Important inputs such as hybrid seed and chemical fertilizers need to be made affordable, as high costs limit their usage. The government actions should be highly polite rather than political to encourage farmers to use chemical fertilizers and herbicides through subsidized rates and other credit policies. In addition to market stability, the prices of maize must be high enough to ensure a better profit for producers. At marketable surpluses the price of maize abruptly goes down, which discourages maize growers. Community-based storage facilities must be created to hold produce until prices rise. Transportation must be adequate to supply timely inputs and transport products to the market to reduce the additional head load charges.

Increased use of hybrid maize and production technology can raise yield levels in the coming decade. The large number of small holders in the state is still an untapped source for increased production. Maize research needs to be given due priority by good financial support for public sector research in a cooperative and complementary mood for socioeconomic development of maize growers. Maize breeding research should encompass public and private institutions

and individuals and look to both short- and long-term goals aimed at economic development of the state in general and socioeconomic development of farming folk in particular.

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Amplification of heterochromatic knob size in callus culture by unequal sister chromatid exchange
— Gardingo, JR; Santos-Serejo, JA; Aguiar-Perecin, MLR

Tissue culture and in vitro plant regeneration systems have provided alternative means for mass proliferation of several plant species. Several reports have given evidence that successful plant regeneration from maize embryo-derived callus cultures is genotype dependent (see Fluminhan and Aguiar-Perecin, 1998). In addition, tissue and cell culture systems have been useful for studies on the effect of stress on chromosome stability. Chromosome breakage associated with heterochromatin regions has been observed in plant species, as for instance the occurrence of breakpoints on chromosome arms containing heterochromatic knobs detected by meiotic studies of regenerated maize plants (Lee and Phillips, Genome 29:122-128, 1987).

In a previous study, we found altered chromosomes in embryo-derived callus cultures from sister lines obtained from a Brazilian flint variety. These materials were homozygous for knobs at the long arm of chromosomes 6 (*K6L2*; *K6L3*), 7 (*K7L*), and 8 (*K8L*), and the short arm of chromosomes 7 (*K7S*) and 9 (*K9S*); in one of these lines, *K9S* was not present. Chromosome changes were detected by C-banding technique applied to callus cells. Chromosome 7 was the most affected, and this was interpreted as a consequence of the presence of knobs on both arms of this chromosome (Fluminhan et al., Ann Bot 78:73-81, 1996). The presence of an altered chromosome 7 with a normal long arm and a duplication on the short arm (displaying two knobs), was explained by the occurrence of a breakage event at *K7S* followed by cycles of breakage-fusion-bridge (BFB). Interestingly, this abnormal chromosome was stable for several months in vitro, giving evidence that healing at the chromosome broken ends had occurred. In fact, the presence of telomeric sequences on the termini of this chromosome was further demonstrated (unpublished). Another change observed in the chromosome 7 was the occurrence of amplification of the knob located on the long arm.

The origin of this amplification was investigated in further experiments by the cytogenetic analysis of R1 progenies resulting from regenerated plants derived from a callus culture designated 12F, obtained from line 13342/5. Figure 1 shows C-banded mitotic prophases of regenerated plants, homozygous for the normal *K7L* (Fig. 1A) and heterozygous for the presence of the amplified *K7L* (Fig. 1B). Fourteen heterozygous plants were selfed, and in the progeny 19 amplified *K7L* homozygotes, 58 heterozygotes, and 39 normal *K7L* homozygotes were recovered. The plants homozygous for the *K7L* amplification survived. Our interpretation is that a BFB event did not result in the amplification of *K7L* in contrast to the change mentioned above involving the terminal knob at the short arm of chromosome 7. The knob on the long arm is not terminal, and a breakage followed by BFB cycles would cause deletion of a significant distal region of the arm.

In a further experiment using 2- to 4-month-old callus cultures derived from sister lines designated 13342/1, 13342/5, 132331, and their hybrids (references on the lines in Fluminhan and Aguiar-Perecin, Ann Bot 82:569-576, 1998), we observed metaphase cells with one of the homologues of chromosome 7 displaying an asymmetric C-band corresponding to *K7L* (Fig. 1C). This gave evidence that unequal sister chromatid exchange at the knob site occurred in culture, and would modify the knob size without disrupting gene linkage in the chromatids involved. The frequency of this event was very low: It was detected in three cells of two lines and one hybrid in an experiment in which 5223 C-banded metaphases were analyzed and 2.35% presented alterations (knob amplification or reduction) on the long arm of chromosome 7.

These results are interesting not only in the context of effects of tissue culture on heterochromatin, but also as evidence of one of the mechanisms that must have occurred during the evolution of maize races. For example, in a classical analysis of maize races, McClintock, Kato, and Blumenschein (Chromosome Constitution of Races of Maize, Chapingo, México, 1981) characterized several genotypes by their knob position and sizes. This size polymorphism might have originated from unequal crossing–over involving germ cells. Callus culture techniques and C-band preparations were carried out as previously described (Fluminhan et al., Ann Bot 78:73-81, 1996).

Figure 1. C-banded mitotic prophases of plants regenerated from a 2-year-old callus culture (A, B) and C-banded mitotic metaphase of a 2-month-old callus culture derived from hybrid 13342/5 x 13342/1 (C). Note the normal size of *K7L* in both homologues in A and a *K7L* amplification in B. The small arrow in C points to chromosome 7 showing an asymmetric band on the long arm and the large arrow indicates normal chromosome 7. Scale bar = 10μm.
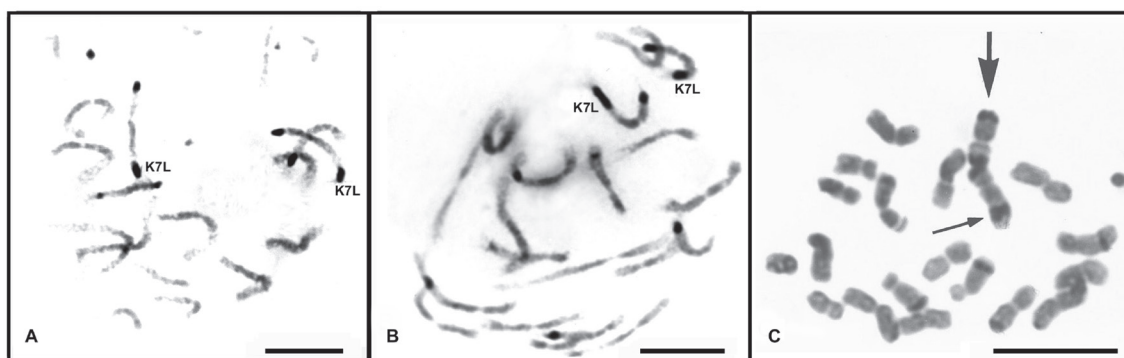
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Application of support vector regression for prediction of Grey Leaf Spot resistance using high-density molecular data

— Ornella, L[1]; Perez, P[2]; Tapia, E[1]; Crossa, L[2]

Grey leaf spot (GLS), caused by *Cercospora zeae-maydis*, is pandemic in Africa, and recognized as one of the most significant yield-limiting diseases of maize worldwide (Ward et al., Plant Sci 83:884-895, 1999). Marker-assisted selection (MAS) for reliable, resistant genotypes can be difficult when resistance is multigenic. A valuable alternative is to use genomic selection (GS) methods to estimate breeding values of individuals using the sum of all marker effects (Heffner et al., Crop Sci 50:1681-1690, 2010). Prediction of genetic values can be carried out using parametric or nonparametric approaches. Parametric models, such as Bayes A (Meuwissen et al., Genetics 157:1819-1829, 2001), Bayesian Lasso (Crossa et al., Genetics 186:713-724, 2010), or Ridge Regression (Piepho, Crop Sci 49:1165-1176, 2009), are the most commonly used. However, they are not flexible enough to incorporate complex gene action (e.g., dominance or epistasis). Support vector regression (SVR) algorithms are very suitable for GS applications. Unlike parametric models, no assumptions are made regarding genotype-phenotype relationships (Long et al., Theor Appl Genet 123:1065-1074, 2011; Maenhout et al., Theor Appl Genet 115:1003-1013, 2007).

In this work we report a test of the ability of SVR to predict GLS resistance. Genotyping data were collected using the Illumina 55K SNP (Single Nucleotide Polymorphism) chip and GLS susceptibility information collected for of a set of 300 inbreds, measured in four different trials, GLS resistance values were assigned using an ordinal scale ranging from 1 (no disease) to 5 (complete infection). The SVR was trained with linear and radial basis function kernels. The Box-Cox transformation was applied to the original trait data to make their distribution more symmetric. Regression was performed using the RegSMOImproved class of the WEKA Workbench and two alternative kernels, linear and radial basis function. Parameters of the algorithms, C (linear kernel) and C, γ (radial base function kernel), were optimized by a base 2 loga-

rithmic grid search over an extensive range of values. Each point of the grid was evaluated by internal 5-CV cross-validation on the training set using Pearson's correlation as a criterion for success. The epsilon parameter was not optimized (default value = 0.001).

Evaluation of predictive capability was performed by means of repeated hold out (Kim, Comput Stat Data Anal 53:3735-3745, 2009). Data was randomly partitioned into a training set (90% of the complete set of lines) and a test set (10% of the set). This was repeated 50 times by sampling lines at random. In order to facilitate the comparison between kernels, we used the same partitions for both linear and RBF kernel. Success of prediction was measure using Pearson's correlation coefficient between observed and predicted values. We also evaluated the predictive mean square error (PMSE). Comparison of performance was statistically validated using the non-parametric Wilcoxon (one tail) matched pairs test, which does not depend on normality assumptions (Daniel, Applied Nonparametric Statistics, Wadsworth, 1989).

The prediction success was highly variable (Table 1). The radial basis function kernel always outperformed the linear kernel in both correlation and PMSE, though this difference was only statistically significant in PMSE. Heffner et al. *(l.c.)* states that if net merit exceeds 0.50, GS could greatly outperform conventional MAS in terms of gain per unit time and cost. An improved method for optimization of parameters or a larger number of training examples should make SVR useful for grey leaf spot GS in breeding programs.

SARATOV, RUSSIA

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Mitotic activity stimulation in apical root meristems of maize at different frequencies of an alternating magnetic field

— Belyachenko, JA; Usanov, AD; Tyrnov, VS; Usanov, DA

For various agricultural crops the effect of mitotic activity (MA) stimulation in apical root meristems of seedlings under the influence of a low-frequency magnetic field (MF) is established. We already marked a stimulating effect of a low-frequency MF with certain parameters in maize apical root meristems (Belyachenko et al., MNL 84:38, 2010). For different maize lines and hybrids various levels of MA stimulation can be observed (Belyachenko et al., MNL 85:33, 2011).

Table 1. Predictive performance, determined as Pearson's correlation coefficient and PMSE, of support vector regression with radial basis function kernel (SVR-RBF) or linear kernel (SVR-Lin) for GLS resistance on four maize datasets using high-density molecular data.

Data	No. of lines	No. of markers	Correlation			PMSE		
			SVR-Lin	SVR-RBF	p-value Wilcoxon*	SVR-Lin	SVR-RBF	p-value Wilcoxon*
GLS1	272	46374	0.1969	0.2097	0.3677	1.2855	0.9809	<0.001
GLS2	280	46374	0.4044	0.4252	0.0105	0.8790	0.8276	<0.001
GLS4	261	46374	0.4959	0.5208	0.2307	0.7937	0.7398	<0.001
GLS6	281	46374	0.2632	0.2776	0.1489	1.0690	1.0300	<0.001

* p-value of Wilcoxon (one tail) matched pairs test.

MF frequency is an important parameter influencing biological response value. The aim was to compare the effects of different MF frequencies on apical root meristems MA of hybrid maize PO 176 seedlings using the following parameters: frequency ranging from 2 to 20 Hz, induction of 25 mT, and 1 hour of exposure. Dry maize seeds were exposed to MF influence. Root tips 1-1.5 cm long were fixed for cytological analysis. Amounts of cells at different stages of a cellular cycle were estimated on temporary acetocarminic squash preparations. No fewer than 3,000 cells and mitotic index values were calculated for each of three replications (Fig. 1).

MA increase under MF influence was observed at all frequencies used. At different MF frequencies the size of stimulating effect varies. The greatest effect is seen at 2 Hz. Frequencies from 6 to 12 Hz as well as 20 Hz give stimulating effect levels above 10%. The rest of the frequencies tested led to lower rise of MA in apical root meristems. Our experiments show the importance of frequency as a biologically significant parameter of MF and show an opportunity to use MF for achievement of maximum levels of MA stimulation in maize meristems.

Figure 1. Stimulating effect of alternating MF on apical root meristems MA of hybrid maize PO 176 seedlings.
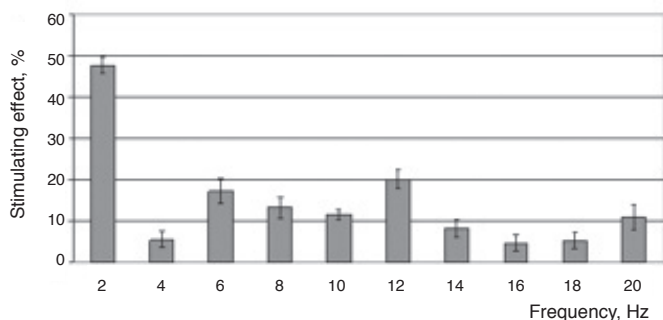
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Maize Genetics Cooperation Stock Center

Yg-N1582* is homozygous viable and has potential for use as a marker in haploid induction
— Sachs, MM; Stinard, PS

Markers used for screening haploids in inducer lines presently include *R1-nj* (which gives a pigmented kernel crown and embryo) and *B1* + *Pl1* (which give pigmented plant tissues). These traits can also result in pigmented seedling roots (Chase, Bot Rev 35:117-167, 1969; Rotarenco et al., MNL 84:21-22, 2010). However, root color is neither strong nor consistent.

We've had several queries about potential alternative markers to use in a haploid inducer, as in some crosses the present markers are not useful. We've tested several of our mutants and recently came up with one we think has excellent potential. We have an uncharacterized dominant Yellow-green mutant (*Yg*-N1582*, an EMS mutant isolated by Gerry Neuffer; see http://maizegdb.org/cgi-bin/displayvarrecord.cgi?id=77818 and Fig. 1) that has excellent expression immediately upon coleoptile emergence (when

Figure 1. Rows 1 and 2, homozygous *Yg*-N1582* seedlings. Rows 3 and 4, seedlings from a selfed ear segregating for *Yg*-N1582*.

light grown); the phenotype persists in the adult plant, and the trait is homozygous viable with no apparent decrease in vigor or in potential seed set (at least at a qualitative level).

So, with this mutant, screening for haploids would be just a bit later than with roots, but the results would be far more predictive. The haploid seedlings would have normal green leaves; the diploids would be yellow-green. The screening can be done in a sand bench or in a thickly planted field row.

Most dominant Yellow-green seedling mutants behave like dominant *Oy1* mutants, in that when homozygous, they produce luteus seedlings that die within two weeks after emergence. The *Yg*-N1582* mutant is unique in that the homozygous mutant apparently has no deleterious effect on the plant, other than it being yellow-green. We are not aware of any other dominant mutants that would be as visible at the germinating seedling stage and have no harmful effect on a potential inducer line when homozygous. Other potential uses for this mutant include use as a border marker and in studies of photosynthesis.

Allele test results for Maize Genetics Cooperation Stock Center "phenotype-only" stocks
— Stinard, PS

We report here the results of tests of allelism of white endosperm/green seedling and white endosperm/viviparous mutants from the Maize Genetics Cooperation Stock Center's collection of mutants characterized by phenotype only. In the case of the new *y1* alleles, homozygous mutant plants were crossed by homozygous *y1* testers; allele tests were considered positive if the resulting ears bore white kernels. In the case of the new *w3* allele, heterozygous mutant plants were crossed by heterozygous *w3* testers, and kernels from the resulting ears were seedling tested for the albino seedling *w3* phenotype. The mutants that gave positive tests of allelism are presented in Table 1.

Table 1. Results of allele tests of mutants from the Maize Genetics Stock Center phenotype-only collection.

Former mutant designation	New allelic designation
y*-73-2	y1-73-2
y*-73-426	y1-73-426
y*-73-2262-1	y1-73-2262-1
y*-73-2262-2	y1-73-2262-2
y*-73-2394	y1-73-2394
y*-73-4035	y1-73-4035
y*-1981	y1-1981
y*-1982-3	y1-1982-3
y*-syn-DOCI	y1-syn-DOCI
y*-Funk-81-13	y1-Funk-81-13
y*-Sprague	y1-Sprague
y*-1981-14	y1-1981-14
pale-y*-83-3382-16	y1-83-3382-16
y*-85-3041-2	y1-85-3041-2
vp*-UFMu-02780	w3-UFMu-02780

Data-mining the B73 genome sequence for carotenoid biosynthesis gene candidates

— Stinard, PS

Many of the genes associated with classical carotenoid-deficient endosperm mutants of maize have been cloned and characterized, e.g., *y1* (phytoene synthase; Buckner et al., Plant Cell 2:867-876, 1990); *vp5* (phytoene desaturase; Li et al., Plant Molecular Biology 30:269-279, 1996); *y9* (zeta-carotene isomerase; Li et al., Plant Physiology 144:1181-1189, 2007); *vp9* (zeta-carotene desaturase; Matthews et al., J Exp Bot 54:2215-2230, 2003); *ps1* (lycopene beta-cyclase; Singh et al., Plant Cell 15:874-884, 2003); and *vp2* (4-hydroxyphenylpyruvate dioxygenase; Matthews et al., 2003). However, to date, many carotenoid-deficient loci have eluded association with steps in the carotenoid biosynthetic pathway. The list of uncharacterized genes includes *lw1*, *lw2*, *lw3*, *lw4*, *w3*, *y8*, *y10*, and *cl1*. We report here the association of these loci (with reasonable confidence) to specific gene products. Our technique was to identify characterized *Arabidopsis* orthologs of carotenoid biosynthetic genes and perform BLAST searches against the maize B73 genome (version 2) using the MaizeGDB genome browser tools. The results are summarized in Figures 1 and 2 and Tables 1 and 2.

With the exception of *vp2*, the characterized genes involve steps in the direct pathway leading from geranylgeranyl diphosphate to beta-carotene. *vp2*, however, is implicated in the biosynthetic pathway for plastoquinone (Fig. 1), an electron receptor involved in the desaturation steps between phytoene and lycopene. We first examined steps in the plastoquinone biosynthetic pathway in *Arabidopsis*. The PDS1 gene in *Arabidopsis* encodes 4-hydroxyphenylpyruvate dioxygenase, involved in the conversion of 4-hydroxyphenylpyruvate to homogentisic acid (Norris et

Figure 1. Plastoquinone biosynthetic pathway. Classical maize gene candidates are listed at the left of each step. *?* = uncharacterized duplicate factor loci. *Arabidopsis* genes are in parentheses.
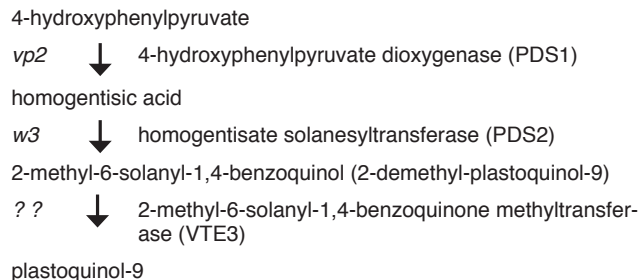
Figure 2. DOXP/MEP pathway. Classical maize gene candidates are listed at the left of each step. *?* = uncharacterized duplicate factor loci. *Arabidopsis* genes are in parentheses.
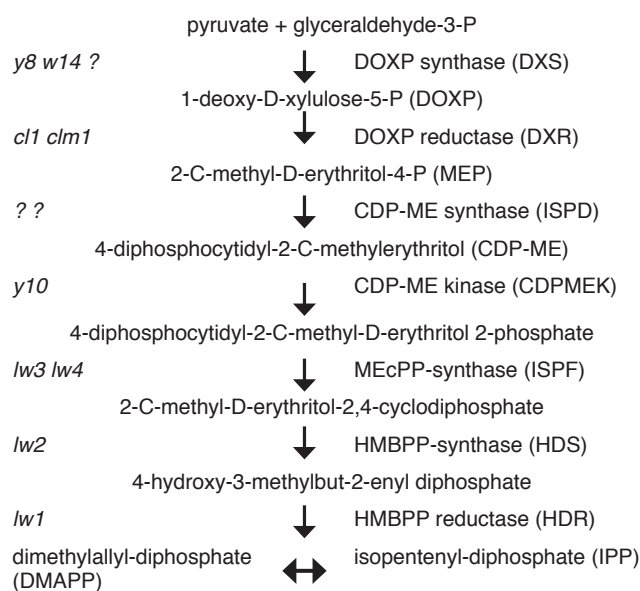
al., Plant Cell 7:2139-2149, 1995). The Genbank sequence for PDS1 (NCBI Reference Sequence: NM_100536.3) was used to BLAST against the maize genome and picked up homology to gene model GRMZM2G088396 (Chr5:83859479..83861633), which is located on 5S near the estimated location of vp2 (Chr5:78386141..80842741), and which encodes a putative 4-hydroxyphenylpyruvate dioxygenase. This is consistent with the data of Matthews et al. (2003).

The PDS2 gene in *Arabidopsis* encodes homogentisate solanesyltransferase, involved in the conversion of homogentisic acid to 2-demethyl-plastoquinol-9 (Tian et al., Planta 226:1067-1073, 2007). The Genbank sequence for PDS2 (NCBI Reference Sequence: NM_001161137.1) was used to BLAST against the maize genome and picked up homology to gene model GRMZM2G113476 (Chr2:206847694..206863769), which is located on 2L near the estimated location of *w3* (Chr2:204481904..205710630), and which encodes a putative prenyltransferase/zinc ion binding protein with high sequence homology to the *Arabidopsis* homogentisate solanesyltransferase gene.

Thus the maize *w3* locus is an excellent candidate for the gene encoding maize homogentisate solanesyltransferase. A UniformMu line (UFMu-02780) carrying an insert (mu1031674) in this gene model segregates for a white endosperm viviparous mutant allele of *w3*. Although this result is suggestive, confirmation that the *w3* locus encodes homogentisate solanesyltransferase will require molecular analysis.

The remaining uncharacterized genes were placed in the biosynthetic pathway leading from 1-deoxy-D-xylulose-5-P (DOXP) to isopentenyl-diphosphate (IPP), part of the plastidial DOXP/MEP pathway (Fig. 2; reviewed in Lichtenthaler, Proc Intl Plant Lipid Symposium 11-24, Budapest, Hungary, 2004). Whereas most of the reduced carotenoid mutations in genes involved in the later, purely plastidial parts of the carotenoid biosynthetic pathway exhibit vivipary due to reduced synthesis of ABA, mutants in genes of the MEP pathway might be expected to exhibit a less severe phenotype due to shuttling of intermediates from the alternative cytosolic MVA pathway (Rodríguez-Concepción, Phytochemistry Reviews 5:1-15, 2006). Thus, mutants in MEP pathway genes might be expected to produce low levels of endosperm carotenoids and exhibit dormancy, i.e., a "lemon white" phenotype. Such mutants include *lw1*, *lw2*, *lw3*, *lw4*, *cl1*, and *y10*.

The DXS gene in *Arabidopsis* encodes DOXP synthase, involved in the conversion of pyruvate and glyceraldehyde-3-P to 1-deoxy-D-xylulose-5-P (DOXP). Vallabhaneni and Wurtzel (Plant Physiology 150:562-572, 2009) and Cordoba et al. (J Exp Bot 62:2023-2038, 2011) report three DXS genes in maize: *dxs1*, *dxs2*, and *dxs3*. These correspond to maize gene models GRMZM2G137151 (Chr6:146378393..146382661), GRMZM2G493395 (Chr7:14077852..14081075), and GRMZM2G173641 (Chr9:20462059..20467072), respectively. Cordoba et al. (2011) indicate that of these three DXS genes, *dxs1* is expressed the most in leaves, and *dxs2* and *dxs3* are expressed the most in yellow endosperms, with *dxs2* expressed more highly than *dxs3*. The *y8* gene is estimated to be at Chr7:14027268..14618739, which overlaps the *dxs2* location and is therefore a candidate gene for *dxs2*. Although *y8* mutants are homozygous viable and therefore not traditional "lemon whites," the expression pattern of the three DXS genes might explain how a knockout in *dxs2* could result in the *y8* mutant phenotype. It is possible that a knockout of *dxs2* might not be fully compensated for by *dxs3* expression in the endosperm, leading to the pale yellow *y8* mutant phenotype. A fully functional *dxs1* gene would allow normal carotenoid production in the rest of the plant (i.e., a fully viable green plant). On the other hand, if only the *dxs1* gene were knocked out, one would expect a yellow endosperm albino seedling mutant. *w14* (estimated to be at Chr6:148253633..148506034) is a possible classical maize gene candidate for the *dxs1* locus.

The DXR gene in *Arabidopsis* encodes 1-deoxy-D-xylulose 5-phosphate reductoisomerase, involved in the conversion of 1-deoxy-D-xylulose-5-P (DOXP) to 2-C-methyl-D-erythritol-4-P (MEP). The Genbank sequence for DXR (NCBI Reference Sequence: NM_125674.2) was used to BLAST against the maize genome and picked up homology to gene models GRMZM2G056975 (Chr3:30226804..30233358) and

Table 1. Classical maize carotenoid genes and predicted gene models.

Classical maize gene	Location	*Arabidopsis* gene candidate	Orthologous maize gene model
vp2	5S (5.04)	AT1G06570[1] (PDS1)	GRMZM2G088396
w3	2L (2.08)	AT3G11945 (PDS2)	GRMZM2G113476
y8	7S (7.01)	AT4G15560 (DXS)	GRMZM2G493395
w14	6L (6.05)	AT4G15560 (DXS)	GRMZM2G137151
cl1	3S (3.04)	AT5G62790 (DXR)	GRMZM2G056975
Clm1	8S	AT5G62790 (DXR)	GRMZM2G036290
y10	3L (3.07)	AT2G26930 (CDPMEK)	GRMZM5G859195
lw3	5L (5.06)	AT1G63970 (ISPF)	AC209374.4_FG002
lw4	4L (4.06)	AT1G63970 (ISPF)	GRMZM5G835542
lw2	5L (5.05)	AT5G60600 (HDS)	GRMZM2G137409
lw1	1L (1.10)	AT4G34350 (HDR)	GRMZM2G027059

[1] TAIR locus name (from www.arabidopsis.org).

GRMZM2G036290 (Chr8:8094442..8101055), both of which encode maize DXR protein and show high homology with each other and the *Arabidopsis* gene. These are excellent candidates for the duplicate genes *cl1* (Chr8:33707329..33742708) and *Clm1* (chromosome 8S, location unknown). Note that mutants at *cl1* lead to a reduction in both endosperm and plant carotenoids. Variants at the Clm1 locus are able to compensate for the reduction in plant carotenoids in *cl1* mutants, but not for the reduction in endosperm carotenoids. This could be due to tissue-specific differences in expression of the two DXR genes.

The CDPMEK gene in *Arabidopsis* encodes 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, involved in the conversion of 4-diphosphocytidyl-2-C-methylerythritol to 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate. The Genbank sequence for CDPMEK (NCBI Reference Sequence: NM_128250.3) was used to BLAST against the maize genome and picked up homology to gene model GRMZM5G859195 (Chr3:187922271..187927591), which is located on 3L and which encodes 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase with high sequence homology to the *Arabidopsis* gene. The maize *y10* locus is estimated to be at Chr3:205199570..205264647, which seems a little far from the location of GRMZM5G859195. However, the genetic map of chromosome 3 places *y10* close to *na1* (Chr3:184214701..185318488). Thus, the maize *y10* locus is an excellent candidate for the gene encoding maize 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase.

The ISPF gene in *Arabidopsis* encodes 2-C-methyl-D-eryth-

Table 2. Predicted duplicate factor maize carotenoid genes and gene models.

Arabidopsis gene	Orthologous maize gene model	Location
AT3G63410[1] (VTE3)	GRMZM2G082998	1L
AT3G63410 (VTE3)	GRMZM2G099206 (pseudogene?)	3S
AT4G15560 (DXS)	GRMZM2G137151	6L
AT4G15560 (DXS)	GRMZM2G493395	7S
AT4G15560 (DXS)	GRMZM2G173641[2]	9S
AT2G02500 (ISPD)	GRMZM5G856881	3L
AT2G02500 (ISPD)	GRMZM2G172032	8L

[1] TAIR locus name (from www.arabidopsis.org).
[2] Data from Cordoba et al., 2011.

ritol 2,4-cyclodiphosphate synthase, involved in the conversion of 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate to 2-C-methyl-D-erythritol-2,4-cyclodiphosphate. The Genbank sequence for ISPF (NCBI Reference Sequence: NM_180640.2) was used to BLAST against the maize genome and picked up homology to gene models *AC209374.4_FG002* (Chr5:196279295..196281037) and GRMZM5G835542 (Chr4:155830779..155832786), both of which encode maize 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase and show high homology with each other and the *Arabidopsis* gene. These are excellent candidates for the duplicate factor loci *lw3* (Chr5:188462959..190607852) and *lw4* (Chr4:155828832..155834753).

