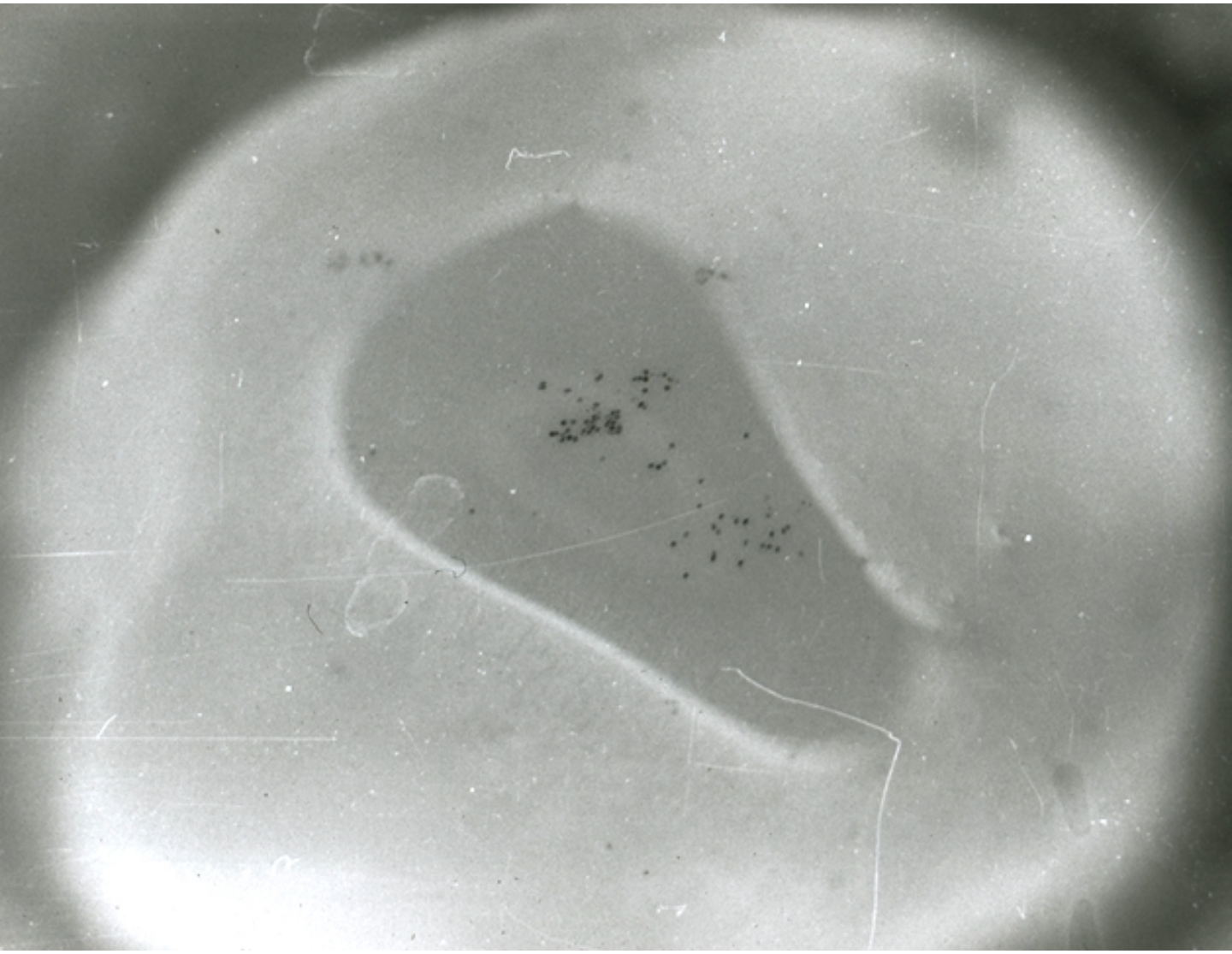
MAIZE GENETICS COOPERATION NEWSLETER

87

DECEMBER 7, 2014



Division of Biological Sciences and Division of Plant Sciences, University of Missouri, Columbia, MO

NOTE: The 57th Maize Meeting will be held at St Charles, IL March 12-15, 2015.
Check MaizeGDB for more details.

Front cover image: highly paramutant *R1*. See E Coe, this issue p 12. “More about curious mottling in highly paramutant R1 kernel”.
Back cover: *Ds-GFP*. See Y Li et al this issue p 20. “A sequence-indexed single gene knockout resource for maize”. Photo courtesy Dr. Yubin Li.

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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 publish studies of general scientific interest in 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 will be posted online without editing. Electronic submission is encouraged and is done by sending your contributions as attachments, or as text of an email, to MaizeNewsletter@missouri.edu. Submissions that would require extensive editing to be understandable will be returned.