The HDS gene in *Arabidopsis* encodes 4-hydroxy-3-methylbut-2-enyl diphosphate synthase, involved the conversion of 2-C-methyl-D-erythritol-2,4-cyclodiphosphate to 4-hydroxy-3-methylbut-2-enyl diphosphate. The Genbank sequence for HDS (NCBI Reference Sequence: NM_125453.6) was used to BLAST against the maize genome and picked up homology to gene model GRMZM2G137409 (Chr5:182124005..182130631), which is located on 5L near the estimated location of *lw2* (Chr5:174149224..175478743), and which encodes 4-hydroxy-3-methylbut-2-enyl diphosphate synthase with high sequence homology to the *Arabidopsis* gene. Thus, the maize *lw2* locus is an excellent candidate for the gene encoding maize 4-hydroxy-3-methylbut-2-enyl diphosphate synthase.

Finally, the HDR gene in *Arabidopsis* encodes 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, involved in the conversion of 4-hydroxy-3-methylbut-2-enyl diphosphate to isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The Genbank sequence for HDR (NCBI Reference Sequence: NM_119600.3) was used to BLAST against the maize genome and picked up homology to gene model GRMZM2G027059 (Chr1:272936836 to 272940502), which is located on 1L near the estimated location of *lw1* (Chr1:271108631..273434076), and which encodes 4-hydroxy-3-methylbut-2-enyl diphosphate reductase with high sequence homology to the *Arabidopsis* gene. Thus the maize *lw1* locus is an excellent candidate for the gene encoding 4-hydroxy-3-methylbut-2-enyl diphosphate reductase.

Gene candidates can be assigned to nearly all of the loci associated with reduced endosperm carotenoids. Mutants, many of which are derived from populations carrying active transposable elements, exist for all of these loci, so it should be a simple matter to determine whether these mutants are due to lesions at the candidate loci. However, there are still genes in the carotenoid biosynthetic pathway for which mutants have not yet been identified. One possible explanation is that some of these genes occur as duplicate loci in maize for which two or more genes would need to be knocked out in order to observe a mutant phenotype. One such example is the genes homologous to the *Arabidopsis* gene ISPD (Fig. 2; NCBI Reference Sequence: NM_126305.2), encoding 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase. The *Arabidopsis* gene picks up homology with maize gene models GRMZM5G856881 (Chr3:170115790..170118780) and GRMZM2G172032 (Chr8:164748939..164752371). These genes encode a putative 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase with homology to each other and to the *Arabidopsis* gene. We predict that if both genes were knocked out, a reduced endosperm carotenoid mutant phenotype would result. This and other examples of predicted duplicate genes are summarized in Table 2. Reverse genetics tools such as the UniformMu project might someday identify knockouts in these individual genes that could then be combined to test this hypothesis.

brachytic3 is allelic to *brevis1*
— Stinard, PS

James Brewbaker recently indicated in an email to the Maize Genetics Stock Center that the phenotypes of the mutants *br3* and *bv1* in an Hi27 inbred background are nearly identical, and he asked us whether we had ever conducted a test of allelism between them. Both mutants map to chromosome 5, but they had never been tested against each other. To resolve this question, we set up crosses to test allelism in our 2011 winter nursery. We included *na2* in our tests as well since it has a short plant stature and also maps to chromosome 5.

The crosses were set up as follows: Known heterozygotes for *br3* in coupling with *pr1*, known heterozygotes for *bv1* in coupling with *pr1*, and known heterozygotes for *na2* in coupling with *pr1* were intercrossed in all possible combinations. Homozygous *pr1* (red) kernels from each ear were planted in the observation block of our 2012 summer nursery, and observed at maturity. Progeny grown from the cross of *br3* heterozygotes by *bv1* heterozygotes segregated for brachytic (brevis) plants in a high proportion due to coupling with *pr1*. Crosses of *br3* heterozygotes to *na2* heterozygotes and crosses of *bv1* heterozygotes to *na2* heterozygotes resulted in nonmutant progeny. We conclude therefore that *br3* and *bv1* are allelic, and *na2* represents a separate locus. Because *bv1* (first report Li, J Hered 22:14-16, 1931) has precedence over *br3* (first report Singleton, MNL 33:3-4, 1959), the *br3* locus has been lapsed and the *br3* data has been merged with the *bv1* data in MaizeGDB. The old "*br3*" reference allele is now called "*bv1-br3*."

New duplicate factor pair for lemon white endosperm/seedling, *lw5* and *lw6*
— Stinard, PS

In the course of propagating the Neuffer pale yellow endosperm/albino seedling (lemon white) mutant *w*-N176* (MGCSC stock number 3507G), we noticed that the trait segregated 15:1 for nonmutant (yellow endosperm/green seedling) : lemon white in F2 selfed ears made following the outcross of the original Neuffer source to our M14/W23 standard. Since the only other known duplicate factor pair for lemon white is *lw3 lw4*, we performed a test of allelism of *w*-N176* by self-pollinating plants grown from yellow kernels from segregating ears of *w*-N176* and outcrossing these plants to known heterozygotes for *lw3 lw4*. All outcrosses of plants carrying *w*-N176* to the *lw3 lw4* heterozygotes produced yellow kernels only, and sand bench seedling tests of kernels from these outcross ears produced only green seedlings. We therefore conclude that *w*-N176* is not allelic to the duplicate factor pair *lw3 lw4* and represents a new duplicate factor pair for lemon white endosperm, which we have named *lw5 lw6*.

To further characterize *lw5 lw6*, we planted yellow kernels from 15:1 segregating ears, self-pollinated the resulting plants, and counted the numbers of yellow and lemon white kernels on the resulting ears. Confirmation of the lemon white phenotype was made by sand bench plantings of samples of separated yellow and lemon kernels from segregating ears. In all cases, yellow kernels gave rise to green seedlings and lemon kernels gave rise to albino seedlings. Kernel counts and chi-square calculations for 3:1 and 15:1 ratios of yellow : lemon white kernels from segregating ears are presented in Table 1. In all cases, the segregation ratios conformed to either a 3:1 or a 15:1 ratio. For a duplicate factor pair, the ratio of ears bearing yellow kernels only to ears bearing a 3:1 segregation ratio to ears bearing a 15:1 segregation ratio is expected to be 7 : 4 : 4. We observed a ratio of 9 : 5 : 10. Although this is a small sample number, it still conforms statistically to the expected ratio (chi-square = 2.763, df = 2, p > 0.1, no significant difference). Thus, the behavior of *w*-N176* in the F3 generation is consistent with duplicate factor inheritance. We have named the two lemon white mutant alleles from the *w*-N176* stock *lw5-N176A* and *lw6-N176B*.

To date, specific gene candidates (based on the B73 reference genome, version 2) have not been assigned to the lemon white loci of maize, but in a separate Maize Newsletter article, we speculate on possible gene candidates for these loci based on a map-based approach (see Stinard, MNL, this issue) and suggest that the other known maize lemon white factors (*lw1, lw2, lw3, lw4, y10,* and *cl1*) correspond to genes in the DOXP/MEP isoprenoid biosynthetic pathway. We were able to associate known maize lemon white loci with all DOXP/MEP pathway genes except for CDP-ME synthase (ISPD), for which we predicted the existence of a duplicate factor pair, GRMZM5G856881 on 3L and GRMZM2G172032 on 8L. We are in the process of isolating stocks of the separated *lw5* and *lw6* factors, and once we have done so, we will attempt to map them with B-A translocations selected to uncover these two gene candidates. Results should soon be forthcoming.

Table 1. Counts of yellow (+) and lemon white (lw) kernels from self-pollinated ears of plants grown from yellow kernels from 15:1 segregating self-pollinated ears of *w*-N176*.

Ear	+	lw	3:1 chi-square	15:1 chi-square
2012-426-1	406	31	74.73	0.53*
2012-426-5	327	92	2.07*	176.42
2012-426-6	481	29	101.46	0.28*
2012-426-9	427	32	79.56	0.41*
2012-426-11	452	31	88.94	0.02*
2012-427-1	358	112	0.34*	247.90
2012-427-3	73	20	0.61*	36.94
2012-427-7	491	31	101.15	0.09*
2012-427-9	429	30	83.46	0.06*
2012-427-10	351	112	0.16*	254.32
2012-427-11	520	35	103.44	0.00*
2012-429-1	365	28	66.97	0.51*
2012-429-7	390	151	2.45*	433.23
2012-429-8	429	28	86.82	0.01*
2012-429-10	140	6	33.98	1.14*

* p > 0.1 (no significant difference from indicated ratio)

Tiny plant mutant *ty*-8446* is allelic to *brd1*
— Stinard, PS

The tiny plant mutant *ty*-8446*, a small seedling mutant that arose following gamma radiation experiments at Oak Ridge Laboratory, originally was described and mapped to chromosome 1 by F. D. Pettem (MNL 30:9-10, 1956). Mutant seedlings of *ty*-8446* do not respond to gibberellic acid (Phinney, MNL 30:11-12, 1956). The *brassinosteroid-deficient dwarf1 (brd1)* mutant, recently described by Makarevitch et al. (PLoS One 7:e30798, 2012), has a similar seedling phenotype and also maps to chromosome 1. In order to resolve the relationship between these two mutants, a test of allelism was performed at the Maize Genetics Stock Center. Plants heterozygous for *ty*-8446* and *brd1-m1* were intercrossed, and kernels from the resulting ears were planted in the sand bench. The emerging seedlings segregated for the tiny seedling phenotype, indicating allelism. Although *ty*-8446* was isolated earlier than *brd1*, it had not been assigned a formal gene symbol. Therefore, the *brd1* locus name is retained, and the *ty*-8446* allele has been reassigned the name *brd1-ty8446*.

The photosynthetic mutant *ppr10* is allelic to the luteus seedling mutant *l15*
— Stinard, PS

The photosynthetic mutant *ppr10 (pentatricopeptide repeat10)* conditions a luteus seedling phenotype, and the locus is located on the long arm of chromosome 6 (Pfalz et al., EMBO J 28:2042-2052, 2009). Other luteus seedling mutant loci (*l10, l12,* and *l15*)

also map to the long arm of chromosome 6, but tests of allelism of *ppr10* with these loci have not been previously reported. The Maize Genome Database placement of *l15* seemed to be closest to the map position of *ppr10*, so we conducted a direct test of allelism of *ppr10* with *l15*. Plants heterozygous for *ppr10* in coupling with *y1* were crossed with plants heterozygous for *l15* in coupling with *y1*, and yellow and white kernels from the F1 ears were separated and planted in the sand bench. Seedlings grown from the yellow kernels were predominantly green, with a few luteus seedling crossovers, and seedlings grown from the white kernels were predominantly luteus, with a few green seedling crossovers. We conclude that *ppr10* is allelic to *l15*, and suggest that the locus name *l15* be retained due to its precedence in the literature (Robertson, MNL 55:115, 1981). An *l15* stock was sent to the Barkan lab for analysis; Western blot analysis of *l15* seedlings with antibodies raised against PPR10 protein showed absence of PPR10 protein (Barkan lab, personal communication), providing further confirmation of *ppr10/l15* allelism.

While my mother and I were going through some of her personal effects, she found an old ILLINOIS ALUMNI NEWS from January 1949. She must have known at the time that her 1-year-old son was destined to be a corn breeder and saved this issue with the article about the University of Illinois Botany department's hiring of Marcus Rhoades and John Laughnan. Remembering reading the letter from John Laughnan written in 1958 and published in the 2008 Maize Genetics Cooperation Newsletter where he proposed "an informal get-together of maize geneticists," I thought perhaps some would enjoy reading about the hiring of Dr. Laughnan and Dr. Rhoades. John Laughnan particularly is a special memory for me as he taught me my first college genetics course. As a sweet corn breeder I also am indebted to him for his development of Illini Chief, the first sweet corn hybrid with the sh2 gene – a type of corn now commonly known as "supersweet."

Dr. Rhoades helped found the Maize Genetics Coop, and I believe was the first curator of the genetic stocks. While a graduate student at Illinois the MGC still met at Allerton Park. At those meetings I had the opportunity to meet Dr. Rhoades and many other well-known corn geneticists and chat informally during the evening cocktail hour.

I hope some of you appreciate the little bit of history presented in this old alumni news.

David Fisher
DeForest, WI
Monsanto

Botanists Study Corn Heredity

Two professors, newcomers to the University's Botany department—Marcus M. Rhoades, who was brought here from Columbia, and John R. Laughnan from Princeton—are working as a team to do research in the hereditary characteristics and the cellular structure of corn. The two scientists, who are experts in two phases of the same subject, are devoting their time to the basic aspects of the corn plant.

Professor Rhoades is one of the nation's foremost cytogeneticists whose main interest is cytology (the study of cell structure) and Professor Laughnan is concerned with chemical genetics. Although what they find out may have no immediate bearing on methods in corn production, the geneticists might well point out that their past work led to the production of hybrid corn—worth millions of dollars.

Actually, the fact that corn is such an important crop in the U. S. economy—and in the economy of the Midwest in particular—is not the primary reason behind the U. of I. study.

It just happens that corn is the plant best suited to the study of genetics. "Irrespective of the value of the corn crop, I'd still choose it," Professor Rhoades declared.

"It's the best plant we have for this type of work," he said. "It's easy to grow, the sexes are separated (the tassel is the male flower, the ear the female flower), it's easy to cross and its chromosomes are very favorable for study."

Corn's economic importance does make it easier for the geneticists to get the necessary laboratories and equipment, however, just as the atom bomb makes it easier for the physicists to get appropriations for their betatrons and cyclotrons.

The U. of I. has allocated some $60,-000 to the development of a cytogenetics laboratory in the Natural History building, and much of the necessary equipment has been ordered.

A small amount of experimental corn is being grown in University greenhouses during the winter, and the agronomy plots will provide a larger amount in the summer, through cooperation between the "theoretical" and the "applied" research workers.

In the meantime, Professors Rhoades and Laughnan are studying the nature of gene mutation from corn samples they already have—corn whose kernels are speckled with black or striped with red like peppermint candy.

The mutations which cause the changes "are the building blocks of evolution," Professor Rhoades noted. "Plants and animals have become diversified only because of gene changes or mutations.

"An experimental attack on the study of the mutation process may yield some information on the nature or structure of genes themselves and also on the way in which genes act in controlling development."

David H. Timothy (1928-2010)

The family of Dave (Tim) Timothy, noted maize cooperator, wishes to celebrate his life by sharing his biographical history with the maize community. Dave's publications can be found on MaizeGDB:http://www.maizegdb.org/cgibin/displaypersonrecord.cgi?id=16845&proberef=1&probestart=1&probeclstart=&probeavstart=1#proberef. The following report, with slight modifications here, was published in the Pittsburgh Post-Gazette on November 17, 2010.

David H. Timothy, 82, died unexpectedly November 14, 2010, at Rex Hospital, Carey, NC. He was born on June 9, 1928, in Pittsburgh, Pennsylvania, but spent most of his life in Raleigh, until he moved to Glenaire Retirement Community in April 2007.

Dr. Timothy received his B.S. and M.S. in Agronomy from the Pennsylvania State University and his Ph.D. in Plant Genetics at the University of Minnesota. In 1956, he joined the Rockefeller Foundation as director of the Corn Program in Colombia, South America. While there, he originated and was curator of the *Tripsicum* World Collection. In 1961, he left the Rockefeller Foundation and joined the faculty of North Carolina State University (NCSU). There he was Professor of Crop Science, Plant Genetics, and Botany until his retirement in 1993.

Professor Timothy was called upon to consult for the World Bank and to serve as the Chief Scientist of the U.S. Department of Agriculture Grants Program while still on the faculty of NCSU. He also served on the Boards of the American Society of Agronomy, Crop Science Society of America, and The National Science Foundation. He was a founding board member of Genetic Resources Communications systems. In 1994, Dr. Timothy was awarded the prestigious Frank N. Meyer Medal for Plant Genetic Resources for his creation of broad interest and support for germplasm conservation.

Dr. Timothy is survived by Marian Whiteley Timothy (formerly of Williamsport, PA), his wife of 57 years; two daughters, Marjory J. Timothy and husband Bob Bollinger of Charlotte, NC, and Elisabeth T. McChesney and husband David of Raleigh; one son, David W. Timothy and life partner Alberto G. Carbonilla of Trenton, NJ; five grandchildren, Robert T. Bollinger, Timothy G. McChesney, James W. Bollinger, Caroline E. McChesney, and William W. Bollinger; a sister, Timmie Blattner of Mount Lebanon, PA; a brother, Edgar J. Timothy and wife Elisabeth of Espyville, PA; and several nieces and nephews. A memorial service was held at 3 p.m. Saturday, November 20, at Highland United Methodist Church, 1901 Ridge Road, Raleigh, NC. The family received friends at the church following the service.

In lieu of flowers, contributions may be made in memory of David H. Timothy to Easter Seals UCP, Horizons Foundation, Inc., ATTN: Bob Frazier, 2315 Myron Drive, Raleigh, NC 27607, telephone number: 919-965-8630 (www.nc.eastersealsucp.com).

— Submitted Jan. 6, 2012, by Lee B. Kass and Marion Timothy

maize gene review
maizegenereview.org

These reviews are an extension of the summaries and images provided by many cooperators for publication in the *Mutants of Maize*, 1997, eds M. Gerald Neuffer, Edward H. Coe and Susan R. Wessler , Cold Spring Harbor, NY, and which were included in the MaizeGDB, prior to publication in hard copy. Unlike the Newsletter notes, the maize gene reviews may be freely cited without prior permission of authors. The initial submissions were published in the vol 83 of this Newsletter. Data from each review is parsed for inclusion in MaizeGDB, and the reviews are highlighted at MaizeGDB locus and person (author) pages, to acknowledge expert contributions to the MaizeGDB. We thank these first submitters for being very generous with their time in participating in this fledgling project. Specifically these are: Alice Barkan (*caf1, caf2, crp1, crs1, crs2, crs4, csy1, ppr2, ppr4, ppr5, ppr10, rnc1, tha1, tha4, tha8, why1*) David Braun (*tdy1*), George Chuck (*bd1, ts4, ts6*), Erik Vollbrecht and Sarah Hake (*ra2*), Rachel Wang et al (*afd1, sgo1*).

We encourage unsolicited submissions. Authors should supply concise summaries of their favorite genes, with images of mutant phenotypes, preferably previously unpublished. We are especially interested in key alleles, regulation, gene products, pathways (metabolic, development), evidence for map locations, and any other key information about the locus. Submissions may be in a text format of author's choosing, or approximate the formats used in the following submissions. Reviews will be edited by Newsletter with author review of final copy. Updates may be added from time to time, similar to the process for the Online Mendelian Inheritance of Man, but where initial authors will be consulted regarding major updates. If you would like to contribute, send your reviews to Mary Schaefer, schaefferm_a_t_ missouri.edu.

This year we thank Paula McSteen for providing a review for the gene bif2 barren infloresecence2.

Author Paula McSteen, University of Missouri, Columbia, MO 65211 USA
Name *bif2 barren inflorescence2*
Chromosome 1 bin 1.06. **Gene Model** GRMZM2G171822
Function: Regulation of auxin transport during axillary meristem and lateral organ initiation

Summary
The *bif2* mutation affects all axillary meristems in the plant. The tassel has fewer branches, spikelets, florets and floral organs (McSteen et al 2001). The ear shoot, if it forms, has very few kernels. The mutant plants also have defects in vegetative development as they make fewer tillers (in a *tb1* mutant background) and makes one or two fewer leaves than normal. The *bif2* gene encodes a serine threonine protein kinase (McSteen et al 2007) that phosphorylates auxin efflux carried ZmPIN1a (Skirpan et al 2009) and bHLH transcription factor BARREN STALK1 (Skirpan et al 2008). Natural variations in *bif2* alleles are associated with tassel branch number and plant height (Pressoir et al 2009)

First report Briggs SP and Johal G (1992) **MNL** 66:51.

Key alleles *bif2-ref* aka *bif2-N2354*, EMS induced missense mutation converts Pro 193 to Leu;
bif2-77, 168-bp insertion in first exon; *bif2-70*, same *as bif2-77*; *bif2-RM::Mu1*, *Mu1* insertion in first intron; *bif2-1504::Mu1*, *bif2-47330::Mu1* have distinct *Mu1* insertions in second exon; *bif2-160*, unknown cause (McSteen et al 2007). EMS induced mutants *bif2-04HI-A632xOh43GN-105*; *bif2-03HI-B73xMo17GN-556*; *bif2-03HI-B73xMo17GN-834*; *bif2-03IL-A619TR-503*; *bif2-03IL-A619TR-59* come from the Maize Infloresence Architecure Project. http://www.maizegdb.org/ems-phenotype.php

Map location: Based on AB translocation and tight linkage to umc67a (Mcsteen and Hake 2001), and agrees with sequence on B73 reference genome sequence AGP v2 (maizesequence.org). InDel genotyping probe defined (Pressoir et al 2009).

Gene Product: Serine threonine protein kinase (McSteen et al 2007); phosphorylates in PIN1a (in vitro, Skirpan et al 2009), and BA2 (in vitro, Skirkpan et al 2008).

Expression: Axillary meristems and lateral organs during vegetative and reproductive development

References:
Skirpan A, Hendrickson Culler A, Gallavotti A, Jackson D, Cohen JD, McSteen P (2009) BARREN INFLORESCENCE2 interaction with ZmPIN1a suggests a role in auxin transport during maize inflorescence development. **Plant & Cell Physiology**, 50: 652-657.
Pressoir G, Brown PJ, Zhu W, Upadyayula N, Rocheford T, Buckler ES, Kresovich S (2009) Natural variation in maize architecture is mediated by allelic differences at the PINOID co-ortholog barren inflorescence2. **The Plant Journal**, 58:618-625.
Skirpan A, Wu X, McSteen P(2008) Genetic and physical interaction suggest that BARREN STALK1 is a target of BARREN INFLORESCENCE2 in maize inflorescence development. **The Plant Journal**, 55:787-797.
McSteen P, Malcomber S, Skirpan A, Lunde C, Wu X, Kellogg E, Hake S (2007) *barren inflorescence2* encodes a co-ortholog of the *PINOID* serine/threonine kinase and is required for organogenesis during inflorescence and vegetative development in maize. **Plant Physiology** 144:1000-1011.
McSteen P, Hake S (2001) *barren inflorescence2* regulates axillary meristem development in the maize inflorescence. **Development**,128:2881-2891.

Links: MGDB bif2 | NCBI bif2 | UniProt A6MW92

Maize Genetics Cooperation • Stock Center

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2011

8,305 seed samples have been supplied in response to 420 requests for 2011. These include 121 requests received from 26 foreign countries. This has by far been a record-breaking year for requests. The previous annual record was 332 requests filled in 2002. Most of the increase is due to the great interest in reverse genetics tools, such as the UniformMu sequence indexed stocks, which are being used as our colleagues are finding sequences of genes that their research is focused on, in the B73 genome data. Other popular stock requests include the NAM RIL populations, Hi-II lines, *ig1* lines, Stock 6 haploid-inducing lines, male sterile cytoplasms, and Maize Inflorescence Project EMS lines.

Approximately 6.0 acres of nursery were grown this summer at the Crop Sciences Research & Education Center located at the University of Illinois. Wet spring weather forced us to plant our crossing nurseries a couple of weeks late. There were sufficient stands for an adequate increase in most instances. During the height of our pollination season in July, 100+ degree temperatures resulted in drastically reduced seed sets on certain critical days; supplemental irrigation and timely rainfall may have helped to mitigate the damage. Because of the spread of maturities in our materials, little material will need to be replanted next year as a result of the excessive heat. Despite late plantings, the increase in heat units this summer allowed for a relatively early harvest. Moderate temperatures and low plant stress following pollination resulted in acceptable yields for most stocks.

Special plantings were made of several categories of stocks:

1. Plantings were made of donated stocks from the collections of Alice Barkan (photosynthetic mutants), Pat Bedinger (male sterile mutants), David Braun (new *tdy1* and *sxd1* alleles), James Brewbaker (Hi27 near-isogenic mutant lines), Inna Golubovskaya (meiotic mutants), Thomas Hartwig (*na1* alleles), David Jackson (*fea2*, *ra3*, and *abph1* alleles), Gerry Neuffer (new dominant EMS-induced mutants), Snook Pataky (*Rp* and *Rpp* variants), and others. We expect to receive additional accessions of stocks from maize geneticists within the upcoming year.

2. We are conducting experiments in collaboration with Jerry Kermicle in order to further characterize kernel color mottling factors. We are also trying to recover instances of the lapsed *y5* locus from PI accessions of orange endosperm tropical flints. We are starting collaborations to identify the specific gene products associated with previously uncharacterized (or incompletely characterized) white endosperm/albino seedling loci.

3. Outcrosses of A-A translocation stocks grown by Janet Day Jackson were grown in 2011 observation to confirm by seed set which ones actually carry the translocation. However, with the loss of Janet's position, keeping up with the propagation of the translocation stocks will become increasingly difficult.

4. Stocks produced from the NSF project "Regulation of Maize Inflorescence Architecture" (see: http://www.maizegdb.org/MIP/) were grown again this summer. Approximately 250 families of M2 materials that were produced in 2007 were grown to increase seed supplies and recover previously observed mutations; this also included previously phenotyped families that had limited seed supplies. In addition, 1,269 families of 2009 and 2010 EMS seed increase materials were grown for adult plant observation and 348 families were screened in sand benches for seedling traits; the materials observed include mutated A619, B73 and Mo17 inbred lines, A619xB73 and B73xMo17 hybrid, and various other inbred lines.

Insufficient funding prevented us from having a winter crop during the 2010/2011 growing season. However, we were able to have a winter nursery planted at the Illinois Crop Improvement Association's facilities in Juana Díaz, Puerto Rico for the 2011/2012 season. Critical plantings of a limited number of stocks were also made in our greenhouse facilities.

We have received 4,608 additional UniformMu sequence indexed lines produced by the Construction of Comprehensive Sequence Indexed Transposon Resources for Maize project (http://www.maizegdb.org/documentation/uniformmu). We presently have 8,273 of these stocks.

Our IT Specialist has continued to make updates and improvements to our curation tools, which are used to maintain data for our collection. These tools input our public stock data directly into MaizeGDB to give maize scientists access to up-to-date information about our collection. The tools are also used for our internal database (e.g., inventory, pedigrees and requests). A new search tool has been written that allows more flexibility in locating specific items in our inventory. A tool to maintain harvest information, with features to quickly go from harvest notes to inventory and cross-referencing harvest information by the parent pedigree is in progress. Importing data from MaizeGDB into our local database has been streamlined. We work with MaizeGDB to make sure our tools continue to interoperate well with MaizeGDB's databases, plus offer suggestions on where to go in the future. Maintenance continues on our web site (http://www.uiuc.edu/ph/www/maize).

2012

7,396 seed samples have been supplied in response to 450 requests for 2012. These include 153 requests received from 27 foreign countries. This has by far been a record-breaking year for requests, exceeding the total number (420) received last year, which was also a record breaker. Before that, the previous annual record was 332 requests filled in 2002. Interest in reverse genetics tools, such as the UniformMu sequence indexed stocks, continues to grow. Presently, requests for UniformMu stocks represent more than 40% of our total requests. Other popular stock requests include the NAM RILs and other mapping populations, Hi-II lines, ig1 lines, Stock 6 haploid-inducing lines, male sterile cytoplasms, and Maize Inflorescence Project EMS lines.

Approximately 6.0 acres of nursery were grown this summer at the Crop Sciences Research & Education Center located at the University of Illinois. Seasonable spring weather allowed us to plant our crossing nurseries in a timely manner, and warmer than normal weather during the growing season accelerated our pollination season. There were sufficient stands for an adequate increase in most instances. However, during the height of our pollination season in early July, 100+ degree temperatures resulted in drastically reduced seed sets on certain critical days; weekly supplemental irrigation helped to mitigate damage due to drought. Because of the spread of maturities in our materials, few stocks will need to be replanted next year as a result of the excessive heat. The increase in heat units this summer allowed for a relatively early harvest. Moderate temperatures and low plant stress later in the season resulted in acceptable yields from the pollinations that were not affected by excessive heat.

Special plantings were made of several categories of stocks:

1. Plantings were made of donated stocks from the collections of Phil Becraft (thk1), James Brewbaker (outcrosses of unique Hi27 near-isogenic mutant lines to B73), Vicki Chandler (mop1, mop2, mop3, rmr1, rmr2, B1, and dwarf variants), Susan Gabay-Laughnan (d1 and emp4 alleles), Andrea Gallavotti (various inflorescence mutants), Sarah Hake (kn1 alleles), Thomas Hartwig (na1, na2, and url1 alleles), David Jackson (fea2 and abph1 alleles), Gerry Neuffer (dominant EMS-induced mutants), Snook Pataky (Rp and Rpp variants), Pat Schnable (A1-b), Nathan Springer (brd1-m1), Clint Whipple (tasselsheath mutants) and others. We expect to receive additional accessions of stocks from maize geneticists within the upcoming year.

2. We are continuing our attempts to recover instances of the lapsed y5 locus from PI accessions of orange endosperm tropical flints and are continuing collaborations to identify the specific gene products associated with previously uncharacterized (or incompletely characterized) white endosperm/albino seedling loci. Through tests of allelism, we have identified new alleles at the y1, brd1, al1, w3, and d1 loci. The photosynthetic mutant ppr10 was found to be allelic to l15, and br3 was found to be allelic to bv1.

3. Due to lack of personnel, we have discontinued active curation of the A-A translocation stocks that were previously maintained by Janet Day Jackson. However, one last attempt was made to increase translocation stocks that had not been grown since 1990. Outcrosses of these translocations were made to standard in our 2011 winter nursery and the outcrosses were grown in our 2012 summer observation to confirm by pollen examination that they actually carry a translocation.

4. Stocks produced from the NSF project "Regulation of maize inflorescence architecture" (see: http://www.maizegdb.org/MIP/) were grown again this summer. Approximately 250 families of M2 materials that were produced between 2003 and 2007 were grown to increase seed supplies and recover previously observed mutations; this also included previously phenotyped families that had limited seed supplies. In addition, 1,510 families of 2010 and 2011 EMS seed increase materials were grown for adult plant observation and 419 families were screened in sand benches for seedling traits; the materials observed include mutated A619, B73 and Mo17 inbred lines, A619xB73 and B73xMo17 hybrid, and various other inbred lines.

We grew a winter nursery at the Illinois Crop Improvement Association's facilities in Juana Díaz, Puerto Rico during the 2011/2012 season. Critical plantings of a limited number of stocks were made in our greenhouse facilities.

We received some Rpp* (resistance to Puccinia polysora; Southern rust) stocks from Jerald (Snook) Pataky. These are factors that are not alleles of Rpp9. All but two of these were given to us as homozygous for the trait, so they can be easily maintained. Unfortunately, Snook was unable to characterize F3 material for these last two before he retired; these two are considered by him to be the most important (Rpp*-Cavalry and Rpp*-Suregold). We made arrangements with Clayton Hollier, at Louisiana State University, to conduct Southern rust inoculation tests on plants grown from the seeds of Snook's F3 ears. However, the plants did not survive the heat and drought he experienced this summer. He will try again next summer.

We currently have 8,273 UniformMu sequence indexed stocks, produced by the "Construction of comprehensive sequence indexed transposon resources for maize" project (http://www.maizegdb.org/documentation/uniformmu). We have also recently received 200 stocks from the "Genome-wide mutagenesis of maize using Ac/Ds transposons" project (http://www.plantgdb.org/prj/AcDsTagging/).

Our IT Specialist has continued to make updates and improvements to our curation tools, which are used to maintain data for our collection. These tools input our public stock data directly into MaizeGDB to give maize scientists access to up-to-date information about our collection. The tools are also used for our internal database (e.g. inventory, pedigrees and requests). A tool for entering and managing harvest notes has been mostly completed. The harvest notes tool allows COOP staff to quickly migrate information from harvest to inventory as appropriate, easily generate harvest tags and makes it easier to find information about the parent pedigrees of harvested plants, or individual pedigrees. A "family tree" tool was written that allows COOP staff to quickly see the ancestors or descendants of any pedigree family, which has been useful for finding and fixing problems in our database as well as providing a new view of existing data. The family tree tool gives COOP staff quick access to pedigrees listed in the ancestors or descendants graphs. Importing data from MaizeGDB into our local database has been streamlined. We work with MaizeGDB to make sure our tools continue to interoperate well with MaizeGDB's databases, plus offer suggestions on where to go in the future. Maintenance continues on our web site (http://www.uiuc.edu/ph/www/maize).

Marty Sachs
Director

Philip Stinard
Curator

Shane Zimmerman
Agric Sci Res Tech (Plants)

Josh Tolbert
Information Tech Specialist

Chromosome 1 Markers
101G ms26
106I thk1
117B bm2 gt1 ^Hi27
132E dbcb1
132F dbcb1 P1-rw
132G dbcb1 P1-vv::Ac
132H nl3
132I nl3 P1-rw
132L emp4-1::Mu3
132M bif2-03IL-A619TR-59
132N kn1-03HI-B73xMo17GN-71
132O brd1-m1
133B Kn1-DL

Chromosome 2 Markers
216H Brta1
216I Brta1 v4
223C B1'
223E B1' Mop2-1
223F B1' mop2-2
227J ms32
5303A B1-PeruIIB1'-pg20
5303B B1-PeruIIB1'-pg27
5303C B1-PeruIIB1'-n1
5303F B1-IIIB1-Peru-D1'
5303H B1-PeruIIB1-I-D1'
5303I B1-PeruIIB1-I-D2'
5303J B1-PeruIIB1-I-D8'
5303K B1-PeruIIB1-I-D10'
5303L B1-PeruIIB1-I-D18'
5303M B1-PeruIIB1'-pg1
5303O B1-PeruIIB1'-pg6
5303Q B1-PeruIIB1'-pg10
5303T B1-PeruIIB1'-pg17
5303V B1-PeruIIB1'-n3
5303W B1-IIIB1-Peru-D1

Chromosome 3 Markers
301J A1-b(Ec) Sh2
305H thi2-blk
310G na1-1
310H na1-4
318BA ba1-03IL-A619TR-996
320EA et1 ^Hi27

Chromosome 4 Markers
401B su1 c2; A1 A2 C1 R1
412J ppr5-2::Mu1
417A fea2-0::Mu8

Chromosome 5 Markers
509P am1-2
509Q am1-pra
509R am1-489
509S am1-6
513H A2 bm1 Ae1-5180::Mu1 pr1
532D cf1-m1::Mu8
532E cf1-m2::Mu1
532F cf1-m3::Mu1
532G cf1-m4::Mu4

Chromosome 6 Markers
616F tdy1-umu
616G tdy1-D46::Mu3
616H tdy1-D24::Mu1
617I why1-2::Mu
617L tdy1-D190::Mu1
617M tdy1-PM
621A y1-84-6024-4
621D tsh1-1
621F rmr1-1 Pl1'

Chromosome 7 Markers
712D sgo1-1::Mu1
725C ra3-fea1
725D ra3-EV
725E ra3-NI

Chromosome 8 Markers
801C ms8-mtm99-56
801D ms8-Stan2
811H wtf1-1::MuDR

Chromosome 9 Markers
901D Wc1-Caragua(PI485411)
901J ppr2263-m1::Mu
904H bz1-rcy::rcy:Mu7; Cy
904I Lgn1-ref
908I phs1-O
928U ms20-BS32
932T wx1 Flta1 y1
932U gl15-779

Chromosome 10 Markers
X04C mac1-1
X04G ms*-775
X07CG y9-87-2422-14
X11C rel2-03HI-B73GN-203
X231OA R1-d(Hopi)
X35X Rp1-E

X35Y Rp1-F
X35ZA Rp1-L
X35ZB Rpp9

Unplaced Genes
U540C dy1-9101
U740L crs4-1::Mu
U740O ppr4-2::Mu1

Multiple Genes
M241D A1 A2 b1 C1 C2 Pl1 Pr1 r1-r
M242Q a2 bt1 a1 sh2 ^Hi27
M242R fl1 v4 y8 ^Hi27
M242S Og1 B1 ^Hi27
M242T R1-nj y1 ^Hi27
M242U a1 sh2 y1 ^Hi27
M242V dbcb1 v4 ^Hi27
M242W su1 A1 A2 C1 C2 Pr1 R1
M242X b1 r1-g

Cytoplasmic-Sterile / Restorer
C437JB Hi27 (C) Restored; cms-C RfC

Recombinant Inbred
MxT RILs Maize-Teosinte RILs (entire set)

Stocks Characterized Only by Phenotype

adherent anthers
6514D ada*-07MO-B73xMo17GN-249

adherent leaf
6513A ad*-04HI-A632GN-40

albescent
3611P al*-04HI-A632xOh43GN-294
3611Q al*-04MO-A619xB73GN-237
3611R al*-07IL-B73GN-56
6602J Alb*-N2522

albino seedling
3512G w*-04HI-A632GN-18

amylose extender
6401E ae*-04MO-A619xB73GN-77

barren inflorescence
6514E ba*-04HI-Mo17xA632GN-157
6516S bif*-03IL-A619TR-503
6516T bif*-03IL-A619TR-799
6602A Bif*-N2616
6606C Bif*-N2623

barren stalk
6516B ba*-07MO-B73xMo17GN-135
6516R ba*-07MO-B73xMo17GN-376

bleached leaf
6603F BlhGr*-N2538
6603G BlhGr*-N2540
6603L PgBlh*-N2550

blotched leaf
6006I blo*-JLB1
6006J blo*-JLB2 P1-wr

brachytic plant
6602H D*-N2520
6602I D*-N2521
6605N Br*-N2641B
6606A D*-N2648

brittle kernel
5812N bt*-04MO-A619xB73GN-58
5812O bt*-04MO-A619xB73GN-69
6404A bt*-04MO-B73xMo17GN-92
6404B bt*-04MO-B73xMo17GN-27
6404D bt*-04HI-A632GN-32
6404L bt*-04HI-A632xOh43GN-4
6404M bt*-04HI-A632xOh43GN-5
6404N bt*-04HI-A632xOh43GN-8

brown kernel
6401H bnk*-04MO-A619xB73GN-55
6401I bnk*-04MO-A619xB73GN-56
6401K bnk*-04MO-B73xMo17GN-24
6401M bnk*-07MO-B73xMo17GN-62

brown midrib
5803J bm*-85-3087-29
5803O bm*-04HI-A632xOh43GN-88
5803P bm*-04HI-A632xOh43GN-227
6510H bm*-07IL-Mo17GN-260

camouflage pattern
4311D tdy*-07MO-B73xMo17GN-422

cherry pericarp
5805N lc*-JLB Pl1

colored leaf
5805M lc*-JLB
5805N lc*-JLB Pl1
6605D WiNcS*-N2608
6605F ChlStk*-N2619

crinkled leaf
4106I cr*-03HI-B73xMo17GN-1036

crossbanded leaf
4311A cb*-07MO-B73xMo17GN-477
6602C BlhCb*-N2510
6603D GrNl*-N2536
6605I PgCb*-N2633

defective kernel
3705O dek*-04HI-A632xOh43GN-78
6407B dek*-04HI-Mo17xA632GN-6
6407C dek*-04HI-Mo17xA632GN-22
6407D dek*-04HI-A632xOh43GN-104
6407F dek*-04MO-B73xMo17GN-24
6407G dek*-06HI-M017GN-20
6407H dek*-07IL-Mo17GN-64

dwarf plant
4402P d*-03HI-B73xMo17GN-120
4402Q d*-07MO-B73xMo17GN-466
4403P d*-07MO-B73xMo17GN-474
6509B d*-07MO-B73xMo17GN-487
6509C d*-07IL-B73GN-230
6509E d*-07IL-B73GN-373
6605C D*-N2605
6605G DPgPtc*-N2622

extended auricle
5809B Eta*-99-1632-7

extra auricles
5804F Wab*-SGL

fasciated ear
6512A fae*-04MO-A619xB73GN-25
6512B fae*-04MO-A619xB73GN-77
6512C fae*-07IL-B73GN-20
6516G fae*-07MO-B73xMo17GN-369

fascicled ear
6512D fas*-03IL-A619TR-179

flecked leaf
6006L lfl*-JLB
6405A flk*-03IL-A619TR-939

floury endosperm
6401J fl*-05HI-RnjxW22GN-5
6405D fl*-04HI-A632TR-11

glossy leaf
5412B gl*-07IL-B73GN-108

gnarled plant
4106J gn*-04HI-Mo17xA632GN-81

green striped leaf
4009Q gs*-04HI-A632xOh43GN-13

hairy sheath
6602E Hs*-N2514
6602K Hs*-N2523
6604B Hsf*-N2559
6604L Hsf*-N2594

leaf epidermis irregular
5808H extra leaf wax*-03IL-A619TR-69

lesion
6507A les*-03HI-B73xMo17GN-500
6507B les*-04MO-A619xB73GN-99
6507C les*-04MO-A619xB73GN-183
6507D les*-07IL-B73GN-51
6507E les*-B73xMo17GN-2
6507F les*-07IL-Mo17GN-2
6507G les*-04MO-A619xB73GN-28
6507H les*-07IL-Mo17GN-45
6507J les*-07MO-B73xMo17GN-284
6507Q les*-07IL-B73GN-209
6507R les*-07IL-Mo17GN-62
6507T les*-06HI-B73GN-137
6602D Les*-N2512
6602L Les*-N2527
6603H Les*-N2541
6603J LesGr*-N2544
6603M Les*-N2551
6604D Les*-N2570
6604E LesGr*-N2576
6604H Les*-N2586
6604K Les*-N2592
6604O Les*-N2599
6605B Les*-N2604
6605J Les*-N2635
6605L Les*-N2638
6605M LesGr*-N2639
6607A les*-07IL-B73GN-41

male sterile
4012LA Ms*-N2629
5405I ms*-07MO-B73xMo17GN-52
5405J ms*-04HI-Oh43xA632GN-56
5406J dsy*-9904
5406L syn*-mtm99-25
5406M syn*-mtm00-03
5406P mca*-mtm00-10
5406Q syn*-IG2007
5407A ms*-MTM3774
5407B dsy*-9905
5407C syn*-mtm99-14
6514B ms*-07MO-B73xMo17GN-133

many tillers
6510D tlr*-07MO-B73xMo17GN-415

miniature kernel
238-38 mn*-MTM5807
6404C mn*-04HI-Mo17xA632GN-16
6404O mn*-04HI-A632xOh43GN-14

nana plant
6509I na*-07IL-B73GN-451
6509K na*-07IL-Mo17GN-119

narrow leaf
6510A nl*-07IL-B73GN-325
6602M GrNl*-N2528
6602O Lxm*-N2530
6604G Morph*-N2585
6604N Nl*-N2598

necrotic leaf
4102Q nec*-07IL-B73GN-235
4103P nec*-07MO-B73xMo17GN-288
6603B YsLes*-N2534

necrotic leaf tips
4101P nec*-07IL-B73GN-230

necrotic stripe leaf
6006K bst*-JLB

oil yellow plant
6009B oy*-03HI-B73xMo17GN-1137
6009D oy*-04HI-Oh43xA632GN-29
6602F Oy*-N2515
6603C Oy*-N2535
6605E Oy*-N2609

opaque endosperm
3902O O*-05HI-A632GN-9
5809C o*-Shaver

pale green plant
6515C pg*-03HI-B73GN-7
6515I pg*-06HI-B73GN-19

pale green seedling
4205P pg*-07IL-B73GN-203

pale sheath
6604C PaSh*-N2562

pale yellow endosperm
6401A y*-04MO-A619xB73GN-9
6401B y*-04MO-A619xB73GN-14
6401C y*-04MO-A619xB73GN-22

6401F y*-04MO-A619xB73GN-12
6401G y*-05HI-A632GN-3
6402Q y vp*-04MO-A619xB73GN-7
6402R y-vp*-04MO-A619xB73GN-8
6402S y vp*-04MO-B73xMo17GN-92
6406A y vp*-04HI-A632xOh43GN-34
6406E y vp*-04MO-B73xMo17GN-75
6406F y vp*-04HI-A632GN-13
6406L y vp*-03IL-A619TR-168

piebald leaf
6604F Pb*-N2583
6606B pb*-N2649

pigmy plant
5504G py*-SGL
6605A PgyV*-N2602

ragged leaf
4106K rg*-04MO-A619xB73GN-402
4601P rg*-06HI-Mo17GN-10

reduced tassel branch number
6514H fbr*-06HI-B73GN-119

resistant to Puccinia polysora
5407J Rpp*-Va59
5407K Rpp*-DS61
5407L Rpp*-B1138T
5407M Rpp*-1416-1

resistant to Puccinia sorghi
5407I Rp*-PI061

rough sheath
6510G rs*-06HI-B73GN-139

semidwarf
6603N SdwWi*-N2552
6603O Sdw*-N2556

short plant
6602G D*-N2516
6603K ShtBaRld*-N2549

shredded leaf
6608A shr*-07MO-B73xMo17GN-31

shrunken kernel
4006Q sh*-04MO-A619xB73GN-57
6403A sh*-04MO-A619xB73GN-40
6403B sh*-07MO-B73xMo17GN-51
6403C sh*-04MO-B73xMo17GN-31
6403D sh*-04MO-B73xMo17GN-60
6403E sh*-04HI-A632xOh43GN-73

6403F sh*-04MO-B73xMo17GN-91
6403G sh*-06HI-Mo17GN-20
6403H sh*-03IL-A619TR-188
6403I sh*-04HI-A632GN-25
6410B sh*-04HI-A632GN-45

slender plant
6006M sky*-JLB lfl*-JLB

small plant
4408P smp*-03HI-B73xMo17GN-1242
6509H smp*-07IL-B73GN-425
6509J smp*-03HI-B73GN-128

striate leaf
3709P sr*-07IL-B73GN-178
3709R sr*-07MO-B73xMo17GN-66
4210O sr*-03HI-B73GN-80
4210P sr*-03HI-B73GN-118
6506S sr*-06HI-B73GN-181
6511F sr*-04HI-Mo17xA632GN-160

sugary kernel
5712P su*-03IL-A619TR-164
6410A su*-04MO-A619xB73GN-67
6410C su*-03IL-A619TR-221
6410E su*-04MO-B73xMo17GN-8
6410F su*-04HI-A632xOh43GN-29

tassel seed
5807L ts*-PI200203
6508A ts*-03HI-B73xMo17GN-655
6508B ts*-03HI-B73xMo17GN-925
6508C ts*-03HI-B73xMo17GN-1011
6508D ts*-03HI-B73xMo17GN-1114
6508E ts*-04HI-A632GN-182
6508F ts*-04HI-A632xOh43GN-233
6508G ts*-04MO-A619xB73GN-155
6508H ts*-04HI-Oh43xA632GN-171
6508I ts*-04MO-A619xB73GN-357
6508K ts*-07IL-Mo17GN-91
6508O ts*-03IL-A619TR-228
6508P ts*-03IL-A619TR-1129

tasselless
6514F tl*-04HI-Oh43xA632GN-199

terminal ear
5806R te*-03HI-B73xMo17GN-406
6602B Te*-N2620

unbranched tassel
6514G ub*-03IL-A619TR-358

upright leaves
6006M sky*-JLB lfl*-JLB

variegated pale green plant
6603A Pgm*-N2531

virescent striped
6604M Vsr*-N2595

viviparous kernel
3607G vp*-04MO-A619xB73GN-1
3607H vp*-04MO-A619xB73GN-5
5902R vp*-04HI-A632xOh43GN-50
6402O vp*-04MO-A619xB73GN-4
6402Q y vp*-04MO-A619xB73GN-7
6402R y-vp*-04MO-A619xB73GN-8
6402S y vp*-04MO-B73xMo17GN-92
6402T vp*-04MO-B73xMo17GN-109
6406A y vp*-04HI-A632xOh43GN-34
6406B vp*-04MO-A619xB73GN-41
6406C vp*-04MO-B73xMo17GN-12
6406E y vp*-04MO-B73xMo17GN-75
6406F y vp*-04HI-A632GN-13
6406G vp*-04MO-A619xB73GN-6
6406H vp*-04HI-Oh43xA632GN-8
6406J y-vp*-04HI-Oh43xA632GN-17
6406L y vp*-03IL-A619TR-168
6406N y-vp*-04HI-A632GN-34
6406O vp*-04HI-A632GN-42
6701A y-vp*-04MO-B73xMo17GN-131
6701B y-vp*-04HI-A619xB73GN-68

white luteus seedling
4111O wl*-07-B73xMo17GN-284

white stripe leaf
6506A wst*-04MO-A619xB73GN-165
6506I wst*-04MO-A619xB73GN-183

white stripe leaf (iojap-like)
4011N ij*-03HI-B73xMo17GN-680
6511A ij*-07MO-B73xMo17GN-339
6511B ij*-07MO-B73xMo17GN-88
6511C ij*-07IL-B73GN-290
6511E ij*-03HI-B73xMo17GN-227

wilted plant
4209O wi*-07MO-B73xMo17GN-454
6505N wi*-03HI-B73GN-174
6505O wi*-04HI-A632GN-180
6505P wi*-04HI-Oh43xA632GN-1
6505Q wi*-03IL-A619TR-769
6506P wi*-03IL-A619TR-501
6604A Pgy*-N2558
6604I WiSdw*-N2587

6605K WiPg*-N2636

yellow and green variegated leaf
4309P yg-zb*-07MO-B73xMo17GN-168

yellow green leaf
4309M yg*-04HI-Mo17xA632GN-80
4309N yg*-04HI-Mo17xA632GN-137
4309O yg*-07IL-B73GN-2
6515A yg*-04MO-A619xB73GN-25
6515B yg*-06HI-B73GN-61
6515D yg*-04HI-A632TR-14
6515E yg*-07IL-B73GN-192
6516F yg*-07MO-B73xMo17GN-276
6604J Yg*-N2588
6605O Yg*-N2642

yellow stripe leaf
6412B ys*-04HI-A632GN-144
6412C ys*-07IL-B73GN-171
6505A ys*-03HI-B73xMo17GN-210
6505B ys*-04HI-A632xOh43GN-18
6505C ys*-04HI-A632xOh43GN-137
6505D ys*-04HI-Oh43xA632GN-187
6505E ys*-07IL-B73GN-279
6603E LesYs*-N2537

zebra leaf
6006N zb*-G232
6006O zb*-Caribbean
6506B zb*-04HI-Oh43xA632GN-72
6506C zb*-04MO-A619xB73GN-28
6506D zb*-07MO-B73xMo17GN-213
6506H zb*-07IL-B73GN-33
6506M zb*-07IL-M017GN-122

zebra necrotic leaf
6006P zn*-CM207
6505F zn*-03HI-B73xMo17GN-151
6505G zn*-03HI-B73xMo17GN-227
6515H zb-nec*-04MO-A619xB73GN-243

zebra striped seedling
6505R zb*-04HI-A632GN-173

MaizeGDB 2012
www.maizegdb.org

Site redesign is on schedule. The redesign for the MaizeGDB web interface (first announced March 2011) was released in preliminary form March 2012 and will be released for public testing March 2013 at the Maize Genetics Conference in Chicago, IL. As explained in the MNL85 report, this effort will result in improved operation, function and overall cosmetic appeal. A number of security features have been added. There will be new tools to permit users to link their papers to genes/gene models in MaizeGDB. These tools provide access to the international standards such as the gene function vocabulary (Gene Ontology or GO) for cellular components, biological process and molecular function. The interface is being alpha-tested (Nov-Jan) by community volunteers (see acknowledgements).

Functional genomics updates.

Sequence-indexed mutations. Some 15,611 new UniformMu insertions were added this year, from data supplied by the UniformMu project. These now number 42,785, representing 14,157 gene models in the filtered gene set and with insertions within 100bp of the start and end positions of genes. The 6130 stocks with the mapped insertions are available at the Maize Genetics Cooperation – Stock Center. To find those for your sequence of interest, use the MaizeGDB BLAST search tools, or look at the UniformMu track on the genome browser track. More details about these mutants and the project are posted at MaizeGDB (http://www.maizegdb.org/documentation/uniformmu/).

Gene Expression. Representation now includes embedded glyphs from the eFP browser at BAR (The Bio-Array Resource for Plant Biology, http://bar.utoronto.ca/efp_maize/cgi-bin/efpWeb.cgi?dataSource=Sekhon_et_al; Winter et al 2007 PloS One 2:e718.) and MaizeGDB histograms for the Maize Gene ExpressionAtlas (Sekhon et al 2011 Plant J 66(4):553-563. See **Figure 1.)** We also provide access to MapMan analysis tools for these data (Thimm et al. 2004 Plant J 37:914-939). Other data included as new tracks on the Genome Browser are listed below.

Metabolism. Metabolic network representations have been computed by two groups, Gramene and the Plant Metabolic Network, both in collaboration with MaizeGDB, and both using the Pathway-Tools of the MetaCyc project (Caspi et al 2011 Nucleic Acids Res D742-753). CornCyc was computed with more stringency, and will not include all the gene models that are in MaizeCyc. A small amount of manual curation has been applied to these datasets. MaizeGDB links to both of these, and plans some manual curation from the literature (Monaco et al. 2013 Maize Metabolic Network Construction and Transcriptome Analysis. *The Plant Genome* 6:1.).

Protein classifications, gene homologs and syntelogs are listed on each gene model page, and with links to offsite resources. These links were made possible through collaboration with Gramene, Phytozome, and efforts from James Schnable and Mike Freeling. Linked protein classifications resources include Panther (http://www.pantherdb.org), PFAM (http://pfam.sanger.ac.uk/), and COG. (http://www.ncbi.nlm.nih.gov/COG). Homolog links are included from Gramene (http://www.gramene.org), TAIR (http://www.tair.org; Arabiodopsis), and the MSU Rice Genome Annotation Project (http://rice.plantbiology.msu.edu). Syntenic orthologs (syntelogs) are provided for *Sorghum bicolor, Setaria italic, Oryza sativa japonica*, and *Brachypodium distachyon*, with links to those gene models at Phytozome. Links also are provided to tools (qTeller, CoGE) that were used to compute and analyze expression for these syntelogs.

New Genome Browser tracks (in brief; more detail is found by clicking on the '?' at each track).
Assembly/Genome Features

 Knobs. Both BLAST identified and genetically mapped. Ghaffari et al. 2012 Maize Meeting Abstract.

 Foreign contamination. A subtrack for the pseudomolecule indicating regions found by GenBank to be sequence that is mitochondrial or from other species.

 Mitochondrial and cholorplast genome sequences have been added for convenience, although they are not part of the B73 RefGen_v2 assembly. They are annotated with their long established genes, rather than computed gene models.

 Nucleosome occupancy predictions from H Bass and J Dennis. See also Gupta et al 2008 PLoS Comput Biol 4:e1000134.

 Reconstructed chromosomes from maize tetraploidy. Provided by J Schnable and M Freeling; see also J Schnable et al PNAS 108:4069-4074; Schnable J and Freeling M 2011 PLoS One 6:e17855.

Diversity

 HapMap1 SNP from Gore et al 2009 Science 326:1115-1117 and http://www.panzea.org.

 ISU SNPs on 291 IBM RILs from Liu et al 2010 Genetics 184:19-26.

Expression and transcripts (see also above)

 IBM SAM eQTL. RNA-SEQ data for SAM from 105 IBM RILS; from Muehlbauer and the Shoot Apical Meristem (SAM) Project (M Scanlon PI)

 5' Methylcytosine methylation in B73 and Mo17 (Eichten et al 2011 PloS One Genet 7:e1002372.)

 miRNA mirBase data from Zhang et al (2009) PloS Genet. 5:e1000716

 KNOTTED1 binding regions from Bolduc et al 2012 Genes and Dev 26:1685-1690.

 Agilent microarray annotations; anther stages and mutants. From Ginny Walbot and Dave Berger. Ma et al 2006 Genome Biol 7:R22; Nan et al 2011 BMC Plant Biol 11:120; Wang et al 2010 Plant J 63:939-951.

Gene models

 Sorghum syntenic orthologs for 24,000 maize genes) identified using SynMap (http://genomevolution.org) in collaboration with James Schnable. For details of methods see also Lyons et al 2008 Tropical Plant Biology 1:181-190; Tang et al 2011 BMC Bioinformatics 12:102, Schnable et al 2011 PLoS One 6:e17855.

 Split Genes. These are putative gene annotation artifacts and determined by Gramene. There are 2 general categories. When two apparently paralogous genes lie on differ strands in the assembly, with no overlap between the gene fragments and (2) when gene fragments are in close proximity on the same strand, but have no overlapping sequence. Details about the methodology are at http://useast.ensembl.org/info/docs/compara/homology_method.html.

Repetitive Elements

 Sirevirus LTR retrotransposns from Bousios A et al 2011 The Plant Journal 69:475-488.

Outreach. Three MaizeGDB "how to" tutorials were given immediately prior to the Maize Genetics Conference 2012 in Portland, OR. With over 60 scientists attending, this was a big success and we will offer this again in 2013. In collaboration with Cathryn Wojcicki at the National Corn Growers Association (NCGA), four podcasts have been produced that highlight functional genomics analysis tools that have been developed at MaizeGDB with support from the NCGA: GBrowse2 (February 2012), eFP Browser (May 2012), MapMan (August 2012) and Alternative Genome Assemblies (November 2012). The target audience is America's farmers with ~600 downloads per podcast. Each podcast introduces a genomics concept in easy-to-follow terms and then explains how MaizeGDB works to make the data more available to researchers worldwide. Podcasts will continue to be produced on a quarterly basis. We also organized a well-attended Plant Bioinformatics Outreach Exhibit Booth at the Plant and Animal Genome XX meetings, San Diego, CA, and which represented 15 public online resources.
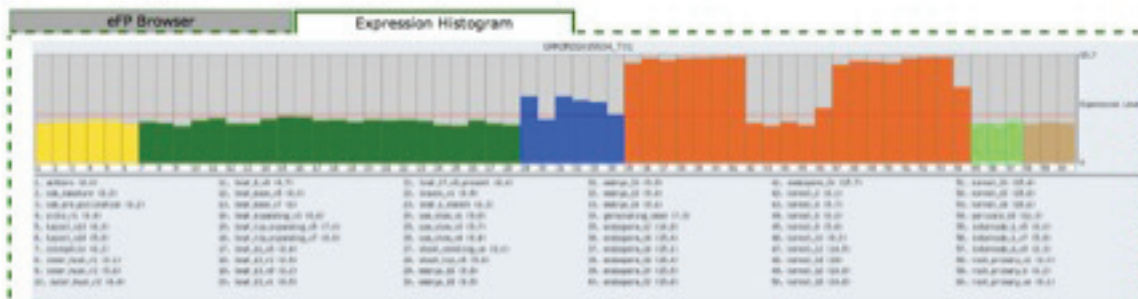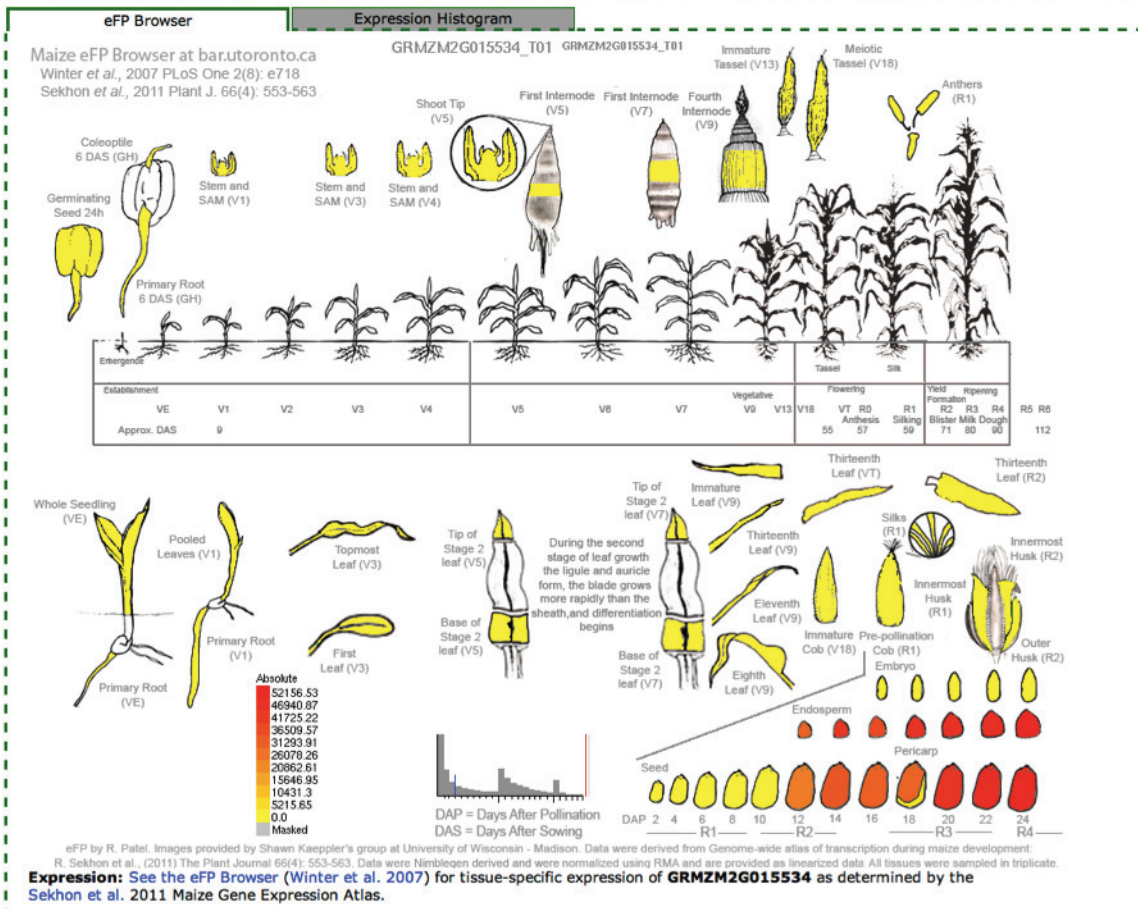
Figure 1. Two expression glyphs embedded in the gene model record GRMZM2G015534, aka *o2 opaque2*. Both represent atlas data from Sekhon et al 2011. The top histogram was developed at MaizeGDB for the Genome Browser and links to tools at PLEXdb and to more detail about tissues stored at MaizeGDB; the second was provided by the BAR project (see text for more detail).

Plans.

A Project Plan for the next 5 years has been drafted for the outside review that is mandated by the ARS Office of Scientific Quality Review (OSQR). Our proposal was drafted with inputs from our Working Group, and with careful consideration of the results of a survey of community needs by the Maize Genetics Executive Committee (http://www.maizegdb.org/mgec-activities2012.php). Top needs were to improve annotations of the assembly; to improve interoperability among databases that serve the community; to represent interactive networks for maize genes; and to document experimentally confirmed phenotypes.

Sorghum community interest. The Sorghum research community recently met to discuss genomics and community needs. Two top needs articulated at that meeting were community support and information systems. There is an interest from the research community as well as from members of the Sorghum Checkoff to consider the services provided by MaizeGDB as a model for their development going forward and to learn from the maize community on how best to address these needs.

A Chinese instance of MaizeGDB. In collaboration with Jinsheng Lai at China Agricultural University, a plan is in place to deploy a Chinese version of the MaizeGDB website in March of 2014 at the Maize Meeting in Beijing. Translations are underway with a plan to host the Chinese site in Ames. This ensures concerted development of functionality for both website instances and enables the continuation of a single resource for maize data. This project is simplified by the fact that the newly redesigned interface infrastructure separates Web content from interface functionality. This project is especially exciting to us because ~25% of MaizeGDB users currently access the site from machines configured to use Chinese as their primary language.

How to provide large datasets to MaizeGDB. Use the feedback form at MaizeGDB (http://www.maizegdb.org/data_contribution.php) to contact us about arrangements to accommodate your data. If possible, it is best to contact us before you begin to generate large datasets, so that a customized pipeline can be created to import your data in a timely and efficient manner.

— Submitted Dec 2012 by Mary Schaeffer, Carson Andorf, Darwin Campbell, Ethalinda Cannon, Jack Gardiner, Lisa Harper, Steven Perez, John Portwood, Jackie Richter, Taner Sen, Kokulapalan Wimalanathan, and Carolyn Lawrence

(redacted from the MaizeGDB MGEC page:
www.maizegdb.org/mgec-activities2012.php)

MGEC activities that occurred over the 2011-2012 year included:

• meeting Sep 2011 with Diane Okamura to discuss opportunities for funding.

• attending the Sep 2011 Plant Science Research Summit towards developing a plan "to invigorate and guide plant science research over the next decade". The report from this summit is posted at http://plantsummit.wordpress.com. Direct and in kind support for this summit were provided by: the American Society of Plant Biologists, the Howard Hughes Medical Institute, the National Science Foundation, the US Department of Agriculture, and the US Department of Energy.

• working with the Maize Meeting Steering Committee to develop the MaGNET (Maize Genetics Network Enhancement via Travel) program to support costs of attending the Maize Genetics Conference. This program seeks to recruit and retain scientists from diverse backgrounds into the maize research community and is described in more detail on the Maize Conference website, http://maizemeeting.maizegdb.org/mm2012/magnet.php.

• meeting with members of Congress and aides to discuss value of plant science to the economy

• conducting a 2012 community survey, summarized in detail online (see URL at top of this note), and presented in open forum at the 2012 maize genetics meeting, and in brief below.

The MGEC 2012 survey was sent to the entire maize genetics community and requested inputs about priorities in research, bioinformatics, education and outreach, Maydica and crop improvement. There were 157 respondents, a number that approximates 1/3 of the number of attendees at the 2012 maize. Respondents included heads of laboratories (35%), research scientists (23%), graduate students (20%), several postdoctorals, educators, and undergraduates. Public and private sectors responded. The top 3 research directions cited were (1) advancement of functional studies of maize gene, gene families, and networks (including reverse genetics resources and developmental atlas RNAseq or proteomic data); (2) simplification of maize transformation and genome engineering capabilities; and (3) increased high-throughput phenotyping capabilities for maize. Top bioinformatics needs were considered to be (1) the improved assembly and annotation of the B73 reference genome sequence, and the genome sequence of diverse inbred lines. (2) improved interoperability of several databases, including but not limited to: MaizeGDB, maizesequence.org, NCBI, PlantGDB, TAIR and Gramene. (3) development of interaction networks for maize genes; and (4) the documentation of confirmed (highquality) phenotype. A number of crop improvement and specific suggestions are provided on the website, and provide examples of how maize research has had impact: http://maizemeeting.maizegdb.org/mgec-survey12/analyze_final_sort.php - Q5 .

In 2005 Virginia Walbot convened the inaugural MaizeGDB Editorial Board, charged with the task of recommending noteworthy maize primary literature. The 2005 board included Hugo Dooner as Chief, Lisa Harper, Erich Grotewold, Ed Buckler and Nathan Springer. A new board is convened each year. Below are the selections for years 2011-2012, sorted by year. Editorial comments are provided by the recommending board members. The information below is accessible from the MaizeGDB homepage button displayed above.

2012 Selections

Barbaglia, AM, et al. 2012. Genetics. 190:965-75. Gene capture by helitron transposons reshuffles the transcriptome of maize.

Editorial Comment: Helitrons are an unusual class of transposable elements that have the propensity to capture fragments of genes and transpose them to new locations in the genome. Some of these gene fragments are transcribed and detected as ESTs. Barbaglia et al. computationally analyzed the B73 genome sequence for Helitrons and made several interesting discoveries: Helitrons compose almost 1% of the B73 genome, many Helitrons show plus/minus variation of being present in the B73 genome, but not in the Mo17 genome, some Helitron-derived ESTs show alternative spliced forms between root and shoot tissues, and in at least one case, an exon outside of a Helitron insertion was joined to the Helitron-derived transcript. These data show that gene fragments captured by Helitrons, as well as flanking exons, can be expressed as chimeric transcripts and may lead to the evolution of new genes. *David Braun, 2012*

Barber, WT et al. 2012. Proc Natl Acad Sci, USA 109:10444-10449. Repeat associated small RNAs vary among parents and following hybridization in maize.

Editorial Comment: Small RNAs have previously been hypothesized to contribute to hybrid vigor or heterosis in plants. Using Illumina sequencing Barber et al. assessed the small RNA population in the seedling shoot apex and developing ear of two maize inbreds, B73 and Mo17 and their hybrid. Very few siRNAs were unique to a single or both parents or to the hybrid. Differences between parents and hybrids resulted from the hybrids inheriting distinct siRNAs from each parent rather than the generation of new siRNAs. These siRNA differences between the parental inbreds were enriched in 21- to 22-nt siRNAs from specific retrotransposon families. Furthermore, a reduction of 24-nt siRNAs by the mop1 mutation did not have an effect on the traits associated with heterosis. In conclusion, genetic variation in the regulation of transposable elements in the maize genome could contribute significantly to hybrid vigor. *Liza Conrad, 2012*

Barbour, JE et al. 2012. Plant Cell 24:1761-1775. Required to

maintain repression2 is a novel protein that facilitates locus-specific paramutation in maize.

Bolduc, N, et al. 2012. Genes Dev. 26:1685-1690. Unraveling the KNOTTED1 regulatory network in maize meristems.

Editorial Comment: The KNOTTED1 (KN1) homeodomain transcription factor functions to establish and maintain stem cells in plant meristems. This study combined expression analysis through RNAseq with chromatin immunoprecipitation (ChIP-seq) to identify genes regulated by KN1. Several thousand loci were shown to be bound by KN1, including both genes and a number of potential enhancer sequences. RNA-seq analysis focusing on only genes bound by KN1 revealed 643 genes that are bound and modulated by KN1, in other words, direct targets of KN1 regulation. These genes were enriched for transcription factors and hormone metabolism. Several transcription factor families known to be important in developmental programs such as homeobox, MADS, auxin response factors, (ARF), YABBY, and basic helix-loop-helix were identified. In addition to KN1 binding to auxin biosynthetic and transporter genes, it was also shown to bind to nearly half of the AUX-IAA and ARF genes in the maize genome. Thus, it appears KN1 regulates the auxin pathway at all levels. This comprehensive look at the KN1 regulatory network provides a solid foundation for broader studies on meristem function in plants. *Liza Conrad, 2012*

Brown, PJ, et al. 2011. PLoS Genet. 7:e1002383. Distinct genetic architectures for male and female inflorescence traits of maize.

Editorial Comment: Maize morphology, particularly that of the reproductive organs, has changed dramatically throughout the process of domestication and subsequent directional selection. This shift in plant architecture was brought about by changes in genetic architecture. This paper investigates the genetic architecture of ear and tassel morphology as compared to that of previously studied flowering and leaf traits. Unlike previous investigations in the NAM population where traits were found to be predominantly controlled by many loci with very small effects, inflorescence traits displayed a shift in genetic architecture toward increased effect sizes, particularly for ear morphology. It is suggested that the larger effect sizes observed in the ear are the result of cryptic variation released after domestication mutations became fixed, leading to instability of ear traits and allowing strong directional selection to occur and be maintained over time. The paper also discusses pleiotropy among the traits, and investigates the proximity of QTL to known inflorescence-implicated mutants and domains. *Addie Thompson, 2012*

Burt, AJ et al. 2012. Crop Sci. 53:554-563. Development and utilization of high carotenoid (HiC) maize germplasm: Proof of concept.

Editorial Comment: Translational genomics is one of those

buzz phrases tossed about by funding agencies, college deans and other decision makers who are looking for ways to move from laboratory to farm to table. Plant breeders have been using translational genomics since the birth of the discipline, applying basic scientific knowledge to improve quality of life for consumers. Plant biotechnology is an area where translational genomics can easily be applied, but we most often think of transgenic crop improvement in this arena. Certainly, transgenic traits that serve producer needs (e.g. herbicide resistance) have been key developments for agriculture at the turn of the 21st Century, but new innovations to better serve consumer needs should be just around the corner. An obvious one is enhancing nutritional quality for key nutrients, such as dietary carotenoids, which can take place either by using transgenic or conventional breeding based approaches. One of the key issues raised in the present study is the use of animal feeding trials to verify that the predicted improvement in nutrient concentration actually translates into enhanced animal health and/or product quality. Here, high carotenoid maize lines developed using conventional breeding are evaluated using feeding studies with laying hens, to validate observations made by eye on kernel color and by HPLC for carotenoid profiling. It was not unexpected that a poultry diet supplemented with carotenoids from marigolds had higher total carotenoids and lutein than any other diet. However, it was very encouraging to see that the experimental maize varieties out delivered zeaxanthins, which together with lutein help protect us from macular degeneration. While this is clearly just a proof of concept, this early test makes clear the promise of translational genomics towards enhancing nutritional quality and human health. Perhaps equally importantly, consumers are already prepared to pay premiums for eggs with enhanced nutritional qualities (e.g. omega-3 fatty acids), such that we know that the marketplace is willing to accept such a product. *Owen Hoekenga, 2012*

Chia, J-M, et al. 2012. Nature Genetics. 44:803-807. Maize Hap-Map2 identifies extant variation from a genome in flux.

Editorial Comment: What do we mean when we talk about the maize genome? Are we referring to the genes, repetitive sequences and other hangers-on found in a single cultivar or speaking more broadly about many or all of the maize cultivars, landraces and other accessions at once? While there has been enormous value in the B73 genome, as per the Bermuda/Fort Lauderdale agreement sequence standards, as people look more widely using less expensive sequencing methodologies it is also clear that the maize genome has tremendous variability between accessions. This picture has become markedly clearer with the update of the maize haplotype mapping project (HapMap2), which now includes 103 inbreds, 23 landraces, 19 teosintes and a single accession of Tripsacum dactyloides, to serve as an out group. Applying independent bioinformatics pipelines identified segregating sites only 33% of the time from the same raw sequencing reads (13 billion reads amounting to ~1 trillion bp). 55 million SNPs were called at high confidence, with polymorphisms located to 21% of the gene models from the B73 reference genome. While nonsense mutations were far less abundant than synonymous or nonsynonymous substitutions, nearly 8% of genes in inbred maize had premature stop

codons. Variation in sequencing depth for individual genes, as an estimate for copy number, indicated that 90% of sliding windows of 10 kb varied at least 2-fold among the study panel; variation in 70% of these sliding windows were found in greater than or equal to 10 accessions. Variation within the gene space of maize varieties is apparently larger than previously estimated, with potentially functional variation occurring at many loci. One of the next great challenges for current and future plant breeders and geneticists is therefore how to best capture and combine these functional variants to continue to enhance the performance of maize into the 21st century. *Owen Hoekenga, 2012*

Cook, J, et al. 2012. Annu Rev Plant Physiol Plant Mol Biol. 158:824-34. Genetic architecture of maize kernel composition in the nested association mapping and inbred association panels.

Editorial Comment: Enhancing sustainability and efficiency of 21st Century agriculture must be mindful of improving quality and yield for crops like maize. While grain yield has been a primary breeding target for many years, tailoring maize grain quality to better meet the needs of the food/feed industry, bioenergy concerns and ultimately the end users will also be very important. These stakeholders are aware of the competition between the carbon and nitrogen sinks of starch, protein and oils in the kernel. Fortunately, the genetic and environmental bases for these traits have been studied for a number of years. Recently, our understanding took a quantum leap forward with the use of the Nested Association Mapping and Inbred Association Panels to dissect these traits. Approximately twenty loci were identified for each trait, explaining 60% of the variance observed with largely additive effects based on seven locations worth of grain samples phenotyped by NIR spectroscopy. Allelic series were common, as 114-136 alleles were observed between starch, protein, and oil quality. Using the Hap-Map v1 SNP data, a causative gene underlying one of the oil QTL was identified. Given the decreasing cost of sequencing and our increasing knowledge of SNP diversity within maize, this phenotypic dataset will continue to be useful for many years to come as one can easily expect many more causative genes will be identified. *Owen Hoekenga, 2012*

Costa, LM, et al. 2012. Curr Biol. 22:160-5. Maternal control of nutrient allocation in plant seeds by genomic imprinting.

Editorial Comment: In plants, parent-of-origin specific gene expression, also known as imprinting, occurs primarily in the endosperm. The main function of the endosperm, similarly to the placenta of mammals, is to provide nourishment to the growing embryo. In the mammalian placenta, imprinted genes regulate the flow of nutrients from mother to fetus. This analysis of the Maternally expressed gene1 (Meg1) is the first report of any imprinted plant gene functioning in maternal nutrient allocation to the embryo. This study establishes that Meg1 is required for transfer cell differentiation, sugar homeostasis and nutrient partitioning during seed development in maize. Precise temporospatial expression of Meg1 promotes differentiation of the endosperm transfer tissue in the developing endosperm. The authors clearly demonstrate that

Meg1 functions in a dosage dependent manner that is regulated through imprinting. Consequently, the loss of imprinting or increase in dosage results in an increased distribution of maternal resources into the endosperm having effects on seed size and sugar partitioning. *Liza Conrad, 2012*

Dahal, D et al 2012. Plant J. 72:70-83. Specific changes in total and mitochondrial proteomes are associated with higher levels of heterosis in maize hybrids.

Editorial Comment: The mechanism underlying heterosis is poorly understood although it has been hypothesized that mitochondrial respiration plays an important part. This study compared total and mitochondrial protein fractions from ear shoots in two higher-heterosis hybrids to a lower-heterosis hybrid. Overall, only 2-3% of proteins observed changed expression between higher- and lower-heterosis hybrids. Changes in energy metabolism proteins involved in glycolysis, the TCA cycle, photorespiration and ETC were correlated with higher-heterosis hybrids. Additionally, a differential abundance of stress-related proteins was detected in these higher-heterosis hybrids. Interestingly, it was demonstrated that the total amount of certain proteins did not change but rather different isoforms correlated to differences in heterosis. These data suggest higher-heterosis hybrids have more efficient energy metabolism and are better adapted to various stresses thus increasing their vigor and level of heterosis. *Liza Conrad, 2012*

Davidson, RM et al. 2012. Plant J 71:492-502. Comparative transcriptomics of three Poaceae species reveals patterns of gene expression evolution.

Editorial Comment: Though not about maize specifically, this article utilizes comparative transcriptome sequencing in multiple tissues of three closely related species from three subgroups of the Poaceae family of grasses: brachypodium, rice, and sorghum. The analysis first focused on the genes in common across the species, identifying a set of around 13,000 common genes. Co-expression networks were investigated, and it was found that of the shared genes, expression was more similar in conserved tissues like anthers and leaves than in reproductive tissues such as seeds and flowers. Interestingly, more shared genes contribute to lineage-specific reproductive expression than lineage-specific genes. The article also investigates regulatory evolution as related to expression levels and gene function, finding that genes with high expression levels are GO-term enriched for housekeeping, and selectively constrained. A shared whole genome duplication event prior to speciation also allows for exploration of the diversification of homologous gene pairs within and among species, with orthologous gene pairs in blocks of genomic colinearity between species showing increased conservation of expression. Many of these concepts have been or are starting to be investigated in maize, and having additional datasets from related species will allow further study into evolutionary implications of genome-wide transcription variation. *Addie Thompson, 2012*

Dong, Z, et al. 2012. PLoS One. 7:e43450. A gene regulatory network model for floral transition of the shoot apex in maize

and its dynamic modeling.

Editorial Comment: Regulation of flowering time is a complex process that involves both genetic and environmental inputs. The accurate prediction and genetic manipulation of flowering time has clear agricultural importance. Empirical models that predict flowering time exist, but these models are unable to make predictions for novel genotypes. The ideal model would incorporate both genetic and environmental factors to make accurate predictions. As a first step, the authors constructed a conceptual gene regulatory network that regulates flowering time in maize, synthesizing information on candidate genes and their relationships from work in maize, other grasses and Arabidopsis. To produce a quantitative, predictive model, they constructed a simplified dynamic gene network model, consisting of four key regulatory genes including the autonomous pathway members, ID1 and VGT1, and the integrators, DLF1 and ZMM4 (required to integrate inputs from multiple pathways that regulate flowering time). I will not (and admittedly cannot) comment on the mathematics, but the model successfully predicted zmm4 expression levels and total leaf number (as a proxy for days to tassel initiation) of novel genotypes. It will be interesting to see how the addition of new genes and environmental inputs impact the model and its predictions. While models that accurately predict flowering time are clearly important from an agricultural perspective, these models also play an important role in the discovery as they often make new (and unexpected) predictions and give experimental biologists new hypotheses to test. *Beth Thompson, 2012*

Eichten, S et al. 2011. PLoS Genet 7:e1002372. Heritable Epigenetic Variation among Maize Inbreds.

Editorial Comment: This study examined the whole genome CG methylation patterns in two different maize inbreds allowing the authors to tease apart genetic from purely epigenetic variation. A total of nearly 700 Differentially Methylated Regions (DMRs) were identified in B73 and Mo17 with a subset of these DMRs occurring in identical-by-decent regions were little or no sequence variation is present. Overall, the authors discovered that the majority of the DMRs occur in intergenic regions however a small number of DMRs do overlap with genes. Genes found within the DMRs are generally hypomethylated, high-confidence genes with higher expression levels, and are in syntenic positions relative to other grasses. Interestingly, there was no detectable difference in methylation between maize subgenome1 and subgenome2 from Schnable, et al 2011 even though subgenome1 is more highly expressed. The use of near-isogenic lines allowed the authors to evaluate the inheritance and whether the methylation in these DMRs is controlled by genetic differences elsewhere in the genome. The majority of DMRs (85%) were stably inherited. Furthermore, a small subset (3/13) of the stably inherited DMRs show evidence of trans-acting control of the DNA methylation. This study demonstrates the presence of purely epigenetic variation and provides a foundation for further research into the phenotypic effects of epigenetic variation in maize. *Liza Conrad, 2012*

Fouquet, R et al. 2011. Plant Cell 23:4280-4297. Maize rough

endosperm3 encodes an RNA splicing factor required for endosperm cell differentiation and has a nonautonomous effect on embryo development.

Editorial Comment: The endosperm is critical to support normal embryo growth and development and is a key determinate of seed size. While we know that the developmental programs of the embryo and endosperm must be carefully integrated, we know relatively little about the signaling that occurs between these two tissues. Here, the authors employed a clever strategy using B-A translocations to find seed mutants with nonautonomous roles in the endosperm and embryo. This work focuses on Rough Endosperm3 (Rgh3), which functions in the endosperm to promote embryo development. Specifically, they found that Rgh3 is required to promote the switch from cell proliferation to differentiation in the endosperm. This observation suggests that endosperm viability per se is not sufficient to promote embryo development, but rather at least one specific endosperm cell type is required to promote normal embryo development. Rgh3 encodes the RNA splicing factor U2AF35, a core splicing factor. Splicing is not globally perturbed in rgh3 mutants, rather splicing defects are limited to a subset of alternatively spliced mRNAs. Analysis of these aberrantly spliced transcripts (and investigation of the normal function of these genes) provides a potential inroad to identify endosperm-embryo signaling molecules and the endosperm cell types that are essential for this signaling. *Beth Thompson, 2012*

Gaudin, AC; McClymont, SA; Raizada, M. 2011. Crop Sci. 51:2780-2795. The Nitrogen Adaptation Strategy of the Wild Teosinte Ancestor of Modern Maize, Zea mays subsp. parviglumis.

Editorial Comment: While the impact of domestication on the form and function of the maize plant has been considered for many years, the majority of attention has been placed above ground. Roots are not an easy system to study and have largely been ignored. An innovative aeroponics apparatus has been designed to allow plants to be grown to maturity using controlled nutrient environments and to have their roots systems easily visualized and sampled for additional study. This aeroponic approach allowed quantitative traits of root architecture, reporter gene expression and nitrogen use efficiency to be examined in teosinte and the W22 inbred. Teosinte and other wild plants are well adapted to irregular soil environments, making more out of less. Modern maize inbreds have been optimized to grow in uniform and nutrient-dense soil environments. However, with increasing fertilizer costs and a wish to decrease the impact of agriculture on the broader environment, having a better understanding of how wild relatives adapt to low nutrient environments has high value. This study establishes a procedure for collecting accurate and reproducible trait data to guide these future efforts in understanding the molecular genetic, physiological, and developmental underpinnings of nitrogen use efficiency. This information will be extremely useful to enhance the sustainability of agriculture. *Owen Hoekenga, 2012*

Gent, JI; Dong, Y; Jiang, JM; Dawe, RK. 2012. Nucl Acid Res. 40:1550-60. Strong epigenetic similarity between maize centromeric and pericentromeric regions at the level of small RNAs, DNA methylation and H3 chromatin modifications.

Editorial Comment: The domain adjacent to the centromere, the pericentromere, is essential for proper chromosome cohesion during cell division. Although the centromere is marked by a distinct histone variant, CENH3, there is no such defining characteristic known for the pericentromeric region. In this study, genome-wide sequencing laid the foundation to compare RNA expression levels, DNA methylation and histone modification between the centromere, pericentromere and the chromosome arms. Surprisingly, this thorough epigenetic profiling revealed very little difference between pericentromeres and centromeres. Rather, it showed that pericentromeres are an intermediate state between centromeres and the chromosome arms with respect to siRNAs, DNA methylation, poly(A)-enriched RNA and four Histone3 modifications. The authors conclude that the two primary properties of the pericentromere, chromosome cohesion and suppressed recombination rates, may result from indirect affects of kinetochore binding and its proximity to the centromere rather than an epigenetic state distinct to the pericentromere. *Liza Conrad, 2012*

Hansey, C, et al. 2012. PLoS One. 7:e33071. Maize (Zea mays L.) Genome Diversity as Revealed by RNA-Sequencing.

Editorial Comment: In this work, the authors used RNA-sequencing to define the transcriptome of seedling tissue from 21 diverse maize inbred from the two major heterotic groups (stiff stalk and non-stiff stalk germplasm). Through this work, they were able to identify a core genome, which includes genes expressed in all the inbreds sampled (50% of all genes) and a dispensable genome, which includes genes that are only expressed in one or a subset of the inbreds sampled (28% of all genes). The dispensable genome is likely be a major contributor to the great phenotypic diversity observed in maize. In addition, the authors identified an additional 351,710 SNPS from genic regions (in 22,830) genes. Because these SNPs are present in genic regions, they have the potential to alter protein function. The authors also identified an additional 1300 novel transcripts, including most of which are likely not present in the B73 genome. Interestingly, 150 of the novel transcripts are only present in one heterotic group, giving support to the attractive hypothesis that presence/absence variation could be a major contributor to heterosis. *Beth Thompson, 2012*

Hiltpold, I et al. 2011. Plant Cell Environ 34:1267-75. Systemic root signalling in a belowground, volatile-mediated tritrophic interaction.

Editorial Comment: When leaf herbivores attack plants, plants release volatile organic compounds locally at the site of damage, and systemically from undamaged shoot tissues. The volatiles serve as chemical attractants to predatory insects of the herbivores. Thus, the plant, herbivore, and predator constitute a tritrophic ecological interaction, which has been well studied in shoots, but not investigated previously in roots. Hiltpold et al examined whether Western corn rootworm (WCR) could induce the production of volatiles from maize roots. They found that upon

WCR feeding, (E)-b-caryophyllene (EbC) was induced locally at the wound site, and to a lesser extent, systemically in undamaged roots. Further, the authors showed that EbC functioned as a chemical attractant to an entomopathogenic nematode that is a parasite of WCR. Additionally, herbivory by WCR on maize roots induced the transcription of a gene that functions in the biosynthesis of EbC. Hence, root herbivory triggers the production of a volatile compound that attracts a predatory insect to help the plant defend itself. Perhaps as one way to mitigate the damage caused by WCR (estimated at more than $1 billion per year), corn breeders can select for plants with roots that produce greater amounts of EbC when attacked! *David Braun, 2012*

Huang, JT; Dooner, HK. 2012. Plant Cell. 24:4149-4162. The spectrum and frequency of self-inflicted and host gene mutations produced by the transposon Ac in maize.

Editorial Comment: The study of transposition has a long and distinguished history in maize, starting with the pioneering work of Barbara McClintock. Transposons are now appreciated as major generators of mutation and driving forces of evolution, although major questions remain about the type and frequency of mutations and chromosomal rearrangements that can be generated by transposons. A recent paper by Huang and Dooner, investigates mutations caused by the autonomous Activator transposon, focusing the less common classes of mutations that are not caused by simple excision. Previous work to estimate Ac mutation rates employ systems in which Ac is inserted in the coding region of a gene. In these systems, imprecise excisions as well as other more complex mutations result in loss-of-function phenotypes. Because simple excisions greatly outnumber more complex mutations, characterizing and estimating rates of these more complex mutations has been challenging. Here, the authors utilize a clever system in which Ac is inserted in the 5 UTR of bz, in which simple excision events create purple revertants, while host or transposon deletions produce stable bronze revertants. They isolated 72 stable bronze derivitives, most of which turned out to be internal Ac deletions that behaved as new Ds elements. Interestingly, mutations rates of Ac to Ds in this system were estimated to be an order of magnitude higher than previous estimates. The second most abundant class of mutations were fractured Ac elements, which in which either the 5 or 3 end of Ac was deleted. The abundance of this class of mutations was somewhat surprising given the few examples present in the literature. They also found several deletions of adjacent host sequences (with or without Ac) and single events of other mutation classes. Characterization of these complex mutations indicate most likely originate by DNA repair synthesis followed by microhomology-mediated end joining. *Beth Thompson, 2012*

Hufford, MB, et al. 2012. Nature Genetics. 0:10.1038/ng.2309. Comparative population genomics of maize domestication and improvement.

Editorial Comment: What changes took place within the maize genome during domestication? The morphological changes regulated by loci such as teosinte branched1 (tb1) and teosinte glume architecture1 (tga1) are well known and easy to appreci-

ate relative to the conversion of teosinte into recognizably modern maize. But what else happened to the genome at domestication and later, during improvement? This paper discusses the use of next-generation sequencing applied to 35 modern maize inbreds, 23 landrace accessions, and 17 accessions of wild relatives to an average depth of 5x coverage. It is now clear that domestication and improvement impacted allelic frequencies and local linkage disequilibrium at 1-2% of the gene space (recognized as the current filtered gene set). Some of the selective sweeps detected were larger in magnitude than those already detected at tb1 and tga1, but the effects of these changes are not immediately obvious to the form or function of the plant. Further, an appreciable fraction of the ~3000 locations in the genome that responded to domestication or selection are not obviously associated with the filtered gene set. This suggests, together with experimental evidence from gene expression profiling, that massive changes in cis regulatory sequences were also consequences to domestication and/or improvement, especially where there were not concomitant changes in the coding sequences. Together, these results indicate that allelic frequencies and identities have been altered at perhaps a far larger fraction of the genome than was previously appreciated. Future breeding efforts could concentrate at the re-introduction of allelic variation at these ~3000 loci to spur continued improvement of maize. *Owen Hoekenga, 2012*

Hartwig, T et al. 2011. Proc Natl Acad Sci, USA 108:19814-19819. Brassinosteroid control of sex determination in maize.

Editorial Comment: This paper adds brassinosteroids (BR) to the growing list of hormones that function in maize sex determination. Previous work has shown that BRs have roles in cell elongation, photomorphogenesis and vascular differentiations, however their roles in sex determination have been speculative. Here, the authors report the characterization and cloning the classical mutant, nana plant1 (na1). na1 exhibits a dwarf phenotype characteristic of BR mutant, as well as feminized tassel florets (the tasselseed phenotype), resulting from lack of pistil abortion in the tassel. The authors found that na1 encodes a DET2 homolog, a key enzyme in the BR biosynthetic pathway. BR levels are reduced in na1 mutants and BR inhibitors phenocopy na1 mutants, confirming the role of BR in maize sex determination. na1 is expressed in anthers throughout anther development, suggesting that local BR synthesis might promote stamen maturation in tassels. Interestingly, BR are steroid-like hormones, illustrating the key roles steroid hormones play in both animals and plants. *Beth Thompson, 2012*

Hung, H-Y, et al. 2012. Proc Natl Acad Sci, USA. 159:1309-18. ZmCCT and the genetic basis of day-length adaptation underlying the postdomestication spread of maize.

Editorial Comment: The south-to-north spread of maize from its center of origin in southern Mexico required a significant adjustment in photoperiod sensitivity. While teosintes and tropical maize delay flowering under long days, temperate maize is less sensitive to day length. Here, Hung et al. investigate the genetic architecture of photoperiod sensitivity by measuring the difference in thermal time to flowering in long versus short day

environments. Both the nested association mapping and diverse association mapping populations were utilized for mapping. A series of genome-wide association mapping and linkage mapping in subsequent populations allowed resolution of a QTL on chromosome 10 to ZmCCT, a gene homologous to rice Ghd7, a photoperiod response regulator. ZmCCT showed increased expression in alleles contributed by teosinte lines, leading to delayed flowering. This gene is thought to be one of a suite of genes that was under selection in postdomesticated maize by early humans to decrease photoperiod sensitivity, allowing for the northward spread of maize. This work provides insight into photoperiod sensitivity, its importance in postdomestication, and how it relates to previously mapped flowering time traits. In addition, the experimental approach outlines a thorough and complete investigation from large-scale genome-wide mapping down to the single gene level. *Addie Thompson, 2012*

Kelliher, T; Walbot, V. 2012. Science. 337:345-348. Hypoxia triggers meiotic fate acquisition in maize.

Editorial Comment: Through confocal microscopy Kelliher and Walbot traced cellular ontogeny through multiple rounds of cell division to construct a three-dimensional model of a growing anther. From these studies, they determined when and where during anther development cells switch from a somatic to germinal developmental program. Using this framework, the authors characterized male development in a couple of mutant backgrounds. The multiple archesporial cells1 (mac1) mutant displays altered cell patterning during anther development, misspecification of cell identity, and male sterility. Another male sterile mutant, male sterile converted anther1 (mcsa1) was found to be epistatic to mac1. MSCA1 encodes a glutaredoxin, which is proposed to function as a redox regulator. During anther development, the tassel is tightly wrapped in ensheathing leaves and the oxygen content in the tissue is hypoxic, with approximately 1.2-1.4% oxygen. By applying nitrogen or oxygen gasses through hoses connected to fine needles directly to the developing tassel, Kelliher and Walbot observed changes in anther cell identity and were able to partially rescue the mcsa1 mutant phenotype. They proposed that the oxygen status experienced by cells within the developing anther serves as a positional cue to specify germ cell fate. This research highlights how the environment (here the oxygen content in a tissue) can trigger cell specification and reprogram plant development. *David Braun, 2012*

Kirienko, DR et al. 2012. Plant Physiol 159:1309-1318. Reliable transient transformation of intact maize leaf cells for functional genomics and experimental study.

Editorial Comment: Determining the subcellular localization of maize proteins via stable transformation is a relatively slow process. To circumvent the time required, transient expression assays are often employed. These approaches have some drawbacks, including heterologous expression systems, such as onion epidermis or Agrobacterium infiltration into a dicot leaf, or the isolation of maize protoplasts. An improved method for determining the cellular location for maize proteins in intact maize cells has been developed. Kirienko et al optimized particle bombardment transformation into developing maize adult leaves and identified the basal blade region close to the ligule as having the highest rates for transformation. The authors tested a constitutive strong promoter and terminator driving a cDNA-fluorescent protein translational fusion, and several genomic DNA constructs, with their endogenous regulatory sequences, exons, introns, and UTRs, fused to fluorescent proteins. Both types of constructs worked well in the assay. Cotransformation experiments, either both constructs being simultaneously transiently expressed, or transiently expressing one protein in a tissue already stably expressing a second fluorescent fusion protein, were successful. During these studies, no instances of mislocalization of the transiently expressed proteins were observed. The improved method of Kirienko et al will greatly expedite functional genomic and cell biological studies of maize proteins. *David Braun, 2012*

Lariepe, A et al. 2012. Genetics 190:795-811. The genetic basis of heterosis: multiparental quantitative trait loci mapping reveals contrasted levels of apparent overdominance among traits of agronomical interest in maize (Zea mays L.).

Editorial Comment: One of the best tools developed to parse out the sources of genetic variance and to examine allelic effects in shared genetic backgrounds is the North Carolina Design III (NCIII) experiment. In this schema, members of a mapping population are backcrossed to both parents to create two derived but highly related mapping populations. Thus, homozygous and heterozygous allelic states can be compared in the two different genetic contexts of the two backcross populations. Lariepe et al (2012) have extended the NCIII design a step further, creating three recombinant inbred populations using parents from different heterotic groups. Instead of creating six derived populations from the three parental RI sets, nine populations were created using both backcrosses and a test cross to the unrelated parent. This expands on the power of the NCIII design to examine allelic effects in a third genetic context, which was used to address the bases for heterosis. The three prevailing theories for how heterosis is achieved are: dominance (that hybrids may have a larger number of effective genes than either parent); overdominance (that heterozygotes are superior to homozygotes at some loci); pseudo-overdominance (that genes of opposite effect are locked in repulsion to each other in low recombination regions of the genome). Epistatic interactions between loci are also thought by many to play large roles in heterosis. The augmented NCIII design allowed for powerful genetic dissection of several traits that are responsive to heterosis. Grain yield showed evidence of overdominance, while grain moisture showed evidence of additive effects (dominance) according to the QTL detected from data collected from multiple farm sites. Epistatic interactions were important for grain moisture, silking date and plant height but not grain yield. Pseudo-overdominance was also observed, as many QTL with opposite effects were located in centromeric regions. *Owen Hoekenga, 2012*

Lazarow, K et al. 2012. Genetics 191:747-756. A Hyperactive Transposase of the Maize Transposable Element Activator (Ac).

Li, X, et al. 2012. Genome Res. 22:2436-44. Genic and non-genic contributions to natural variation of quantitative traits in maize.

Editorial Comment: The genetic control of plant morphology has long been investigated through the study of mutants displaying abnormal phenotypes. But what sorts of genetic changes lead to the natural levels of phenotypic variation observed in maize? With more sequencing data being released on an almost daily basis, many recent studies have focused on using large SNP datasets to associate genotypes with phenotypes. These studies lead to more questions: what proportion of phenotypic variation is controlled by changes in SNPs, and how are these SNPs distributed throughout the genome, including genic vs non-genic regions and distribution among promotors, introns, and exons? This paper attempts to answer these questions in an unbiased way using a collection of SNPs from the maize HapMap project, as well as an RNA-seq experiment on dissected shoot apices in the NAM founders that were then projected onto the RILs. Three leaf architecture and two flowering time traits were measured in the NAM population. A two-stage process was then used to identify SNPs associated with the traits. First, a low-resolution QTL mapping was conducted to identify rough regions of the genome using ~1100 tagging SNPs. Then the full set of 1.01 million SNPs was utilized in the target regions to identify specific SNPs associated with the trait. This approach was used instead of a whole-genome high-resolution scan in order to minimize false positives. The merits of the individual use of each SNP set in mapping are also presented and discussed. Although trait-associated SNPs were enriched for non-genic regions, these regions were most frequently within 5kb upstream of genes, and SNPs from the RNA-seq dataset frequently identified the genes implicated by HapMap SNPs. In addition, 79% of the explained variation (which was 44-59% of the total phenotypic variation) came from SNPs in genes or within 5kb upstream. In addition to suggesting increased efficiency in genic- and promoter-targeted GWAS experiments in other complex genomes, these results indicate that promoter regions, and thus gene expression regulation, are playing major roles in natural phenotypic variation. *Addie Thompson, 2012*

Lin, Z et al. 2012. Nature Genetics 44:720-724. Parallel domestication of the Shattering1 genes in cereals.

Editorial Comment: This paper utilizes a variety of mapping techniques to identify the genes responsible for the evolution of the non-shattering seed phenotype in cereals. The gene, named Shattering1 (Sh1), was identified via mapping in sorghum as a YABBY transcription factor. Domesticated sorghum showed three haplotypes that each harbored a mutation that either lowered the expression or truncated the transcript of the gene, suggesting three separate origins of the non-shattering trait. Rice and maize orthologs of sorghum Sh1 were found to be located under shattering QTLs. An insertion in the rice ortholog caused decreased expression as well as a non-shattering phenotype. A whole-genome linkage analysis in maize showed strong narrow peaks over the two maize orthologs, which also contained insertions, structural changes, and frameshift

mutations in domesticated maize inbreds relative to teosinte. This paper tells a tidy story of how different approaches to mapping can be combined with syntenic orthologous gene data across species to help link phenotype to genotype for an evolutionarily important trait. *Addie Thompson, 2012*

Liu, S et al. 2012. PLoS One. 7:e36406. Gene Mapping via Bulked Segregant RNA-Seq (BSR-Seq).

Editorial Comment: Bulk Segregant Analysis (BSA) is a method commonly utilized to map the genomic location of a mutant gene. RNA-Seq is a frequently employed approach to analyze gene expression between treatments or genotypes. Liu et al combined the two methods and developed Bulk Segregant RNA-Seq (BSR-Seq) to simultaneously map the genomic position of a mutant to a chromosomal locus and to analyze transcriptional changes between the mutant and wild-type populations. As a proof-of-concept, they mapped and cloned glossy3 (gl3), a gene controlling epicuticular waxes on juvenile leaves in maize. From a polymorphic segregating F2 population, RNA was independently isolated from a pool of 30 gl3 mutants and a pool of 30 wild-type siblings and analyzed by RNA-Seq. Bioinformatic analyses identified single nucleotide polymorphisms (SNPs) that distinguish alleles of expressed genes. These were mapped back to the B73 reference genome and used to look for enrichment of SNPs that clustered with the gl3 pool, narrowing the gl3 interval to ~2 Mb. By analyzing genes mapped in the interval whose expression differed between mutant and wild-type pools, a candidate gene was identified. Analysis of independently derived Mutator insertion alleles demonstrated that the correct gene was cloned. In addition to mapping and cloning a gene, the method has the added benefit of providing global expression data on changes resulting from the mutation. However, in order to clone the gene from the expression data, the causative mutation has to result in differences in transcript levels and must be expressed in the tissues collected for RNA isolation. Nonetheless, BSR-Seq will be a facile new approach to quickly map mutants in maize. *David Braun, 2012*

Lung'aho, MG, et al. 2011. PLoS One. 6:e20429. Genetic and physiological analysis of iron biofortification in maize kernels.

Editorial Comment: Some have joked that the three most important breeding targets of the last several decades have been yield, yield, and yield. The success of the hybrid seed industry and the gains made by the Green Revolution both speak to the importance of yield in a breeding program. However, the fraction of the world's population that do not have their nutritional needs met by cereal staple-centered diets suggest that a Second Green Revolution must add nutritional quality to the target list. Iron should be on that list as ~2 billion people around the world have iron deficiency or anemia, largely due to inadequate diets brought on by poverty. Iron nutritional quality is a combination of iron concentration and bioavailability, the fraction that is easily absorbed and utilized by the consumer. Unfortunately, the fraction of bioavailable iron in cereals is small (<10%) and the genetic and chemical bases for this trait are just beginning to be understood. The authors used a human cell culture based bioassay to phenotype iron bio-

availability in the IBM RI set per se, without preconceptions as to the potential causes. Iron bioavailability was not well correlated with either iron or phytic acid concentration, suggesting that novel factors were at work. More, larger QTL were detected for iron bioavailability than iron concentration, suggesting that while more difficult to phenotype that iron bioavailability may have a simpler genetic basis than iron concentration. NILs evaluated at five sites outside of the author's home site indicated efficacy beyond central New York. This report marks an important first step towards the biofortification of maize with iron. *Owen Hoekenga, 2012*

Morohashi, K, et al. 2012. Plant Cell. 24:2745-64. A genome-wide regulatory framework identifies maize pericarp color1 controlled genes.

Editorial Comment: Pigments offer powerful reporter systems for genetic research. Pigments, such as the phenylpropanoid-derived phlobaphenes, are highly visual such that small changes to regulatory or structural genes are obvious but often dispensable to the health of a plant. The Pericarp color1 (P1) locus regulates both phlobaphene pigments in the pericarp and structurally related insecticidal maysins in silks. Thus, the P1 regulon represents study systems of both basic and applied biological interest. Here, a systems biology approach was used to more completely describe the P1 regulon, combining next generation sequencing based approaches to examine gene expression and where transcriptional regulators such as P1 bind within chromatin. Taken together, these lines of evidence point to the genes that P1 regulates (or is at least physically associated with) using functional (P1-rr) and null (P1-ww) alleles in both pericarp and silk tissues. P1 had a much larger regulatory impact than anticipated by visual inspection of the isogenic mutant and wild-type stocks. More than 3300 genes were differentially regulated in pericarp between P1-rr and P1-ww sibling lines, with 16/18 representative genes confirmed by qRT-PCR. More than 2100 genes were differentially regulated in silks. These targets included the expected phenylpropanoid-related genes, but also included carbohydrate and lipid metabolic genes and other even less obviously connected pathways. Chromatin immunoprecipitation experiments identified many of the same genes identified by RNA-Seq; however, the number of genes in common to both analyses was similar to that fraction expected by chance. Perhaps more importantly, the canonical transcription factor-binding motif for P1 was highly enriched in both positively and negatively regulated loci, suggesting that the systems biology outcomes were consistent with our existing body of knowledge. This study demonstrates the clear potential of systems biology approaches, to integrate transcriptomic, biochemical and genetic lines of inquiry into a single comprehensive view of a question that has been under examination for more than 100 years. *Owen Hoekenga 2012*

Nelissen, H et al. 2012. Curr Microbiol 22:1183-7. A local maximum in gibberellin levels regulates maize leaf growth by spatial control of cell division.

Editorial Comment: Plant organ growth and size is regulated by a combination of cell division and cell expansion. In the maize leaf, cell division and cell expansion occur in physically distinct regions. Cell division occurs at the base of the leaf in the division zone, while cell expansion occurs in the expansion zone, located adjacent to the division zone. The transition between the division zone and expansion zone is a major determinate of leaf growth. While plant hormones are known to be important regulators of plant growth, the mechanistic details of how the transition from cell division to expansion is still unclear. In this paper, Nelissen et al find that gibberellin (GA) has a key role in determining the boundary between the division and expansion zones and thereby regulating leaf growth. GA levels are highest at the boundary between the division and expansion zones and altering active GA levels shifts the position of the transition. In the leaf, high levels of GA are required to promote cell division in the distal portion of the division zone. This is in contrast to other plant tissues where GA primiraily promotes cell expansion, indicating hormones can have tissue-specific functions. Not surprisingly, the spatial regulation of GA levels is under tight control, and both GA biosynthesis and GA catabolism play important roles in restricting high GA levels to the division zone/expansion zone border. *Beth Thompson, 2012*

Riedelsheimer, C, et al. 2012. Nature Genetics. 44:217-20. Genomic and metabolic prediction of complex heterotic traits in hybrid maize.

Editorial Comment: The term genetical genomics was coined a number of years ago to express the combination of traditional genetic analyses with systems biology approaches such as transcriptomic analysis. Common genetical genomics experiments examine quantitative trait loci that regulate patterns of gene expression or other similar hybrids of old and new. In a similar vein, genetical metabolomics integrates a combination of approaches to dissect the bases of traits of interest using small molecule profiling. Investigators used a tried-and-true approach of evaluating performance of a large test cross panel (285 diverse inbred Dent lines with two different testers), looking for correlates for yield components such as dry matter, plant height and starch content. What sets this work apart is the use of whole genome prediction (sometimes also called genomic selection), where all available genetic marker data are used to create a statistical model to explain and predict the trait(s) of interest. Here, marker information was obtained using a 56K SNP chip to genotype the study panel. Further, authors expanded beyond the simple biochemical traits of sugar, starch and lignin content to using gas chromatography/mass spectrometry to phenotype the metabolome in leaves 33 days after sowing. This snapshot of metabolism and plant performance was included in the whole genome prediction models to help explain plant performance at maturity. If a leaf metabolome from young plants could be useful to predict final dry weight or other yield components, this could represent a significant savings in time and effort and allow more rapid selection of improved germplasm within a breeding context. The authors found that for some traits, the leaf metabolome did nearly as good a job as the genome wide SNP data set for predicting outcomes such as female flowering time, dry matter concentration and starch content. SNPs did better at predicting at lignin content, dry matter yield and plant height. How-

ever, it is worth noting that only 130 metabolites were phenotyped (a 400-fold difference in scale) such that genetical metabolomics represents a powerful new approach for plant improvement. *Owen Hoekenga, 2012*

Riedelsheimer, C, et al. 2012. Proc Natl Acad Sci, USA. 109:8872-8877. Genome-wide association mapping of leaf metabolic profiles for dissecting complex traits in maize.

Editorial Comment: How do we sustain or even accelerate the pace of improvement that is so crucial for our bio-based economy? Genetics has carried us from pre-historical times (with plant domestication) into the historical (with plant improvement), and now leverages statistics and biology information in the present era (with biotechnology). As discussed previously, one of the new statistical approaches for plant improvement is genomic selection or whole genome prediction, which uses as many molecular genetic markers as available to enhance the accuracy of prediction models for agronomically important traits. However, genomic selection is a black box approach as no attempt is made to assign genetic markers to biological processes. Metabolomics can shine light into this black box, associating compounds and pathways of known or suspected biological significance with the genetic markers and agronomic traits. Adding genomics on top of metabolomics allows us to build both comprehensive explanations for complex traits, and also to create testable hypotheses to delve deeper into the mechanisms of plant growth and development. Here, a somewhat structured diversity panel was examined using metabolomic profiling. Previously, agronomic traits were correlated with particular metabolites (see April 2012 MEB). Now, due to the significant degree of linkage disequilibrium (r2 = 0.1 at 500 kb), metabolites were correlated with particular genomic regions. Twenty-six metabolites detected by mass spectrometry (including 17 unknowns) could be associated with SNPs at a particular significance threshold. As this diversity panel is genotyped at higher density or other varieties are included in this style of analysis, the number of associations between SNPs and metabolites will only go up and allow us to produce a far more complete understanding of the biochemical genetic bases of agronomically important traits. *Owen Hoekenga, 2012*

Schnable, J et al. 2012. Genome Biology and Evolution. 4:265-77. Genome-wide analysis of syntenic gene deletion in the grasses.

Editorial Comment: An ancient whole-genome duplication event occurred in the grasses prior to the divergence of the major lineages. Following this polyploidy, many duplicate gene copies were lost through fractionation. After identifying syntenic blocks, rates of duplicate gene loss can be compared. This has been done in maize, where a more recent WGD event has taken place since the all-grass duplication discussed here. Gene retention has been shown to be more frequent for protein complexes, transcription factors, and conserved non-coding regulatory elements. A bias in gene loss between duplicated regions is also present. Here, a method for identifying syntenic blocks and lost orthologs and assigning fractionation regions and bias is detailed by example of the ancient grass duplication. Analysis showed that ongoing fractionation in the grasses is still biased, and furthermore, that reciprocal duplicated gene loss does not seem to have caused the adaptive radiation of the grass lineages. Not only does this work provide a resource for scientists seeking ways to study ancient polyploidy and subsequent fractionation, but it provides a dataset of high confidence syntenic orthologs in other grasses which could be helpful to maize researchers. *Addie Thompson, 2012*

Sekhon, RS et al. 2012. PLoS Genetics. 8:e1002980. Maize Unstable factor for orange1 is required for maintaining silencing associated with paramutation at the pericarp color1 and booster1 loci.

Editorial Comment: Paramutation is the heritable expression change of one allele due to the epigenetic influence of another allele, leading to non-Mendelian inheritance of the affected traits. Though the epigenetic mechanisms of allelic diversity are just starting to emerge in recent research, several factors have been implicated in contributing to the presence and regulation of paramutation in maize, including upstream tandem repeats and RNA-mediated silencing mechanisms. By characterizing one paramutagenic and one non-paramutagenic epiallele of the pericarp color1 gene, Rajandeep et. al. demonstrate that asymmetric (CHH) and not symmetric (CG, CHG) methylation differ in an enhancer element of the gene. This supports the idea that RNA-mediated silencing pathways play a role in paramutation at p1. A dominant mutation at unstable factor for orange1 (ufo1) leads to reactivation of the silenced epialleles, as well as the loss of H3K9me2, a suppressive histone methylation that may be maintaining silencing. ufo1 is also shown to be required for silencing at another paramutable locus in maize, booster1. This paper serves to further implicate RNA-mediated silencing mechanisms in paramutation, as well as to characterize mutations and epialleles of genes involved in epigenetic silencing and paramutation. *Addie Thompson, 2012*

Shen, Y; et al. 2012. PLoS One. 7:e32237. Genome Expression Profile Analysis of the Immature Maize Embryo during Dedifferentiation.

Editorial Comment: Genetic transformation of maize is a challenging process requiring the production of embryonic callus. The efficiency of inducing embryonic calli from immature embryo-derived cells is strongly genotype-dependent and varies greatly across maize inbred lines. Shen et al. characterized the transcriptional changes that occur during three stages of dedifferentiation of maize immature embryos. 251, 324 and 313 differentially expressed genes were identified at stages I, II, and III of the maize inbred line 18-599R, respectively. Genes significantly changed during dedifferentiation were involved in amino acid and carbohydrate transport and metabolism, cell wall/membrane/envelope biogenesis and signal transduction mechanism. This study moves towards understanding the underlying mechanisms influencing embryo dedifferentiation with potential future implications in transformation efficiency in maize. *Liza Conrad, 2012*

Slewinski, TL et al. 2012. Plant Cell Physiol. 53:2030-2037.

Scarecrow plays a role in establishing Kranz anatomy in maize leaves.

Editorial Comment: The C4 photosynthetic pathway is characterized by the separation of CO_2 fixation and the Calvin cycle into two distinct cell types, the mesophyll (M) and bundle sheath (BS). The arrangement of BS and M cells in concentric rings around the veins is known as the Kranz anatomy. This study implicates the SCARECROW (SCR) transcription factor in the structural specification of the Kranz anatomy in maize leaves. Loss of SCR results in abnormal BS and veins in maize leaves. Previously, SCR has been shown to function in endodermal differentiation in roots and shoots. Evidence suggests conserved pathways may be involved in the differentiation of both endodermis in roots and BS cells in leaves. This includes the shared expression pattern of the PIN1 auxin transport gene in both the root endodermis and BS cell. The authors suggest that it is possible ZmSCR functions in developing leaf primordia to restrict the movement of SHORT-ROOT to the cells that will become BS. *Liza Conrad, 2012*

Slewinski, TL et al . 2012. Plant Physiol. 160:1540-50. Tie-dyed2 encodes a callose synthase that functions in vein development which affects symplastic trafficking within the phloem of maize leaves.

Editorial Comment: Mutant leaves of tie-dyed2 (tdy2) plants are variegated and exhibit distinct regions of either normal-appearing green tissue, or yellow tissue that hyperaccumulates starch and soluble sugars. To understand the basis for this carbohydrate partitioning defect, Slewinski et al. phenotypically characterized the phloem transport capacity of the tdy2 mutant and cloned the corresponding gene. Tdy2 encodes a callose synthase that is expressed in leaves, roots, stems, tassels, and ears. RNA in situ hybridization experiments determined that the gene is expressed in the procambium of the earliest initiated leaves, and throughout the developing veins in slightly older leaves. To characterize the impediment to sucrose transport in tdy2 mutant leaves, 14C-labeled sucrose was applied to tdy2 yellow leaf regions and wild-type leaves. These experiments showed that the mutant was defective in phloem export. However, application of 14C-sucrose to green regions of tdy2 leaves located distal to yellow tissue demonstrated that long-distance transport through the phloem was not impaired. Because Tdy2 is expressed during early vein development and the tdy2 mutant is disrupted in sucrose phloem export, transmission electron microscopy was used to investigate vein ultrastructure. These experiments revealed that tdy2 yellow leaf regions have incomplete vein maturation, but normal plasmodesmata ultrastructure and frequency. Furthermore, because the companion cells in tdy2 yellow leaf regions contain massive oil droplets, a storage form of carbon, these studies implicated a defect in cell-to-cell solute transport from the phloem companion cells to sieve elements. Hence, Tdy2 function is required for proper vein development and ultimately for symplastic trafficking into the phloem translocation stream. *David Braun, 2012*

Studer, A et al. 2011. Nature Genetics. 43:1160-1163. Identification of a functional transposon insertion in the maize domestication gene tb1.

Editorial Comment: Maize was domesticated from its wild relative teosinte. One of the key domestication traits between maize and teosinte was a change in plant architecture, which is controlled by the teosinte branched1 (tb1) locus. Previous work had shown that changes in expression levels of tb1 in maize correlate with alterations in branching. Additionally, the sequences controlling the expression changes were mapped to the upstream regulatory region of tb1. In the current paper, the authors map polymorphisms between maize and teosinte in the tb1 promoter and identify a Hopscotch retrotransposable element insertion into the maize sequence. Promoter:reporter expression assays in maize leaf protoplasts show that the Hopscotch element causes a two-fold increase in expression, which is similar to the differences in tb1 expression assayed between maize and teosinte. Interestingly, the authors determined that the Hopscotch insertion predated domestication, demonstrating that the tb1 allele selected by early maize farmers was already present in teosinte. Hence, this work is an elegant illustration of the power of transposons to cause variation and the evolution of genetic novelty. *David Braun, 2012*

Studer, AJ; Doebley, JF. 2012. Genetics 191:951-958. Evidence for a natural allelic series at the maize domestication locus teosinte branched1.

Editorial Comment: Teosinte branched 1 (tb1) played a major role in the domestication of teosinte to maize and controls multiple developmental processes important for both plant architecture and ear morphology. Here, Studer and Doebley ask if tb1 also contributes to morphological variation in teosinte. To this end, the authors introgressed nine teosinte alleles into the W22 inbred background and measured 4 morphological traits known to be controlled by tb1 (including both ear morphology and plant architecture traits). They found three classes of teosinte tb1 introgressions: class I resembles maize in both ear morphology and plant architecture traits, class II produces teosinte-like plant architecture traits, but maize-like ear traits, and class III produce both teosinte-like ear morphology and plant architecture traits. Interestingly, these phenotypic classes correspond to the taxonomic origin of the alleles. There was no correlation between phenotype and the length of the introgressed chromosome segment, suggesting that tb1 and not a linked locus is responsible for the morphological variation. Although the authors were unable to identify the causal polymorphisms in tb1, they provide compelling evidence a natural allelic series that controls complex morphological traits in maize. *Beth Thompson, 2012*

Swanson-Wagner, R, et al. 2012. Proc Natl Acad Sci, USA. 109:11878-11883. Reshaping of the maize transcriptome by domestication.

Editorial Comment: In this study, expression profiling was performed on 38 maize and 24 teosinte genotypes to investigate how the maize transcriptome has changed through domestication. In addition to identifying more than 600 regions corresponding to genes that were most likely targets of selection during domestication, they also found five cases where selection may be acting on

regulatory regions. Co-expression network analysis was used to reveal more than 1,100 genes with altered co-regulation during domestication regardless of whether their average expression changed for the individual genes. Furthermore, 45 genes were identified with altered expression in inbred maize relative to outcrossing teosinte. The majority of these genes have higher expression in maize and are enriched for involvement in chitin metabolism and defense response. This research goes beyond simply identifying genes selected during domestication and provides a more global picture of transcriptional changes due to: selection on regulatory regions, co-regulated gene networks, and changes in reproductive mechanism. Additionally, this in-depth transcriptome analysis adds more evidence to the impact that regulatory changes have on phenotypic diversity during evolution. *Liza Conrad, 2012*

Takacs, EM, et al. 2012. Plant Cell. 24:3219-34. Ontogeny of the Maize Shoot Apical Meristem.

Editorial Comment: Plants continuously produce organs throughout their life cycle due to the activity of the shoot apical meristem (SAM), which both initiates new lateral organs and maintains a population of stem cells. The SAM originates in the embryo although relatively little is known about the molecular events that precede and are required for its specification. Here, the authors use a combination of lasercapture microdissection and transcriptomic profiling both before and after SAM initiation (collecting both embryonic and seedling tissues) to identify genes that function during SAM initiation and are critical for SAM function. The authors were able to globally identify genes expressed at specific stages of SAM development, as well as in lateral organs. Interestingly, they found that genes required for lateral organ specification are expressed before genes that function instem cell maintenance, indicating that SAM organogenesis function is established before the stem cell maintenance function. This study also sheds light on the homology of specific organs including the scutellum and coleoptile, both of which appear to be leaf-like lateral organs based on gene expression patterns. In addition to providing key insights into the ontogeny of this critical structure, this study provides a wealth of molecular markers that can be used to dissect specific stages of SAM development and is a great resource for the community. *Beth Thompson, 2012*

van Heerwaarden, J et al. 2012. Proc Natl Acad Sci, USA 109:12420-12425. Historical genomics of North American maize.

Editorial Comment: Genetic improvement in maize has been a remarkable success, accounting for much of the roughly doubling of maize yields in the last 80 years. Heerwarden et al. analyzed genome-wide SNP variability from 400 maize accessions - including open pollinated landraces, early inbred lines, advanced public inbred lines, and elite commercial inbred lines- to investigate the genomic changes that have occurred as a result of this selective breeding. Breeding increasingly differentiated the heterotic groups, genetically distinct breeding pools that are crossed to make hybrid seed. However, overall allele frequencies have remained remarkably constant across breeding history. Some loci show strong shifts in frequency indicative of directional selection across time. However, the selection candidates did not have long, shared haplotypes, reduced haplotype diversity, or evidence of different ancestry relative to other regions of the genome. The authors conclude that directional selection has led to shifts in population structure, but dramatic genomic change has not accompanied the remarkable genetic improvements in crop productivity. *Lewis Lukens, 2012*

Wahl, R et al. 2010. PLoS Biol. 8:e1000303. A novel high-affinity sucrose transporter is required for virulence of the plant pathogen Ustilago maydis.

Editorial Comment: Corn smut, Ustilago maydis, is an important pathogen of maize. As a biotrophic fungus, U. maydis obtains carbohydrate resources from its host plant. To do so, U. maydis hyphae form contact zones with the plasma membrane of infected host cells. Uptake of extracellular sucrose from the cell wall (apoplast) allows growth of the fungus and results in tumor formation on the infected plant organ. Wahl et al. identify a protein, Srt1, from U. maydis distantly related to plant sucrose transporters (SUTs), which transport sucrose from the apoplast into the cytoplasm. The authors show that deletion of Srt1 reduces the virulence of the fungus. Additionally, they demonstrate that Srt1 localizes to the fungal plasma membrane, is induced by sucrose in developing tumor tissue, and has a higher affinity for sucrose than previously characterized plant SUTs. These data indicate that the fungus can outcompete the host for apoplastically localized sucrose. Furthermore, by using Srt1 to import sucrose directly, rather than cleaving it into hexoses via cell wall-bound invertases, the fungus avoids triggering the host hexose-induced defense responses. Hence, Srt1 functions to provide sucrose to the fungus, and it provides a mechanism to bypass production of signaling molecules that initiate plant defense responses. *David Braun, 2012*

Wang, H; Bennetzen, J. 2012. Proc Natl Acad Sci, USA 109:21004-9. Centromere retention and loss during the descent of maize from a tetraploid ancestor.

Editorial Comment: Comparative analysis of genetic and physical maps of the grasses has established that the modern maize genome derives from an ancient tetraploid ancestor. In the ~12 million years that have elapsed since this tetraploid event, large scale rearrangements have returned the genome to a diploid form, while events at the gene level have resulted in the loss of one copy of many duplicate genes, a process known as fractionation. The maize tetraploid event occurred soon after the split between the maize and sorghum lineages. Consequently, the availability of sequenced maize and sorghum genomes provides a wonderful opportunity to capture a genome in flux as it reorganizes following tetraploidy. Schnable et al. (Proc Natl Acad Sci, 2011) have exploited previously genomic resources to follow fractionation in maize, and to define the ancestry of the modern genome with respect to the two ancestral genomes of the tetraploid ancestor. Here, Wang and Bennetzen address a fundamental process required for a tetraploid to return to the diploid state, namely, reduction of chromosome number driven by reorganization of the centromeres. Making use of single-copy pericentromeric sequences conserved

between maize and sorghum, the authors provide evidence for whole chromosome insertion near, but not necessarily into, other centromeres, along with translocation and fusion events, and generate a scenario for the establishment of modern maize genome organization. While we now have a far better understanding of the end-point if this process, the authors acknowledge the potential mechanistic problems of passing through the intermediate steps: notably, stability of dicentric chromosomes would require rapid inactivation of one of the two centromeres. Furthermore, what might we expect of the many intermediate forms between tetraploidy and the modern diploid state? And, what are the forces that have driven this remarkable return to the ancestral karyotype? *Ruairidh Sawers, 2012*

Wang, P et al. 2012. Planta 237:481-95. Evolution of GOLDEN2-LIKE gene function in C3 and C4 plants.

Editorial Comment: C4 plants, such as maize and Sorghum, have dimorphic chloroplasts, bundle sheath (BS) and mesophyll (M) cells, while C3 plants have a single chloroplast type. The two paralogous GOLDEN2-like (GLK) genes in maize, ZmG2 and ZmGlk1, are expressed in a BS or M cell-specific manner. This study investigates the evolution and sub-functionalization of the GLK gene family. Phylogenetic analysis including 50 GLK genes revealed that the last common ancestor of flowering plants had a single GLK gene therefore gene duplications have occurred in a lineage specific manner. However, all C4 species have two GLK genes while C3 plants can have either one or two copies. Expression of the two GLK genes present in sorghum is compartmentalized similarly to maize although strong compartmentalization is not found in C4 eudicot Cleome gynandra that has morphologically similar BS and M cells. To determine whether the sub-functionalization present in maize and sorghum is a common feature of all Poales, GLK single and double mutants were generated in rice, a C3 plant. Phenotypic analysis demonstrated both GLK genes in rice regulate chloroplast development in both BS and M cells in a functioning redundantly manner. Taken together with lack of compartmentalization in C. gynandra, this suggests GLK compartmentalization may be restricted to only C4 species with dimorphic chloroplasts. The authors hypothesize that duplication of GLK genes allows for sub-functionalization leading to to cell-specific function of BS and M cells in C4 plants with dimorphic chloroplast. *Liza Conrad, 2012*

Waters, Amanda J., et al. 2011. Plant Cell. 23:4221-33. Parent-of-Origin Effects on Gene Expression and DNA Methylation in the Maize Endosperm.

Editorial Comment: Imprinting, or expression dependent upon the parent of origin, is an important mechanism in the regulation and development of the triploid plant endosperm. Here, Waters et al. investigated imprinting of endosperm and embryo tissues 14 days after pollination using RNA sequencing. By comparing allele dosage of expressed genes with parental sequence polymophisms, they identified 100 putatively-imprinted genes in the endosperm. About half of these imprinted genes were found to be preferentially expressed in the endosperm relative to other tis-

sues. Since DNA methylation and chromatin changes are thought to play a role in imprinting, methylation in the tissues was also investigated. Hypomethylation of the maternal allele in the endosperm was observed in all cases tested. Putative imprinted genes were compared to those previously identified in rice and Arabidopsis, identifying 10 imprinted genes conserved among species. *Addie Thompson, 2012*

Weise, SE; et al. 2012. Plant Biotechnology Journal. 10:545-54. Engineering starch accumulation by manipulation of phosphate metabolism of starch.

Editorial Comment: Surplus carbohydrates assimilated during daylight can be transiently stored as starch in leaves. As opposed to kernel starch, phosphorylation of leaf starch is critical for proper metabolism. Transitory starch is phosphorylated by a glucan, water dikinase (GWD); mutations in this gene result in a starch excess (SEX) phenotype in Arabidopsis leaves. Weise et al identified a maize homolog of GWD and used RNAi to knockdown the gene's expression with the goal of increasing starch content in leaves. Starch levels in the leaves of RNAi plants were increased approximately 20-40-fold over controls, and starch accumulated to 15-26% of the dry weight of the leaves in the RNAi plants. The starch over-accumulating plants grew normally and accumulated above-ground biomass similar to the controls. As found in wild-type plants, even though the transgenic plants hyperaccumulated starch, the excess starch accumulated only in the bundle sheath cells. Plants engineered to contain high levels of modified leaf starch may be valuable for the production of biofuels, for silage, and for industrial applications. *David Braun, 2012*

Whipple, C et al. 2011. Proc Natl Acad Sci, USA 108:E506-E512. grassy tillers1 promotes apical dominance in maize and responds to shade signals in the grasses.

Editorial Comment: One of the major domestication genes in maize, teosinte branched1 (tb1), plays a role in suppressing tiller development and lateral branching. Whipple et al. describe the cloning and characterization of grassy tillers1 (gt1), a class I homeodomain leucine zipper gene that significantly reduces tillering and lateral branching. Both gt1 and tb1 show evidence of selection in domestication, and map to tillering and branching quantitative trait loci. Expression of gt1 is dependent on tb1, and is regulated by light in sorghum and teosinte. These results indicate that gt1 acts in the shade avoidance response pathway to reduce tillering and lateral branching, contributing to domestication traits and plant architecture in modern maize. Regulation of shade avoidance and tiller growth have have both been implicated in the modification of important agronomic traits such as increased biomass and yield in other grasses, making characterization of genes in these pathways potentially useful to crop improvement. *Addie Thompson, 2012*

Wingen, LU et al. 2012. Proc Natl Acad Sci, USA 109:7115-20. Molecular genetic basis of pod corn (Tunicate maize).

Editorial Comment: Pod corn is a classical morphological mutant in maize, in which the glumes are enlarged and grow over

the kernels. Based on this phenotype, pod cord was suggested to be an ancestral form of maize and has long been of significant interest to the maize community. Even though recent efforts to map genes involved in maize domestication have ruled out maize ancestor hypothesis, pod corn remains an intriguing mutant because of its striking phenotype. Pod corn is the result of a dominant mutation at the Tunicate (Tu) locus, which early genetic experiments suggested is complex. Until now the molecular basis of Tu locus has remained elusive. Here, Wingen et al provide evidence that the Tu locus is caused by a rearrangement of the promoter region and duplication of the ZMM19 gene. Interestingly, one ZMM19 duplicate has been inactivated in partial Tu revertants, and ZMM19 expression levels correlate with severity of the Tu phenotype. ZMM19 encodes a STMADS11-like MADS-box transcription factor. In contrast to most MADS-box TFs that function in floral development, ST-MADS11-like genes function in vegetative development. The authors show that ZMM19, which is normally not expressed in the inflorescence, is present in the inflorescence of Tu mutants. Furthermore, Tu mutant glumes exhibit more leaf-like traits and overexpression of ZMM19 in Arabidopsis transforms sepals into more leaf-like organs, suggesting that ZMM19 promotes development of leaf features. In addition to uncovering the molecular basis of a classic mutant, analysis of the Tu locus also illustrates the importance of gene copy number in regulating gene expression and morphological traits. *Beth Thompson, 2012*

Zerjal, T et al. 2012. Theor Appl Genet. 124:1521-37. Maize genetic diversity and association mapping using transposable element insertion polymorphisms.

Editorial Comment: Transposable elements make up the majority of the maize genome, and active transposition can lead to changes in gene structure, number, and function. In this article, a small set of MITES were used as markers in 26 diverse inbreds, an association panel of 367 individuals, and a 322-member landrace panel. This allowed the researchers to investigate population structures, yielding results similar to those found using SSRs and RFLP markers. Genetic distance and geographic distance were found to be correlated, especially for landraces within 500km of each other. In addition, the association panel was utilized for association mapping using the MITE markers, where one rare tandem insertion was found to be linked to male flowering time. The insertion was found to be in a location not previously identified as associated with flowering time, close to a cytochrome P450 gene and in high LD with a mutation in the second exon. Several hypotheses for mechanisms of how the transposon itself might be interfering with gene expression are also discussed: the tandem insertions may form hairpin loops and restrict access to the gene, or the insertions themselves may contain siRNA sequences that serve as targets or producers of siRNA, altering gene expression. Studies like these provide insight into how transposable elements continue to alter the structure and regulation of the maize genome. *Addie Thompson, 2012*

Zhang, X et al. 2012. Plant Physiol 159:1453-1462. PUNC-TATE VASCULAR EXPRESSION1 (PVE1) is a novel maize gene required for leaf pattern formation that functions downstream of the ta-siARF pathway.

Editorial Comment: Trans-acting siRNAs (ta-siRNAs) are a plant specific class of small RNAs that result from the intersection of the miRNA and siRNA pathways. miRNA-directed cleavage of a the non-coding TAS3 RNA is carried out by a specialized RISC complex, which contains AGO7, and generates ta-siRNAs that negatively regulate ARF3a. rgd2 contains a mutation in AGO7 and has severe developmental defects, including leaf growth and patterning. To identify genes that function downstream of rgd2/ago7 in leaf development, the authors isolated shoot apical meristems from rgd2 and normal plants using laser capture micro dissection, and looked from genes that were up or down regulated in rgd2 compared to normal. The authors identified a novel gene, PUNCTATE VASCULAR EXPRESSION1 (PVE1) that is significantly down regulated in rgd2/ago7 SAMs. PVE1 RNA is is expressed in developing vascular bundles and analysis of PVE1 mutants indicate that PVE1 is required for vascular development as well as leaf patterning. PVE1 promotes ta-siARF function and negatively regulates AGO1, and the authors propose that PVE1 functions in a distinct pathway that intersects and interacts with the ta-siARF pathway. *Beth Thompson, 2012*

Zhang, Z et al. 2012. J Plant Physiol 169:797-806. Characterization and expression analysis of six MADS-box genes in maize (Zea mays L.).

Editorial Comment: MADS-box transcription factors are essential regulators of a wide range of developmental processes including meristem and floral organ identity in plants. In this study, six MADS-box genes belonging to the SEP1/AGL2-like clade where characterized. Quantitative RT-PCR revealed 5 of the genes are highly expressed in silks, ears, seeds 5 DAP and tassels implying these genes may function in flower and fruit development. Yeast one hybrid results indicated that all six proteins are capable of functioning as transcriptional activators. One gene, ZMM7-L was further characterized to be nuclear localized and displayed expression changes in response to various abiotic stresses such as NaCl, PEG and ABA. The authors speculate ZMM7-L may be a negative transcription factor responsive to abiotic stress in maize. *Liza Conrad, 2012*

2011 Editorial Board Selections

Allen, A et al. 2011. Plant Biotechnology Journal. 9:857-64 Transgenic maize plants expressing the Totivirus antifungal protein, KP4, are highly resistant to corn smut.

Editorial Comment: I decided to go with a more applied paper this time. Commercial transgenic maize lines on the market to date address input traits, making maize resistant to insects or herbicides, thereby increasing yield and ease of production. Input traits benefit the public by decreasing the cost of food production, but this benefit is often not readily apparent. This paper demonstrates a transgenic approach to controlling fungal infections which can cause a reduction in yield, so fungal resistance can be considered an input trait as well. However, some fungi produce dangerous tox-

ins so controlling these fungi would improve crop safety. Fungal resistance can therefore be considered an output trait as well, with a direct benefit to the public in the form of increased food safety. The approach taken by these authors exploits a viral gene called KP4 that kills the fungus Ustilago maydis or corn smut. Several transgenic lines were produced that express varying levels of the KP4 transcript and its corresponding protein. Resistance to Ustilago maydis infection correlated with the levels of KP4 transcript and protein in transgenic maize plants inoculated with the fungus. Further, leaf extracts from transgenic plants inhibited the growth of Ustilago maydis in culture. While Ustilago maydis is does not have as large an economic impact as some other fungi, this work is important because it establishes an additional method that can be used in addition to traditional methods such as breeding and improved management practices for control of fungal infections. *Paul Scott, 2011*

Amien, S, et al. 2010. PLoS Biol. 8:e1000388. Defensin-Like ZmES4 Mediates Pollen Tube Burst in Maize via Opening of the Potassium Channel KZM1.

Editorial Comment: Angiosperms deliver non-motile sperm cells via the male gametophyte or pollen. Following pollination, sperm cells travel down the growing pollen tube and are only released as the pollen tube comes in contact with the female gametophyte. The synergid cells of the female gametophyte are known to excrete a mobile signal important for the final stages of pollen tube guidance towards the female gametophyte. The authors of this paper show that synergids also produce a signal that is required to both stop pollen tube growth and rupture the tip to release the sperm. A subfamily of four short cysteine-rich defensin-like proteins (ZmES1-4) are expressed in in the embryo sac, particularly in the synergids. Knockdown of these genes by RNAi results in female sterility due to the inability of the pollen tube to release sperm after encountering the female gametophyte. Defensin and defensin-like proteins have diverse functions including anti-fungal or anti-microbial activity in other plants, and as venom components in animals. Unlike other plant defensins, ZmES1-4 has weak anti-microbial activity. However, ZmES4 is sufficient to induce pollen tube bursting by an interaction with KZM1, a potassium channel in the membrane of the pollen tube. The ability of ZmES4 to induce pollen tube rupture appears to be species specific. It remains to be determined if this pathway is conserved in other angiosperms. *Clinton Whipple, 2011*

Bousios, A, et al. 2012. Plant J 69:475-88. The turbulent life of Sirevirus retrotransposons and the evolution of the maize genome: more than ten thousand elements tell the story.

Editorial Comment: The maize genome has been described as a series of gene-rich islands floating in a sea of retrotransposons. This paper is about the sea. About three quarters of the maize genome is made up of LTR retrotransposons. In maize, copia is one of the largest families of LTR retrotransposons. The authors of this paper exploit a new method of identifying one type of copia elements, the sireviruses, to examine the relationship among copia family members in maize. Their approach reveals new relationships

and allows classification of many previously unclassified elements. These results clarify how this family of retrotranposons evolved and suggests that the sireviruses are the only copia family members to successfully proliferate in maize. Even dedicated island dwellers will appreciate this well-written and illustrated paper about the maize genomic sea. *Paul Scott, 2011*

Brown, NJ et al. 2011. Science 331:1436-1439. Independent and parallel recruitment of preexisting mechanisms underlying C4 photosynthesis.

Editorial Comment: Many plants have evolved mechanisms to cope with the tradeoffs between carbon dioxide fixation and water loss caused by gas exchange through stomata. One of the most effective mechanisms is C4 photosynthesis, in which carbon dioxide is captured in the mesophyll cells, then diffuses via plasmodesmata to adjacent bundle sheath cells where carbon dioxide is released near the primary carbon fixing enzyme of photosynthesis RuBisCO. A key to this carbon dioxide concentration mechanism is the differentiation of mesophyll and bundle sheath cell identities, which express distinct components of the C4 photosynthesis pathway. C4 photosynthesis has evolved independently from diverse angiosperms that employ C3 photosynthesis. In this paper, Brown et al. demonstrate that bundle sheath specific expression of one C4 pathway enzyme, NAD-dependent malic enzyme (NAD-ME), requires a specific 240 nucleotide sequence found in the 5' end of the transcript. Interestingly, this sequence is conserved in both C3 species (including Arabidopsis and rice) and closely related C4 species (cleome and maize). Furthermore, C4 species show bundle sheath specific expression of NAD-ME, even when the sequence comes from a C3 species so long as the conserved 240-nt region is present. Thus it appears that a conserved post-transcriptional regulatory mechanism has been independently recruited to direct bundle sheath specific expression in C4 species as diverse as maize (a monocot) and cleome (a eudicot). It remains unclear what mechanism is regulating bundle sheath specific expression of NAD-ME, but these results suggest that C3 species already have both enzymes and a conserved but latent regulatory mechanism that can potentially facilitate a shift to C4. *Clinton Whipple, 2011*

Chuck, G et al. 2011. Proc Natl Acad Sci, USA 108:17550-17555. Overexpression of the maize Corngrass1 microRNA prevents flowering, improves digestibility, and increases starch content of switchgrass.

Editorial Comment: Few topics are hotter than the search for alternative energy sources that are renewable, efficient and cost-effective. The myriad ways plant material could be used as a source of biofuel to replace or supplement petroleum-based fuels are the focus of several national research initiatives. Many sound ideas are being tested but, to-date, commercial success appears a distant promise. Chuck and co-authors report a significant advance in overcoming a barrier for one biofuel strategy — the production of ethanol from the biomass of perennial grasses. They used the maize Corngrass1 (Cg1) tandem microRNA gene to extend the juvenile phase of development in several plant species, including switchgrass (Panicum virgatum), favored as a potential bioenergy

crop. Overexpression of Cg1 affected several plant characteristics, including increased branching, prolonged juvenile growth and delayed flowering. Although driven by the maize ubiquitin promoter, Cg1 overexpression in switchgrass produced three phenotypic classes: severe, moderate and weak. The weak class had increased biomass, up to 250% increased starch content in stems and never flowered. Modification of all these traits allowed for an increased and more efficient production of glucose that would be used for fermentation. This paper illustrates the power of maize as a model system to test the hypothesis that modulating juvenile development could improve the biofuels properties of a feedstock species moving it a step closer to commercial viability. *Mike Muszynski, 2011*

Djamei, A. et al., 2011. Nature. 478:395-8. Metabolic priming by a secreted fungal effector.

Editorial Comment: Usitlago maydis is a well-known fungus known causing corn smut. Maize plants infected with U. maydis undergo dramatic developmental alterations induced by the fungal parasite resulting in large tumors. Infection begins when a fungal hyphae injects effector proteins into the plant cell that promote virulence by suppressing the natural defenses of the plant. This paper demonstrates that U. maydis employs a chorismate mutase as an effector protein. Chorismate is a metabolite produced by shikimic acid pathway that can act as a precursor in the production of essential aromatic amino acids (prephenate pathway), or alternatively is used in other pathways including the production of salicylic acid. Chorismate mutase takes chorismate down the prephenate pathway. Upon injection of the fungal chorismate mutase it is able to move, presumably via plasmodesmata, to neighboring cells and prime the host cellular metabolism to favor the prephenate pathway and as a result compromise the production of the defense hormone salicylic acid. The authors note that many plant parasites express chorismate mutase, suggesting that this may be a common mechanism to promote virulence. *Clinton Whipple, 2011*

Eichten, S, et al. 2011. Plant Physiol. 156:1679-1690. B73-Mo17 near isogenic lines (NILs) demonstrate dispersed structural variation in maize.

Editorial Comment: The Intermated B73-Mo17 (IBM) population is widely used population for QTL mapping and studies on the genomic architecture of maize. These authors developed a population of near-isogenic lines from the inbreds B73 and Mo17 to compliment the IBM population. One hundred lines were derived from using B73 as the recurrent parent, and 50 lines were derived from using Mo17 as the recurrent parent. Careful analysis of array-based genotype data revealed some interesting findings with respect to the genomic architecture of maize. First, many genomic regions that showed an elevated number of B73 introgressions also showed fewer Mo17 introgressions. Likewise, genomic regions that showed an elevated number of Mo17 introgressions also showed fewer B73 introgressions. This result, combined with segregation distortion data in the IBM population, indicates loci with bias towards either the Mo17 or B73 allele. Secondly, the amount of residual heterozygosity was greater than expected, particularly in

the centromeric regions of low recombination. This finding agrees with the observation in the NAM population of McMullen et al. (2009) and provides further evidence for the Hill-Robertson effect on heterosis. Finally, the authors determined if Mo17-specific amplifications mapped to the same location as the original B73 sequence, or if they mapped to unlinked regions. They discovered a preponderance of unlinked copy number variants. This characteristic of maize genomic variation likely stems from the ancient genome duplication event and subsequent fractionation of gene pairs, or transposon-mediated duplication. This study further characterizes the vast genomic variability of maize, which gives rise to our ability to improve maize through breeding and selection. *Aaron Lorenz, 2011*

Fornale, S et al. 2010. Plant J 64:633-644. ZmMYB31 directly represses maize lignin genes and redirects the phenylpropanoid metabolic flux..

Editorial Comment: The Phenylpropanoid pathway leads to important plant metabolites such as anthocyanins and flavonols. Recent interest in biofuels has caused tremendous interest in the phenylpropanoid product lignin. The brown midrib genes have been studied for many years because they alter lignin content and structure and can improve the digestibility of ruminant feed. Brown midrib genes characterized thus far encode enzymes in the phenylpropanoid pathway. In spite of years of study, little is known about genes that regulate the phenylpropanoid pathway. This paper demonstrates that a myb transcription factor binds to the promoter of a phenylpropanoid pathway gene and adds to a growing body of evidence that the R2R3 family of MYB genes is involved in regulating this pathway. *Paul Scott, 2011*

Gallavotti, A et al. 2011. Plant Cell 23:1756-1771. BARREN STALK FASTIGIATE1 Is an AT-Hook Protein Required for the Formation of Maize Ears.

Editorial Comment: This paper adds to the growing number of genes identified in maize that are critical for axillary meristem initiation. Interestingly, several of these genes are not known from Arabidopsis mutants, including Barren stalk1 (Ba1) and Barren stalk fastigiate1 (Baf1). baf1 mutants fail to produce ears in some genetic backgrounds, and when ears are produced in permissive backgrounds they are fused to the stalk suggesting that Baf1 is involved in both axillary meristem initiation and proper boundary formation. The authors show that Baf1 encodes a protein with an AT-hook DNA binding domain. The AT-hook family is present throughout the land plants, but is poorly characterized functionally. The BAF1 protein appears to be nuclear localized and can homodimerize, suggesting that it functions as a transcription factor. Baf1 is expressed in a narrow stripe of cells adaxial to initiating meristems, in a domain that is identical to Ba1. Interestingly, Baf1 expression in this domain requires Ba1 activity, suggesting that Ba1 and Baf1 act in a common pathway required for meristem initiation. However, baf1 mutants are much less severe that ba1 mutants indicating that other redundant factors are required to promote axillary meristem initiation downstream of Ba1. That orthologous mutants are not known in Arabidopsis demonstrates

the utility of maize genetics in spite of high levels of redundancy. It will be interesting to further link Baf1 and Ba1 function with what is known about auxin-mediated axilary meristem initiation. *Clinton Whipple, 2011*

Gao, Z et al. 2011. Cell Chromosome Res. 19:755-61. Inactivation of a centromere during the formation of a translocation in maize.

Editorial Comment: Decades ago a dicentric chromosome was formed due to a translocation between chromosomes 1 and 5. Subsequently, this translocation was stabilized by the spontaneous inactivation of one of the two centromeres. Gao et al. have demonstrated that the inactive centromere still retains the CentC satellite repeats and CRM centromere-specific retrotransposon sequences, but does not condition a functional centromere. The authors found that this inactivated centromere does not form a constriction on the chromosome, is not bound by CENP-C, and does not contain the histone H3 modification of phosphorylation on serine 10. This finding reiterates the point that the presence of CentC and CRM sequences are not sufficient to condition the formation of a functional centromere, even in the same nucleus where other centromeres with the same sequences are functional. Therefore, the specification of the kinetochore and incorporation of CENP-C to regions of chromosomes that contain CentC and CRM sequences involves a sequence-independent epigenetic mechanism that cannot be specified in trans by another centromere. *Keith Slotkin, 2011*

Ghareeb, H et al. 2011. Plant Physiol 156:2037-2052. Sporisorium reilianum Infection Changes Inflorescence and Branching Architectures of Maize.

Editorial Comment: Biotrophic fungal pathogens often alter developmental fate of their host plants in order to survive and reproduce. This is the case with Sporisorium reilianum, which causes head smut in corn and may be familiar to maize researchers who have seen the stunningly deformed tassels and ears on infected plants. To better understand the mechanisms by which this fungus alters development, the authors performed detailed morphological and transcriptional analyses on infected plants. They found fungal infection altered inflorescence development in specific ways leading to (1) a loss of apical dominance in axillary branches, (2) conversion of floral organs into leaf-like organs (phyllody) leading to complete reversion of the inflorescence to a vegetative state and (3) loss of meristem identity and determinacy, resulting in spikelets initiating inflorescence meristems. Transcript profiling of inflorescences early in the infection process, prior to obvious developmental alterations, identified expression changes in developmentally important transcription factors, hormone biosynthetic genes and genes responsive to increases in reactive oxygen species. Although this work is a first step to dissect the mechanisms that alter developmental fate of the host by this pathogen, since both the maize and S. reilianum genomes have been sequenced, both species offer a unique opportunity to study plant-fungal interactions at the genomic level. *Michael G. Muszynski, 2011*

He, S, et al. 2011. Biomaterials. 32:5471-5477. One-to-one quantum dot-labeled single long DNA probes.

Editorial Comment: In this manuscript PCR products were amplified while linked to inherently fluorescent quantum dots (QDs) in a one-to-one ratio. PCR with QDs was a technical challenge, which was overcome by attaching one PCR primer to the QD prior to amplification of a long (400-500bp) DNA strand. The QDs have brighter and more stable fluorescence compared to organic fluorophores, and these probes have great potential for quantitative measurement of nucleic acid in FISH, Southerns, Northerns, etc.. The authors demonstrated this utility by attaching a 480bp fragment of the maize fatty aldehyde dehydrogenase 1 (rf2eI) gene to the QDs and performing FISH on maize prometaphase, metaphase and interphase chromosomes. The QD probe detected two brightly fluorescing loci at the known location of rf2eI at each of these stages. *R. Keith Slotkin, 2011*

Humphries, JA, et al. 2011. Plant Cell. 23:2273-84. ROP GTPases act with the receptor-like protein PAN1 to polarize asymmetric cell division in maize.

Editorial Comment: Establishing cell polarity is critical in many aspects of plant development, including asymmetric cell division and polar tip growth in root hairs or pollen tubes. In maize and other grasses, the stomatal complex is formed by a series of asymmetric divisions that produce the guard cells and subsidiary cells that surround them. Previously, the maize leucine-rich repeat receptor-like kinase PANGLOSS1 (PAN1), was shown to play a key role in this process. This paper significantly adds to our understanding of the mechanism of PAN1-dependant polarization of maize subsidiary cells. The authors show that plant Rho GTPases (ROPs), which are known to mediate polarity in other developmental contexts, also regulate polarity of subsidiary cells downstream of PAN1. Interestingly, ROPs were shown to localize to the same domain as PAN1, and this localization was PAN1 dependent, suggesting that these ROPs polarize downstream of PAN1. In spite of this, rop mutants enhance the pan1 phenotype indicating that ROPs can still polarize subsidiary cells independently of PAN1. These findings raise interesting questions regarding the evolution of novel polarity establishing pathways. The asymetric division of subsidiary cells is a relatively recent innovation in the Poaceae. Was PAN1 involved in other polarity processes before subsidiary cells arose? If not, how did PAN1 integrate with ROPs to gain a novel polarity role? As more pieces of the subsidiary cell polarity pathway emerge, such questions can begin to be addressed. *Clinton Whipple, 2011*

Javelle, M, et al. 2011. Plant Physiol. 157:790-803. Genome-wide characterisation of the HD-ZIP IV transcription factor family in maize: preferential expression in the epidermis.

Editorial Comment: You have cloned your favorite mutant and find the gene is a member of a larger gene family. What's your next step? One option is to characterize the entire family. This paper describes the comprehensive characterization of a particular sub-family of transcription factor that contains a homeodomain and leucine zipper, the HD-ZIP IV family. This gene family has

been found in all land plants and several members have epidermal-related function and/or expression. Javelle and co-authors characterized the phylogeny, synteny, gene structure, expression and regulation of the 17 HD-ZIP IV genes from maize. As with most maize genes, about half of the HD-ZIP IV's are paralogs, duplicated after maize and sorghum last shared a common ancestor. Of the members where expression was detected, most are expressed in immature reproductive organs and many show a preference for expression in the epidermis. Interestingly, 13 of the 17 genes have two short conserved motifs in their 3' UTRs, indicating they may be regulated by small RNAs or secondary structure formed by these motifs. This paper exemplifies how to characterize a gene family in maize and lays the foundation for analysis of individual member function. *Mike Muszynski, 2011*

Koo, DH et al. 2011. Genome Res. 21:908-914. Distinct DNA methylation patterns associated with active and inactive centromeres of the maize B chromosome.

Editorial Comment: The maize supernumerary B chromosome provides an excellent opportunity to investigate changes in the epigenetic nature of the centromere, as this centromere is found in both functional (associated with the centromere specific histone CENH3) and non-functional states. However, determining the epigenetic status of the centromere core is not trivial, as microarray and deep sequencing approaches have difficulties assessing changes from sequences of highly repetitive DNA. In this paper, immunofluorescence assays are used to determine DNA methylation states on stretched pachytene chromosomes. The authors confirm previous reports that the centromere cores in maize, rice and Arabidopsis are typically hypomethylated and associated with CENH3, distinguishing this functional region from the neighboring pericentromeric regions. Using a translocation line with an inactivated B centromere, Koo et al have demonstrated that the inactivated centromere core in this line is associated with DNA hypermethylation. This result suggests that the epigenetic modification of DNA methylation is associated with centromere function and activity. However, it remains unknown if this DNA methylation effect is a cause or consequence of centromere inactivation and CENH3 disassociation. *Keith Slotkin, 2011*

Kump, KL et al. 2011. Nature Genetics 43:163-8. Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population.

Editorial Comment: NAM strikes again! Nested association mapping is a method for identifying genes that control traits. It has been used previously to dissect flowering time and in this publication it was applied to resistance to the maize pathogen southern leaf blight (SLB). The key to NAM is a set of nearly 5000 recombinant inbred lines derived from 25 diverse inbred lines crossed to B73 to form families of F2-derived lines. The authors scored these lines for resistance to SLB and identified 32 significant QTL. Thanks to the availability of high resolution genotypes for the NAM population, it was also possible to carry out genome wide association tests at 1.6 million polymorphisms. This analysis led to the identification of 245 significant polymorphisms. The authors

then combined the QTL and genome-wide association results by developing a model to explain the phenotypic variance. This model contained terms from both analyses. QTLs were replaced by SNPs where possible to achieve the highest possible resolution. The final model explained 74% of the variation for SLB resistance and contained 51 single nucleotide polymorphisms and three QTL. Many of the genes identified in this study have functions related to plant defense and/or map to positions of QTL identified in other studies. While this is a satisfying result, it is not surprising because most of the previous studies involved populations derived from B73 and some were subsets of NAM. The genes identified in this study are attractive targets for studies of disease resistance at the molecular level. It will be interesting to see how these results are translated into breeding strategies. *Paul Scott, 2011*

Lawit, S et al. 2010. Plant Cell Physiol. 51:1854-68. Maize DELLA proteins dwarf plant8 and dwarf plant9 as modulators of plant development.

Editorial Comment: Dominant dwarfing mutations within DELLA proteins have been responsible for increasing yields through alteration of the harvest index in many crops and thus ushering in the "green revolution." The dwarf plant8 (d8) gene is one such protein and the authors describe the isolation and functional characterization of the dwarf plant9 (d9) gene, the paralog of d8. Although only one dominant, dwarfing D9 allele is described (D9-1), this allele was confirmed as causing alterations to plant stature and flowering time by using transformation of maize and Arabidopsis with both functional and dominant-dwarfing versions of d8 and d9. Interestingly, the D9-1 allele differs from normal d9 by a number of missense mutations and a small indel mutation. The causative polymorphism was localized to the C-terminal E600K mutation through analysis of a comprehensive domain swapping study. Most dominant DELLA mutations affect the N-terminal DELLA or VHYNP domains but the E600K mutation is located in the GRAS domain. Dominant dwarfing mutations in the GRAS domain are rare and are thought function by preventing strong interactions with the F-box SLY1 protein required for degradation by the 26S proteasome. *Mike Muszynski, 2011*

Li, H et al. 2010. Proc Natl Acad Sci, USA. 107:22184-22189. Epigenetic reprogramming during vegetative phase change in maize.

Editorial Comment: The regulation of the timing of phase change, the shift from vegetative to adult growth, has been shown to require the opposing activities of two small RNAs, miR156 and miR172. In turn, each miRNA represses the expression of a subset of two types of plant-specific transcription factor, SPB and AP2-like, respectively. In this report, the authors connect vegetative phase change to another small RNA pathway and to epigenetic silencing of transposons. The authors show that phase change and silencing of the MuDR transposon are associated with reduced expression of leaf bladeless1 (lbl1) and an increase in the levels of the trans-acting small interfering RNA (tasiRNA) target arf3a. They demonstrate that there is a transient loss of lbl1 expression in transitional leaves which they suggest causes coordinated changes

in both MuDR silencing and the tasiRNA silencing pathway. The authors suggest this coordination offers plants an opportunity to monitor and silence the potentially dangerous proliferation of transposons as they prepares to enter their reproductive phase of growth. *Mike Muszynski, 2011*

Editorial Comment: The regulation of transposable elements (TEs) through the lifecycle of an individual is not static, but rather a dynamic process between the TE and host organism. In maize, Li et al have found that the levels of LBL1/SGS3 in leaves affect freshly silenced MuDR TEs during the transition from juvenile to reproductive growth. LBL1 regulation itself is a key component in the tasiRNA-induced phase change, and the affect of LBL1 regulation is manifested on the MuDR TE as reduced DNA methylation, reduced repressive histone modifications, and transient expression. In addition, in cg1 mutants that prolong developmental phase change, efficient TE silencing is also delayed. Together, this data demonstrates that there is crosstalk between the different small RNA pathways that regulate developmental timing and TE silencing. *Keith Slotkin, 2011*

Mayer, KFX; Stein, N. 2011. Plant Cell 23:1249-1263. Unlocking the Barley Genome by Chromosomal and Comparative Genomics.

Editorial Comment: In this paper the authors take a radical approach to the assembly and ordering of genetic elements in a plant genome. They utilize technically diverse data types to order contigs and sequences in Barley. Flow sorting of chromosomes, next generation sequencing, SNP mapping, classical cytogenetics, and leveraging of other complete genomes in the cereals are used to provide a first pass at he order of genes in Barley. The article was startling not only for the value the research provides for genetic mapping and genome-enabled biology in Barley, but also for the implication that a similar approach (or subset of approaches) coudl be taken to improve the annotations of maize and other cereal genomes. Indeed, the approach could be used to generate a set of testable hypothesis for genome organization in any group of organisms for which multiple co-linear genomes are available. As crop genomics moves forward, the sort of genetic and bioinformatic flexibility shown in this paper may well lead to comparatively improving genome assembly and contig ordering. *Brian Dilkes, 2011*

Meng, X et al. 2011. Plant Cell 23:942-960. The FT-Like ZCN8 Gene Functions as a Floral Activator and Is Involved in Photoperiod Sensitivity in Maize.

Editorial Comment: Plants flower in response to a combination of internal and external cues that regulate production of a mobile floral-promoting signal called florigen. Key experiments in tomato, rice and Arabidopsis in the last several years have produced convincing evidence that proteins with homology to phosphatidylethanolamine binding proteins (PEBPs) encoded by the FLOWERING LOCUS T (FT) gene in Arabidopsis and related genes in other species have florigenic activity. FT-like genes with floral-promoting activity have been identified in a growing number of plant species but, until recently, maize was not counted

among them. This is no longer the case. Meng and co-authors have identified one of the 16 maize FT-like genes, called Zea mays CENTRORADIALAS (ZCN), as possessing florigenic activity using a number of experimental criteria. Their systematic approach showed ZCN8 has all the characteristics expected for a florigenic gene. Moreover, their analysis of the floral transition and ZCN8 expression in day-length sensitive tropical lines compared to insensitive temperate lines indicates the diurnal expression of ZCN8 plays a role in how flowering is controlled in response to photoperiod. An illuminating study, indeed! *Mike Muszynski, 2011*

Myers, AM et al. 2011. Plant Cell 23:2331-2347. Maize opaque5 encodes monogalactosyldiacylglycerol synthase and specifically affects galactolipids necessary for amyloplast and chloroplast function.

Editorial Comment: Maize kernels are normally translucent, so if you put them on a light box, light comes through them. In opaque mutants, light is not transmitted through the kernels. Many mutants have an opaque phenotype, suggesting that there are a lot of ways to make opaque kernels. Several mutations with opaque phenotypes have been characterized molecularly and the majority of them are involved in some aspect of seed storage protein deposition. This paper caught my eye because it describes molecular characterization of an opaque mutant (o5) that encodes a monogalacotsyldacylglycerol (MGDG) synthase, an enzyme involved in lipid biosynthesis. The authors present a body of evidence supporting the hypothesis that plastid membrane lipid composition is perturbed in this mutant and these perturbations result in altered starch granules that may explain the opaque phenotype of the kernels. The story is complicated by developmental aberrations in several tissues, emphasizing the importance of membrane structure in development. An explanation of the results was not immediately apparent to me, however the discussion section was particularly satisfying because it draws the results together with previous results into a detailed and reasonable hypothesis that will undoubtedly be a valuable in future studies. Thus, another mechanism for generation of opaque kernels has been characterized, illustrating the molecular complexity of this visually simple trait. *Paul Scott, 2011*

Parentoni, SN et al. 2010. Maydica 55:1-15. Inheritance and breeding strategies for phosphorus efficiency in tropical maize (Zea mays L.).

Editorial Comment: As maize yields are pushed to the limit, resource utilization efficiency traits increase in importance because yield is increasingly likely to be limited by a specific resource. Water use efficiency and nitrogen use efficiency are hot topics these days. The manuscript I selected this month seeks to develop an understanding of the genetics controlling phosphorous use efficiency, a resource use efficiency trait that receives relatively little attention. One of the biggest challenges researchers face when studying a resource use efficiency trait is developing growing conditions in which the trait can be observed. In this study, low and high phosphorous sites were identified for the experiment and the high phosphate sites were fertilized with additional phosphate to obtain

contrasting growing conditions. It appears that these conditions were adequate because significant differences in grain yield and other agronomic traits were observed. The six inbred lines used were well suited to this study because they were selected from a phosphorus use efficiency breeding program to create set of inbreds with a range phosphorus use efficiencies. The authors carried out a generation means analysis a powerful design in which the inbreds, their F1s, F2s and Backcrosses are all compared. In addition to grain yield, grain phosphorous content, stover phosphorous content and anthesis silking interval were measured. Using these traits together with information about the soil phosphorous content, the authors derived additional traits related to phosphorous use efficiency. The resulting data are analyzed using standard quantitative genetics methods and the authors interpret their results in terms of specific recommendations for breeders interested in improving phosphorous efficiency utilization. On the whole, I found this paper to be a thorough treatment of difficult subject matter and an excellent example of how a well-designed experiment can lead to specific recommendations with real-world value. *Paul Scott, 2011*

Phillips, K et al. 2011. Plant Cell 23:550-566. vanishing tassel2 Encodes a Grass-Specific Tryptophan Aminotransferase Required for Vegetative and Reproductive Development in Maize.

Editorial Comment: The juggernaut continues in the molecular identification of maize auxin pathway genes by the Auxin EvoDevo project. The vt2 gene can now be added to the ever growing list of auxin biosynthesis or signaling genes identified that function in organogenesis in the maize shoot. Unlike Arabidopsis where many auxin pathway genes function redundantly, in maize, mutations in single genes have dramatic impacts on vegetative and reproductive development. vt2 mutants, similar to another auxin biosynthetic mutant sparse inflorescence1 (spi1), have reduced shoot growth and an almost completely barren inflorescence lacking most axillary meristems. The vt2 gene was shown to encode a co-ortholog of TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA1), involved in Trp-dependent auxin biosynthesis. Double mutant analysis with spi1 indicated that, unlike previously thought, spi1 and vt2 likely function together in the same auxin biosynthesis pathway. Cloning of vt2 adds another gene to the rapidly expanding maize auxin pathway and adds to our understanding of the evolution of how auxin shapes plant development in different species. *Mike Muszynski, 2011*

Pouvreau, B et al. 2011. Plant Physiol 156:674-686. Duplicate maize Wrinkled1 transcription factors activate target genes involved in seed oil biosynthesis.

Editorial Comment: Researchers often use model species to answer biological questions. One question that arises from this practice is: How relevant is information from a model species to other species? For example, the model species Arabidopsis thaliana is a dicotyledonous species of no agricultural importance, while maize is a monocotyledonous species that produces more than $20 billion worth of grain each year in the US. In this paper, information from Arabidopsis was used to design transgenic plants with increased oil content, a modification of potentially great commercial value. The Wrinkled1 transcription factor was initially characterized in Arabidopsis and shown to be involved in controlling oil deposition in seeds. This manuscript reveals an interesting comparison and contrast between oil biosynthesis in Arabidopsis and maize. In Maize, two wrinkled1 homologs exist and both of these genes functionally complement the Arabidopsis gene. Overexpression of one of these genes in maize has been shown to increase oil content of the seed (Shen et al., 2010 Plant Physiol 153:980-987). In this new work, transgenic maize plants overexpressing one of the wrinkled1 homologs from maize (ZmWri1a) transgenic plants are analyzed in more detail, revealing effects on metabolites and genes involved in oil biosynthesis. Intriguingly, many of the genes identified as having altered expression in the transgenic plants carry a DNA motif called the AW box in their promoter. This sequence has been shown to be the Wrinkled1 binding site in Arabidopsis. Thus it seems that Arabidopsis is an excellent model for oil biosynthesis in maize and insights can be readily transferred to achieve beneficial results. *Paul Scott, 2011*

Reyes, F, et al. 2011. Plant Cell. 23:769-784. Delivery of Prolamins to the Protein Storage Vacuole in Maize Aleurone Cells.

Editorial Comment: I think about grain tissues and proteins in these tissues a lot, and I was fairly comfortable with my understanding of the roles of these tissues and proteins until I read this paper. Starchy endosperm provides nutrition to the germinating seedling by accumulating starch and seed storage proteins such as zeins. On germination, the (usually) single cell layer on the outside of the endosperm called the aleurone makes hydrolytic enzymes that degrade these storage compounds to provide energy and metabolites to the germinating seedling. The authors of this paper demonstrate that like starchy endosperm, aleurone cells accumulate seed storage proteins (although at a lower level than starchy endosperm). What are these proteins doing in there? A reasonable explanation proposed by the authors is that they serve as a source of reduced nitrogen and carbon for the synthesis of hydrolytic enzymes by aleurone cells. The cell biology resulting in accumulation of seed storage proteins in aleurone cells is particularly interesting. In starchy endosperm, seed storage proteins accumulate in endplasmic reticulum-derived protein bodies, while in aleurone they accumulate protein storage vacuoles. An elegant set of micrographic experiments involving fluorescently-tagged proteins and antibody markers to subcellular marker proteins suggests that seed storage proteins arrive at aleurone protein storage vacuoles by a novel pathway. This pathway may help explain how the storage proteins of other cereals are deposited. *Paul Scott, 2011*

Roudier F. et al., 2011. EMBO J. 30:1928-38. Integrative epigenomic mapping defines four main chromatin states in Arabidopsis.

Editorial Comment: There are dizzying arrays of post-transcriptional histone modifications that can influence the regulation of a gene. These histone modifications often do not work independently, but rather occur in a limited number of specific combinations. Roudier et al produced epigenomic maps of eight histone

modifications in Arabidopsis, and combined these with analysis of three additional previously performed histone modifications and DNA methylation. From this analysis, four main plant chromatin types were elucidated. These four chromatin states are: Actively transcribing genes, developmental stage-specific polycomb-regulated genes, heterochromatin-associated epigenetically silenced transposable elements, and a category of genomic regions not associated with any specific chromatin mark. It will be interesting to determine if all maize chromatin can be categorized into one of these four chromatin states, or if the increased size and complexity of the maize genome will translate into additional major classes of chromatin states. *R. Keith Slotkin, 2011*

Sanchez G, JJ et al. 2011. Amer Jour Bot 98:1537-1548. Three new teosintes (Zea spp., Poaceae) from Mexico.

Editorial Comment: Maize was domesticated from the wild grass teosinte, which grows natively in Mexico. There is, however, significant diversity among the Mexican teosintes including annual, perennial, diploid and tetraploid taxa. Current taxanomy includes five teosinte species: Zea perennis (tetrapoloid perennial), Z. diploperennis (diploid perennial), Z. luxurians (diploid annual), Z. nicaruagensis (diploid annual), and Z. mays (diploid annual). Maize (Z. mays ssp. mays) was domesticated from the annual diploid teosinte species Z. mays. This paper adds to this diversity by describing three new populations of teosinte that are different enough from currently described species to be considered new species. The potentially novel species include a diploid annual from Oaxaca, a diploid perennial from Nayarit, and a tetraploid perennial from Michoacan. While the phylogenetic analyses were not quite large enough to fully resolve all the relationships among Zea species, this study further underscores the diversity that exists among teosintes and the need for more extensive analyses. As the authors point out, maize experienced a genetic bottleneck during domestication and many potentially useful alleles are likely harbored among the teosintes. Resolving the relationships among the teosintes will be key to preserving this important germplasm. *Clinton Whipple, 2011*

Schaeffer ML, et al. 2011. Database (Oxford). 2011:bar022. MaizeGDB: curation and outreach go hand-in-hand.

Editorial Comment: It seems particularly fitting to end my year on the MEB with a paper celebrating the 20th anniversary of the MaizeGDB. This article provides a brief historical overview of the MaizeGDB and how it has evolved into the essential community resource it is today. Although initially focused on maps, markers and literature, today the MaizeGDB primarily focuses on the integration of reference genome sequences and sequence-based expression datasets through user-friendly query interfaces and data displays. The centerpiece to this effort is the MaizeGDB Genome Browser which hosts tracks for genome annotation from genome projects (e.g., PlantGDB, maizesequence.org) and sequence-indexed tracks from individual community research projects. As a roadmap to ensure future success and significance, this article outlines several ways the maize research community can collaborate with MaizeGDB. The longevity of the cooperative nature of our community is illustrated by a picture in this article of two meetings at Allerton, IL, separated by a mere 48 years. *Mike Muszynski, 2011*

Schnable, J et al. 2011. Proc Natl Acad Sci, USA 108:4069-4074. Differentiation of the maize subgenomes by genome dominance and both ancient and ongoing gene loss.

Editorial Comment: The maize and sorghum genomes are both functionally diploid and contain ten chromosomes. However, the maize genome underwent a tetraploidy event sometime after the divergence of the maize and sorghum lineages. Many of the duplicate genes (homeologs) in maize have not been maintained. This gene loss combined with chromosomal rearrangements have created a dynamic maize genome that has winnowed the genes and chromosomes back to the ancestral chromosomal number. Interestingly, the gene loss did not occur equally among the subgenomes produced by the maize tetraploidy event. By comparison of syntenic regions of the maize and sorghum genomes, the authors of this paper show that the process of gene loss has been concentrated in one of the maize subgenomes. Furthermore, presence absence variation for genes in diverse maize and teosinte lines shows that polymorphism for gene loss appears to be more frequent in one genome suggesting that the process of gene loss in maize is ongoing. Finally, the authors show that among duplicate genes that have been maintained there are frequent expression differences among the subgenomes, with the same subgenome that frequently loses genes showing reduced expression levels. They suggest a mechanism whereby deletion rates are equal among both subgenomes, but purifying selection maintains genes from the dominant subgenome that exhibits higher expression. *Clinton Whipple, 2011*

Sekhon, RS et al. 2011. Plant J 66:553-562. Genome-wide atlas of transcription through maize development.

Editorial Comment: Who could resist browsing through an atlas of transcription through maize development? Certainly not I. These authors undertook the yeoman's task of obtaining transcript profiles from 60 different tissue/developmental stage combinations. A data set like this could be approached from many different directions and I was eager to see what direction these authors would choose. The mandatory "overview" information was all there - most of the predicted genes are expressed in at least one tissue and a bunch of them are tissue specific. The large number of tissues and developmental stages sampled allow observation of relationships in expression patterns among tissues and organs as well as gene expression changes that occur in the course of development. The authors focus on expression patterns in leaves and seeds and present detailed results for genes in the lignin biosynthetic pathway. Tissue- and organ- expression patterns of different paralogs of lignin biosynthetic gene yielded some surprising results. This paper reveals the tip of the iceberg in terms of information that can be extracted from this data set, which will soon be released to the community. The data will be a great resource, so get on your favorite database and start mining! *Paul Scott, 2011*

Setter, TL, et al. 2011. J Exp Bot. 62:701-716. Genetic associa-

tion mapping identifies single nucleotide polymorphisms in genes that affect abscisic acid levels in maize floral tissues during drought.

Editorial Comment: Expediting the development of crops with increased tolerance to limited water conditions is critical in the face of increased competition for water and climatic variability. Setter et al. (2011) took a candidate gene association mapping approach to identifying SNPs correlated with favorable response to drought. The association mapping panel used was diverse enough to allow resolution at the gene level. The authors evaluated 350 tropical and subtropical inbred lines for metabolite traits under well-watered (WW) and water-stressed (WS) conditions. Candidate genes were chosen based on their putative involvement in metabolic pathways and regulatory systems controlling reproductive development during drought. A negative correlation between abscisic acid (ABA) and ear and silk dry weight was found, in agreement with ABA's role in energy conservation in response to plant stress. One of the strongest SNP-trait associations was between a SNP within an aldehyde oxidase gene and ABA concentration in the silk under WS conditions. Other associations between SNPs within candidate genes and metabolite traits were identified. This study shows how dissecting response to drought, a highly complex trait, into component metabolite traits and associating those traits with marker variants can help identify genes with potential for improving drought tolerance. More studies such as this are needed to provide the knowledge needed to most effectively mine maize germplasm collections for rare alleles conferring enhanced drought response. *Aaron Lorenz, 2011*

Sharma, M, et al. 2011. Genetics. 188:69-79. Identification of the Pr1 Gene Product Completes the Anthocyanin Biosynthesis Pathway of Maize.

Editorial Comment: Several of the most important advances in genetics made use of maize kernels containing anthocyanin pigments. For example, studies of spotted kernels led to the discovery of transposons. When these mobile genetic elements jump into or out of a gene required for anthocyanin synthesis, the result is a sector of the kernel with different pigmentation than the rest of the kernel (i.e. a spot). Anthocyanins are a family of purple or red pigments that accumulate in the aleurone layer of maize kernels. The anthocyanin biosynthetic pathway has been studied extensively at the genetic and biochemical levels and is an excellent model for understanding gene regulation. Mutants are available for every step in the pathway, all of the metabolic Intermediates are known and the genes for every step in the pathway have been isolated and characterized, except one. This paper describes the isolation and characterization of the only uncharacterized gene in the anthocyanin biosynthetic pathway. The Pr1 gene encodes a flavonoid 3'-hydroxylase (F3'H) that catalyzes the conversion of red anthocyanins to purple ones, so kernels lacking Pr1 activity are red. The authors took advantage of the maize genome sequence to identify a putative F3'H gene and characterized several mutations in this gene to establish that it is responsible for Pr1 activity. Further, this gene complements an Arabidopsis mutant lacking F3'H activity and is regulated by genes known to regulate the anthocyanin

biosynthetic pathway. Taken together, these experiments provide convincing evidence that the gene characterized in this paper is Pr1 and constitutes the last step to be characterized at the molecular level of this pathway. *Paul Scott, 2011*

Singh, M, et al. 2011. Plant Cell. 23:443-458. Production of Viable Gametes without Meiosis in Maize Deficient for an AR-GONAUTE Protein.

Editorial Comment: Apomixis is a form of asexual reproduction where meiosis is avoided and an embryo develops without fertilization. To study this process, which could significantly improve current crop breeding practices, Singh et al performed a clever forward genetic screen where only mutant plants that dominantly bypass meiosis produced seeds. Putative mutants were tested by flow cytometry and Dnr4 was identified as a single locus responsible for promoting entry into meiosis. Dnr4 encodes the AGO104 ARGONAUTE protein, which is likely similar to the Arabidopsis AGO9 protein, although there are important differences in the interpretations of their phenotypes as well as their regulation (Olmedo-Monfil et al., 2010 Nature v.464). Dnr4/ago104 mutant plants have reduced CHH and CHG DNA methylation of centromeric repeats, increased expression of these repeats and transposable elements, and a loss of centromeric condensation before entry into meiosis. Together, these data demonstrate that ARGONAUTE proteins, and presumably small RNA pathways, are responsible for regulating chromosome condensation and progression into meiosis. This paper is the latest in a series that demonstrates that in plants, epigenetic regulation, chromatin condensation and small RNA pathways are responsible for the entry into sexual reproduction (Nonomura et al., 2007, Plant Cell v.19)(Olmedo-Monfil et al., 2010 Nature v.464)(Garcia-Aguilar et al., 2010, Plant Cell v.22). *Keith Slotkin, 2011*

Steinhoff, Jana, et al. 2011. Crop Sci. 51:November-December. Multiple-line cross quantitative trait locus mapping in European elite maize.

Editorial Comment: QTL effect-by-genetic background interaction can hinder marker-assisted breeding because marker effects are not consistent across breeding populations and therefore need to be estimated for each breeding population separately. The authors find QTL-by-genetic background interactions for grain yield and grain moisture within a series of connected European bi-parental breeding populations. They found that the most powerful way to detect QTL was to model the marker effect as a nested effect within breeding population, effectively allowing each allele to have a different effect in each population. A model simply ignoring breeding population was inferior for detecting QTL. Both models were better than performing a simple QTL analysis within each bi-parental population separately. Combining data across populations improved power as well as resolution. This study displays the benefits of multiple-population (or "multiple-line cross" as in the title) QTL analysis relative to simple bi-parental QTL analysis. Also, this paper highlights the fact that advanced marker-based selection methods such as genomic selection may benefit from capturing allele-by-genetic background information. *Aaron Lorenz, 2011*

Tabuchi, A et al. 2011. Plant J 68:546-559. Matrix solubilization and cell wall weakening by β-expansin (group-1 allergen) from maize pollen.

Editorial Comment: The start of another pollinating season is upon us in the Midwestern USA and in honor of this occasion I selected a paper relevant to pollination. Maize cells are surrounded by a rigid cell wall, yet when a pollen grain lands on a silk a pollen tube grows between the cells of the silk to deliver the pollen nuclei to the egg. How does the pollen tube work its way between the rigid cell walls of the silk? The hypothesis tested in this paper is that a family of proteins in pollen called beta-expansins modifies the cell walls of the silk so they can be deformed by the pollen tube as it travels down the silk. Expansins are known to function in cell growth by altering the cell wall to allow cells to expand. Beta-expansins are expressed at high levels in pollen, making this hypothesis a reasonable one. The authors carried out an extensive set of biochemical experiments in which they treated silk cell wall preparations with beta expansin purified from maize pollen. Characterization of the resulting products allows the authors to conclude beta expansins alter cell wall structure. Moreover, grass cell walls seem to be altered in a specific way. Several physical properties of cell walls treated with beta expansins were examined as well with results consistent with a role for beta expansins in creating flexibility in the cell wall. Intriguingly, the mechanism of beta-expansin- induced modification does not appear to be enzymatic. This seems reasonable, since some aspects of cell wall formation (polymerization of lignin for example) proceed by non-enzymatic mechanisms. The biochemical details of the effect of beta-expansin treatment of cell walls provide important insights into the mechanism of pollen tube growth in maize silks. *Paul Scott, 2011*

Tenaillon, M et al. 2011. Genome Biology. 3:219-229. Genome Size and Transposable Element Content as Determined by High-Throughput Sequencing in Maize and Zea luxurians.

Editorial Comment: Transposable elements (TEs) can rapidly increase in copy number, generating genome size differences between individuals of the same species, prime examples of which are cotton and rice. Zea luxurians and maize separated ~140,000 years ago, prior to maize domestication. The Z. luxurians genome is ~1.5 fold larger than the maize B73 genome. To determine if this recent evolutionary size difference is due to TE activity, Tenaillon et al used paired-end Illumina sequencing to survey the composition of the maize B73 and Z. luxurians genome. They found that both genomes have roughly ~85% TEs, and the types of different TEs and their relative distribution in the genome are highly conserved. TEs account for 70% of the size difference between the two genomes, with the other 30% still unaccounted for. The similarity in TE number and distribution is surprising, as TEs are the most dynamic part of the genome and it was expected that one or several TE families would show rapid amplification responsible for the genome size polymorphism. This study demonstrates that short-read deep sequencing can be a powerful tool in accessing the genic and TE composition of a genome. This powerful approach can be used to explore the genome size and TE content changes upon domestication and inbreeding in the maize lineage. *Keith Slotkin, 2011*

Tian, F et al. 2011. Nature Genetics 43:159-162. Genome-wide association study of leaf architecture in the maize nested association mapping population.

Editorial Comment: In another demonstration of genome-enabled biology in maize, the nested association mapping population (NAM) was used to examine leaf architecture. Genes controlling leaf angle, strongly associated with plant density tolerance, as well as leaf length and width were mapped by joint QTL analysis. This approach netted 30, 34 and 46 QTL for the three traits with very little overlap for QTL between traits as ascertained by position estimates and trait correlations. In an effort to further localize QTL and leverage the nested population, 1.6million SNPs were catalogued in the parents of the NAM and the genotypes of each RIL estimated based from the 1100 linkage markers used to construct the genetic maps. This approach leverages information from the parents and projects very high resolution genotype data onto the low recombination density RIL. This allowed the researchers to use the historical recombinations present in the founders and attempt association mapping of traits to particular SNP. The approach was remarkably powerful, identifying a large number of SNPs present within the QTL windows detected by joint mapping. More associations, and greater precision and power, may be possible with yet more markers. Still association analysis detected clusters of significant SNPs associated with QTL positions in more than one case suggesting that QTL detected by linkage analysis may have complex genetic bases. Demonstrating the added value of building and utilizing resources such as NAM in maize, the authors detected major associations with two genes known to affect leaf angle in classical maize genetics studies: liguleless1 and liguleless2. The detection of strong QTL at these loci segregating in cultivated maize, may allow the considerable information about their molecular nature and partners to be harnessed in future improvement. The supplementary data for this manuscript are not to be missed and provide a wealth of information linking SNPs to yield data, genome annotation, and demonstrating the sufficiency of an additive model to explain parental phenotypes when the genotypes at detected QTL are fit to the observed phenotypes. *Brian Dilkes, 2011*

van Heerwaarden, J, et al. 2010. Proc Natl Acad Sci, USA. 108:1088-1092. Genetic signals of origin, spread, and introgression in a large sample of maize landraces.

Editorial Comment: A clear consensus has emerged that maize (Zea mays ssp. mays) was domesticated a single time in Mexico from annual teosinte (Zea mays ssp. parviglumis). However, genetic and archeological evidence have been inconclusive about the region of domestication within Mexico. The most ancient remains of domesticated maize have been found in lowland Mexico, and this is also where current parviglumis populations are found. However, previous genetic analyses suggested that domesticated maize from the highlands is more closely related to parviglumis than is maize from the lowland varieties, pointing toward a highland origin for maize. This paper reexamines the genetic evidence for the region of maize domestication by analyzing a large SNP (single nucleotide polymorphism) dataset obtained from maize (Z. mays ssp. mays),

and two teosinte subspecies, Z. mays ssp. parviglumis and Z. mays ssp. mexicana. Their analysis indicated a large amount of introgression between highland maize and ssp. mexicana, which also occurs in the highlands. Thus it is possible that the apparent close relationship of highland maize to ssp. parviglumis may be an artifact of later introgression of teosinte alleles from ssp. mexicana. Indeed, when this introgression is controlled for, maize from the lowlands is the closest to ssp. parviglumis. Thus it appears that when introgression of ssp. mexicana is taken into account, the genetic evidence confirms archeological and biogeographical evidence that maize was domesticated in the lowlands. *Clinton Whipple, 2011*

Virlouvet, LC, et al. 2011. Plant Physiol. 157:917-936. The ZmASR1 protein influences branched-chain amino acid biosynthesis and maintains kernel yield in maize under water-limited conditions.

Editorial Comment: For those that experienced both record heat and drought during this summer pollinating season, you have first-hand knowledge of how critical heat and water stress can be on having a successful crop. Virlouvet and co-authors take a full bore approach to dissect a portion of the molecular mechanisms underlying how the maize plant responds to water deficits by characterizing both the Zea mays abscisic acid- (ABA), stress- and ripening-induced (ZmASR) gene family and how over expression of a key family member, ZmASR1 leads to increased biomass accumulation and grain yield under normal and water limited conditions. ZmASR1 was initially indentified as a candidate protein underlying a QTL controlling leaf senescence and anthesis-silking interval in a RIL population grown under a water deficit. Using the complete B73 reference genome, a total of 9 ZmASR genes were identified with several members responding to both water deficit and other stress treatments as measured by transcript and protein accumulation. Constitutive expression of ZmASR1 in transgenic maize affected several traits leading to drought tolerant yield gains. Comparative analysis of transcriptomic, proteomic and metabolomic profiling results from transgene plus and minus sib plants under normal and water-limiting conditions indicated a link between the biosynthesis of specific amino acids and other metabolites with growth rate. Such results are encouraging but merit further analysis of this transgene in commercially elite germplasm. Overall, this paper exemplifies a well balanced approach that leads to increased understanding of the basic biology of stress responses in maize and also an application of this new knowledge that may protect yield in a crop under water-stress. *Mike Muszynski, 2011*

Wang, X et al. 2011. Plant Cell. 23:27-37. Seventy Million Years of Concerted Evolution of a Homoeologous Chromosome Pair, in Parallel, in Major Poaceae Lineages.

Editorial Comment: Whole genome duplications have been correlated with diversification in several plant lineages including the grass family Poaceae. The Poaceae duplication is estimated to have occurred about 70 million years ago, before the divergence of the rice and sorghum/maize lineages. In the majority of cases, only one of the duplicate genes has persisted, but in about 17% of the cases a paralogous pair of genes has been maintained. These

paralogous genes accumulated independent mutations following the duplication, creating distinct gene lineages. This paper investigates an interesting duplicate region of the grass genome (Rice Chromosomes 11 and 12/Sorghum Chromosomes 5 and 8), in which duplicate pairs have diverged significantly less than would be expected. This similarity appears to be due gene conversion mediated by recombination among the homeologous chromosome pairs. Interestingly, the similarity is graded along the length of the chromosome with the least divergence on the distal arms, and becoming more divergent towards the pericentromeric region, creating "strata" in which the paralogs appear progressively younger as you approach the distal end. Homologous chromosomal regions were identified in maize, although the more recent whole genome duplication and subsequent genomic rearrangements in the maize lineage have obscured the synteny with the more obvious case in rice and sorghum. The authors discuss possible mechanisms by which this unusual duplicate region was created maintained. *Clint Whipple, 2011*

Welcker, C, et al. 2011. Annu Rev Plant Physiol Plant Mol Biol. 157:718-29. A common genetic determinism for sensitivities to soil water deficit and evaporative demand: meta-analysis of quantitative trait Loci and introgression lines of maize.

Editorial Comment: Reduced leaf expansion in response to water deficit has two components: response to evaporative demand, or relative humidity, and response to soil water deficit. Leaf expansion slows or ceases in response to either condition. The authors set out to build evidence to answer an important question: Are the mechanisms controlling response to soil water deficit and response to evaporative demand common, or do completely separate mechanisms exist? The authors used a QTL mapping approach and looked for overlapping QTLs detected under each condition. To achieve separation of conditions, highly controlled greenhouse experiments were conducted and leaf growth was monitored every 15 minutes with a specialized greenhouse phenotyping platform. Three mapping populations were used along with several sets of introgression lines. First of all, the authors found an abundance of variation in response to these conditions. For some RILs, growth ceased under water deficit values three-fold greater than for other RILs. Many QTL (50% of QTLs) were common to both types of sensitivities, suggestion overlap in mechanisms controlling leaf growth in response to both types of water deficit. *Aaron J. Lorenz, 2011*

Wisser, R, et al. 2011. Proc Natl Acad Sci, USA. 108:7339-7344. Multivariate analysis of maize disease resistances suggests a pleiotropic genetic basis and implicates a GST gene..

Editorial Comment: There is very good evidence that alleles conditioning resistance to multiple plant pathogens exist and can be an important, stable source of disease resistance. Wisser et al. (2011) used a maize diversity panel and mixed models approach to show that a substantial proportion of the genetic variation for resistance to three maize fungal diseases is generated by multiple disease resistance alleles. After correcting for days to anthesis and populations structure, the genetic correlation between these three

diseases was still greater than 0.50 for all three disease pairs. Linkage disequilibrium decays within 1500 bp for most genic regions in this diversity panel, which is shorter than the average length of a maize gene. For this reason, the authors inferred that the relationship between these different disease resistances is caused by pleiotropy rather than linkage. An association analysis was performed using only 858 SNPs along with a multi-variate model in order to detect SNPs associated with multiple disease resistance. Surprisingly, three associations were made that exceeded the significance threshold adjusted for multiple testing. The strongest of these associations occurred with a SNP located within a glutathione S-transferase gene family member. These genes have been previously implicated in general disease and stress resistance. Re-sequencing of this gene for 139 to 185 panel members confirmed this association. The allele substitution effect of the mostly strongly associated SNP was quite small, only being ~6% of the range of the disease rating scale used. This paper provides more valuable knowledge in the area of stable disease resistance, which is desperately needed to maximize crop yields and stave off losses from increasing pathogen pressures. *Aaron Lorenz, 2011*

Xu, XM, et al. 2011. Science. 333:1141-1144. Chaperonins facilitate KNOTTED1 cell-to-cell trafficking and stem cell function.

Editorial Comment: The KNOTTED1 protein of maize is an important transcription factor for shoot apical meristem maintenance and acts non-cell autonomously, trafficking to neighboring cells. However, the KNOTTED1 protein is too large to diffuse through the plasmodesmata size exclusion limit, and therefore it was previously demonstrated that selective trafficking occurs to move particular proteins or other substrates of large size through plasmodesmata to neighboring cells. Xu et al. have made important progress in understanding selective trafficking of KNOTTED1 and other transcription factors through plasmodesmata. They engineered an Arabidopsis reporter system of plasmodesmata trafficking based on the non-cell autonomous rescue of a trichome mutant phenotype using fragments of the maize KNOTTED1 protein. Once established, they mutagenized this system and found that a chaperonin subunit, CCT8, is necessary for selective trafficking of KNOTTED1 and other transcription factors. Their data suggests that this protein is required to unfold the selectively trafficked protein upon arrival through the plasmodesmata in the recipient cell. This data provides an important step into understanding how selective trafficking occurs, and why some proteins are selectively trafficked while others are not. *R. Keith Slotkin, 2011*

Yandeau-Nelson, M et al. 2011. Plant Physiol 156:479-490. Starch Branching Enzyme IIa is required for proper diurnal cycling of starch in leaves of Zea mays.

Editorial Comment: The synthesis and degradation of starch in plant leaves is a dynamic process that follows a daily cycle. During the day (light phase) starch is synthesized from the sucrose produced by photosynthesis and during the night (dark phase) the starch is degraded and used for both metabolism and export to sink organs. In this paper, the authors investigated the role of one of the isoforms of the starch synthesis enzymes, starch branching enzyme IIa (sbe2a), on transitory starch accumulation in leaves. The transitory starch in maize leaves is a branched polymer of glucose units mainly composed of amylopectin. The synthesis of amylopectin requires the action of starch branching enzymes (SBEs), of which, maize has three: SBEIa, SBEIIa and SBEIIb that show differential accumulation in leaves and endosperm. In this work, SBEIIa was shown to be the primary SBE responsible for the production of transitory starch granules in leaves that can be efficiently degraded during the night. In sbe2a mutants, the starch polymer is improperly branched leading to the formation of irregular granules. The authors hypothesize that the abnormally shaped granules are not properly degraded leading to hyperaccumulation of starch in leaves. Either due to the increased accumulation of starch or metabolic changes associated with more starch, sbe2a mutants show premature senescence and many hallmarks of programmed cell death. Thus SBEIIa is required for proper starch granule structure allowing for efficient diurnal cycling of transitory starch in leaves. *Mike Muszynski, 2011*

Yi, G et al. 2011. Plant Physiol. 156:1826-1836. The thick aleurone1 Mutant Defines a Negative Regulation of Maize Aleurone Cell Fate That Functions Downstream of defective kernel1.

Editorial Comment: Development of the maize endosperm and single-cell layer aleurone offers a model system in which to study the network of cell specification signals controlling differentiation of these two tissues. Yi et al. add to this network by identifying the thick aleurone1 (thk1) mutation and characterizing its function in aleurone cell specification. Kernels mutant for thk1 produce additional layers of aleurone cells and are embryo lethal. This is in contrast to dek1 mutant kernels that lack any aleurone cells but also have aborted embryos. How does one study two mutations with contrasting phenotypes that cannot produce viable mutant plants? This manuscript showcases the richness of genetic tools available in maize that were used to study thk1 function and its interaction with dek1. Using B-A translocations for mapping, chromosome breaking Ds and Ac lines for sector analysis, epistatic interactions and double-mutant sector analysis, the authors were able to show thk1 defines a negative regulator that functions downstream of dek1 in aleurone specification. The thk1 mutation was caused by a deletion encompassing about 2 megabases and so its molecular nature has yet to be identified. *Michael G. Muszynski, 2011*

Zhang, GQ et al. 2011. Plant Physiol. 156:2155-2171. Cell Wall Modifications in Maize Pulvini in Response to Gravitational Stress.

Editorial Comment: Working in our summer nursery a couple of weeks ago, I noticed that the plants were a little dry, so I turned on the drip irrigation for the night and went home. I woke up in the early hours of the morning to a loud thunderstorm with a lot of wind. When I arrived at my field the next day, my worries were confirmed as most of the corn had been knocked flat during the night. I knew that the stalks will eventually recover, and I have

watched them gradually return to an upright position. I had not given much thought to how maize plants manage this fortunate recovery until reading this paper. Lodging is a source of significant yield losses, not just for maize but also other cereals. Zhang et al. investigate how differential growth of pulvini, the bulbous region at the nodes of the stalk, respond to gravity and return the plant to an upright posture. The pulvini of nodes 9-12 respond to gravity by differentially elongating their cells, with more elongation in the lower region of the pulvinis, which then pushes the stem back towards the sky. This paper takes a detailed look at changes in upper and lower pulvini regions in response to gravistimulation. This includes an analysis of cell wall composition, hormone content, as well as transcript and metabolite profiles. The authors found siginificant changes in cell wall components, particularly lignin, heteroxylan, xyloglucan and heteromannan. They also found significant changes in the levels of the hormones auxin, GA and ABA. These changes were corroborated by the transcriptome and metabolome profiles. The implication of auxin is perhaps not surprising since it is classically known to regulate differential growth in response to environmental stimulation. Interestingly maize mutants like baf1, fail to make analogous pulvini in the tassel, where they function to increase the angle of tassel branches. It would be interesting to investigate pulvinus response to gravistimulation in baf1 and other known maize auxin mutants. *Clint Whipple, 2011*

Zhao, Y et al. 2011. Theor Appl Genet 124:769-76. Accuracy of genomic selection in European maize elite breeding populations.

Editorial Comment: Genomic selection is a marker-based selection method that strives to maximize prediction accuracy for highly complex traits, such as grain yield. High marker densities available now at reasonable costs allow the development marker-based prediction models that are potentially useful across biparental breeding populations. Zhao et al. used real yield data gathered at 10 Italian locations to empirically evaluate genomic selection. They found that marker-based prediction accuracy for yield was approximately equivalent to the prediction accuracy of phenotypes consisting of means across three to four environments with one rep. The cost of genotyping is about the same as the cost of phenotyping at this level, but genotyping and genomic selection can be performed year round. The most interesting thing I found about this paper was that data from different, but highly related breeding populations, did not boost the prediction accuracy within populations despite a six-fold higher population size for effect estimation. This could be caused by 1) epistasis, 2) different QTL alleles segregating across populations, and 3) different QTL-marker linkage phases across populations. More work is needed to leverage data from across an entire breeding program to maximize the prediction accuracy within individual bi-parental breeding populations. *Aaron Lorenz, 2011*

Zhou, L et al. 2011. Cell Res. 21:1267-1270. Two transposable element insertions are causative mutations for the major domestication gene teosinte branched 1 in modern maize.

Editorial Comment: Roughly 10,000 years ago the plant that we know today as maize was being domesticated from wild teosinte (Zea mays ssp. Parviglumis). Previously, the work of John Doebley and others have identified just a few major QTLs responsible for the seemingly large morphological differences between maize and teosinte. One of the morphological differences is in the number of axillary branches that grow out, with maize having increased apical dominance compared to teosinte. The gene that controls this phenotype is teosinte branched 1 (tb1), a TCP family transcription factor that is not mutated in maize, but rather expressed at twice the rate compared to teosinte. The regulatory region responsible for the transcriptional differences in tb1 were previously mapped ~60-70kb upstream of the coding region. Zhou et al have investigated this region from hundreds of maize, teosinte, and association panel diversity lines and found a striking connection between apical dominance and DNA sequence. These authors identified two transposable element insertions that correlate with the transcriptional differences of the tb1 gene between maize and teosinte. These insertions are present in some teosinte lines, and were selected for due to their promotion of apical dominance in maize. This manuscript provides and powerful example of how genetic diversity produced by a transposable element-generated gain-of-function allele was utilized and selected for by the domesticators of modern maize. *R. Keith Slotkin, 2011*

This newsletter shares current research on genetics, cytogenetics, molecular biology, and genomics of maize. Information is shared by Cooperators with the understanding that it will not be used in publications without their specific consent. The maize gene reviews are an exception to this practice.

Send your notes for the Maize Genetics Cooperation Newsletter anytime. Your MNL Notes will go on the Web <u>verbatim</u>, promptly, and will be prepared over the months of June-August for printing in the annual issue. Be concise, not formal, but include specific data, tables, observations and methods. Notes that require extensive editing will be returned. Check MaizeGDB for the most current information on submission of notes. <u>Send your notes as attachments or as the text of an email</u> addressed to MaizeNewsletter@missouri.edu (we will acknowledge receipt, and will contact you further if necessary). Please follow the simple style used in this issue (city /institution title/ --authors; tab paragraphs; give citations <u>with authors' initials</u> --e.g., Maizer, BA et al., J Hered 35:35, 1995, or supply a bibliography). Figures should be supplied in final electronic form. To separate columns in tables, please <u>tab</u> instead of using spaces, to ensure quality tabulations on the web. Mailing address:

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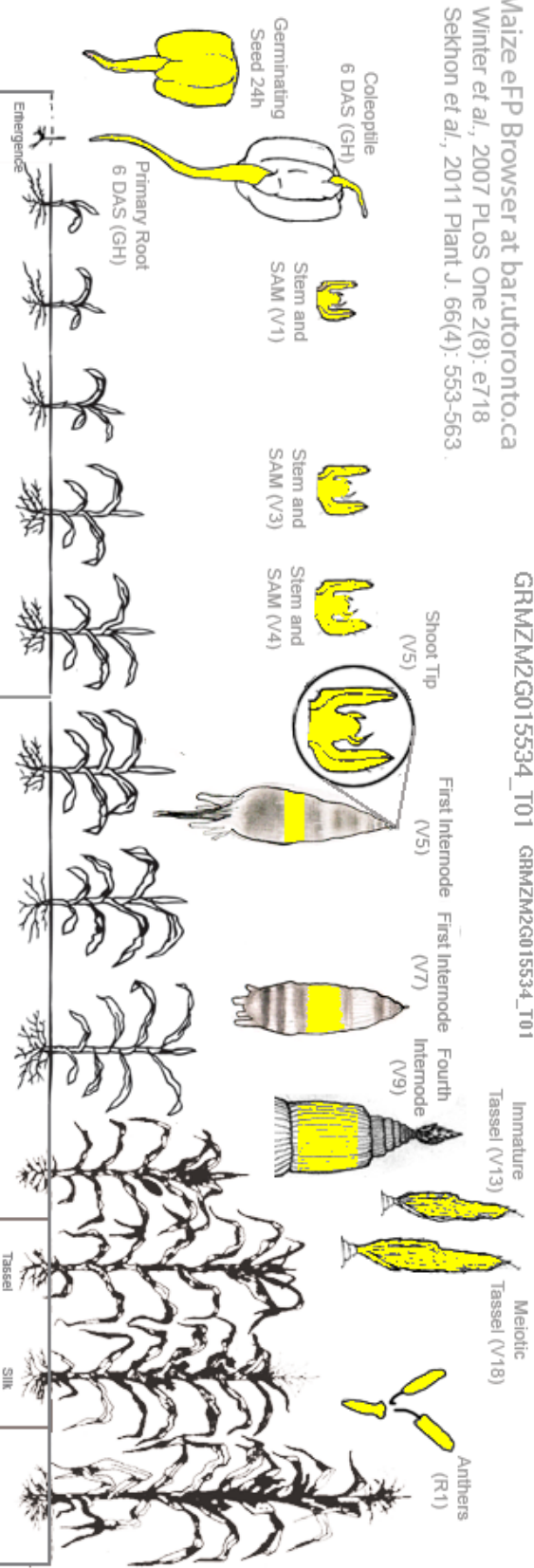|	
MNL 51ff. on line	MaizeGDB - http://www.maizegdb.org
Author and Name Indexes (and see **MaizeGDB**)	
Nos. 3 through 43	Appendix to MNL 44, 1970 (copies available)
Nos. 44 through 50	MNL 50:157
Nos. 51 to date	Annual in each issue
Symbol Indexes (and see **MaizeGDB**)	
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Rules of Nomenclature (1995)	MNL69:182; MNL82:84: and **MaizeGDB** (2006 update)
Cytogenetic Working Maps	MNL 52:129-145; 59:159; 60:149 and **MaizeGDB**
Gene List	MNL69:191; 70:99 and **MaizeGDB**
Clone List	MNL 65:106; 65:145; 69:232 and **MaizeGDB**
Working Linkage Maps	MNL 69:191; 70:118; 72:118; 77:137; 78:126; 79:116; 80:75; 82:87; 83: 103 (Map tutorial) and **MaizeGDB**
Plastid Genetic Map	MNL 69:268 and **MaizeGDB**
Mitochondrial Genetic Maps	MNL 70:133; 78:151 and **MaizeGDB**

Cooperators (that means you) need the Stock Center.
The Stock Center needs Cooperators (this means you) to:

(1) Send stocks of new factors you report in this Newsletter or in publications, and stocks of new combinations, to the collection.
(2) Inform the Stock Center on your experience with materials received from the collection.
(3) Acknowledge the source, and advice or help you received, when you publish.

MaizeGDB needs Cooperators (this means you) to:
(1) **Contact Carolyn Lawrence if you are preparing a grant that will generate large data-sets that you wish to be stored at MaizeGDB. Do this before submission to allow appropriate budgeting.**
(2) New genes? Send email to MaizeGDB [http://www.maizegdb.org/web_newgene.php] with details of **NEW GENES**.
(3) Look up "your favorite gene or expression" in **MaizeGDB** and send refinements and updates via the public **annotation** link at the top of all MaizeGDB pages.
(4) Link your papers to gene models and genes using the annotation tools on the new interface. http://alpha.maizegdb.org/ to replace main interface Fall 2013.
(4) Compile and provide mapping data in full. If not published, submit a note to this Newsletter, along with data for inclusion in **MaizeGDB**.
(5) **Contribute to the community genome annotation effort**. See **MaizeGDB** for updates.
(6) Contribute to the *MNL maize gene review* (www.maizegenereview.org). These data will be transferred to MaizeGDB with credit provided to contributors.
(7) Acknowledge the source, and advice or help you received, when you publish.

GRMZM2G015534_T01

GRMZM2G015534_T01

Germinating
Seed 24h

Coleoptile
6 DAS (GH)

Primary Root
6 DAS (GH)

Stem and
SAM (V1)

Stem and
SAM (V3)

Stem and
SAM (V4)

Shoot Tip
(V5)

First Internode
(V5)

First Internode
(V7)

Fourth
Internode
(V9)

Immature
Tassel (V13)

Meiotic
Tassel (V18)

Anthers
(R1)

Whole Seedling
(VE)

Primary Root
(VE)

Pooled
Leaves (V1)

Primary Root
(V1)

Topmost
Leaf (V3)

First
Leaf (V3)

Tip of
Stage 2
leaf (V5)

Base of
Stage 2
leaf (V5)

During the second
stage of leaf growth
the ligule and auricle
form, the blade grows
more rapidly than the
sheath, and differentiation
begins

Tip of
Stage 2
leaf (V7)

Base of
Stage 2
leaf (V7)

Immature
Leaf (V9)

Thirteenth
Leaf (V9)

Eleventh
Leaf (V9)

Eighth
Leaf (V9)

Thirteenth
Leaf (VT)

Immature
Cob (V18)

Immature Pre-pollination
Cob (R1)

Silks
(R1)

Innermost
Husk (R1)

Embryo

Innermost
Husk (R2)

Outer
Husk (R2)

Thirteenth
Leaf (R2)

Endosperm

Pericarp

Seed

Emergence		Establishment					Vegetative				Flowering			Silk		Yield Formation			Ripening		
		VE	V1	V2	V3	V4	V5	V6	V7	V9	V13	V18	VT	R0	R1	R2	R3	R4		R5	R6
	Approx. DAS	0											55	57	59	71	80	90		112

Anthesis: 57
Silking: 59
Blister: 71
Milk: 80
Dough: 90

Absolute
52156.53
46940.87
41725.22
36509.57
31293.91
26078.26
20862.61
15646.95
10431.3
5215.65
0.0
Masked

DAP = Days After Pollination
DAS = Days After Sowing

DAP 2 4 6 8 10 12 14 16 18 20 22 24
R1 R2 R3 R4