This volume, 87, represents the final print copy of the Newsletter. Volume 88 and succeeding volumes will be accessible in electronic format only; there will be no printed and mailed copy. The closing date for a given volume will be Dec 31. From time to time, new submissions will be described under the "What's New" on the MaizeGDB homepage, and in the MaizeGDB social media (Facebook, Twitter). Please contact the editors: MaizeNewsletter@missouri.edu if you have any questions.

Highlights this year include a contribution from Gerry Neuffer entitled: "Chromosome Breaking Ds Sites in Maize Part II" (submitted as a maize gene review). Images and interpretations are provided, and most stocks are available at the Stock Center. A historical note submitted by Sherret S. Chase, about his interview of Dr Chin C. Li (population geneticist), regarding renowned corn breeder, Dr. C.H. Li (also known as Li Jing Xiong) is also included along with a photograph provided by Ming-Tang Chang.

The maize gene review section is moving to a community wiki, maintained at MaizeGDB and available to all cooperators. Wiki accounts are freely given for adding new gene information that may be linked to MaizeGDB with credits to authors. See this Newsletter for more details.

This year, as last, 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, and much of the redaction of the remaining copy. She has performed this task with precision, considerable patience and good humor, and much communication with authors. The maize community owes her much gratitude for her service.

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

ALLEGHENY, NEW YORK

Maize "super leafy"

— Karl, JR

A strain was created by placing the "Leafy" mutation into the tallest tropical maize of the subspecies, to show the fullest effect of the mutation in concert with the extreme short-night reactivity of the natural background (Karl, MNL 86:4, 2012). In the third back-cross generation (2012; now 93% tallest background), one of at least eight plants had 76 leaves (New York; a new subspecies maximum), 40 of which were above the ear tab (Fig. 1). The other plants were ~9.15 m tall, two of which had ear shoots (one of them permitted to silk) at 6.1 m/leaf 31 and eight leaves below the tassel. The 76-leaf plant had a normal, undeveloped tassel (22 cm) and was permitted to reach a height of 10.3 m. Forty leaves perchance represent the extreme expression. The 76-leaf plant and the other plants were cultivated in the ground of a tall greenhouse in New York (seasonal short night) with artificial short night of constant length (natural, decreasing night length until the summer solstice when the termini of the day (sunrise, sunset) were set at those seasonal extremes, plus a three-hour midnight light exposure). Plants were covered with two layers of (4 mil) opaque plastic sheeting to extend the night length.

In addition to the short-night plants, 14 more of that generation were cultivated in the field and given 68 consecutive 13-hour-long nights beginning when the fifth leaf tip began protruding from the whorl (crotch of previous two leaves). When the long-night treatment ended (the first short night being June 20, summer solstice), the plant height was 1.38 m, 23.5 leaf tips were protruded, 17 leaf collars were protruded on the (pure Chiapas 234) short-internode version of the strain, and 15.5 leaf collars were protruded on the (25% Montana race accession Ecuador 689) long-internode version. Later, five of the 14 plants exhibited leafy, having 12.5 leaves above the ear (20 below), except for one plant whose leaf quantity was greater — so much greater that the quantity could not be determined when the whorl was dissected at the time of comparison to the four normal leafy plants whose tassels were fully protruded (a *delayed flowering* N2461 x tallest tropical F2 mutant had 6-8 leaves above the ear and 18.5 below, 24-27 total; the wild type had 18.5 total).

The odd cases of extreme leafy happen less frequently than the *Leafy* mutation, but with a frequency more similar to that of short-night leafy (the etiology of short-night leafy has presumably been a difference in critical night length between the ear and tassel meristems such that a certain night length is registered by the lateral meristems as a long night, thus causing ear initiation; yet, that same night length is registered by the apical meristem to be a short night and thus permits the apical meristem to continue manufacturing leaves). However, both the *Leafy* mutation and short-night leafy evoke a moderate and ostensibly equal quantity of leaves above the ear (~10-20), unlike the extreme case ("super leafy"). Thus, super leafy may be the additive result of the *Leafy* mutation + short-night leafy (1/2 x 1/4 = 1/8 frequency; 6 + 17 + 17 = 40 leaves above ear). The *Leafy* mutation may delay tassel initiation enough so that the 68 13-hour nights are inadequate to prevent short-night leafy. Thus, there are two doses of leafy.

In the literature, there have been discrepancies over whether "indeterminate" is i) present in a wild-type form in some (e.g. racy tropical) varieties; ii) inactivated by greenhouse cover (filtration of UV wavelength); and iii) night-length dependent. Perhaps some events of the wild-type indeterminate have been short-night leafy, as the *indeterminate* mutation rarely indicates ear position.

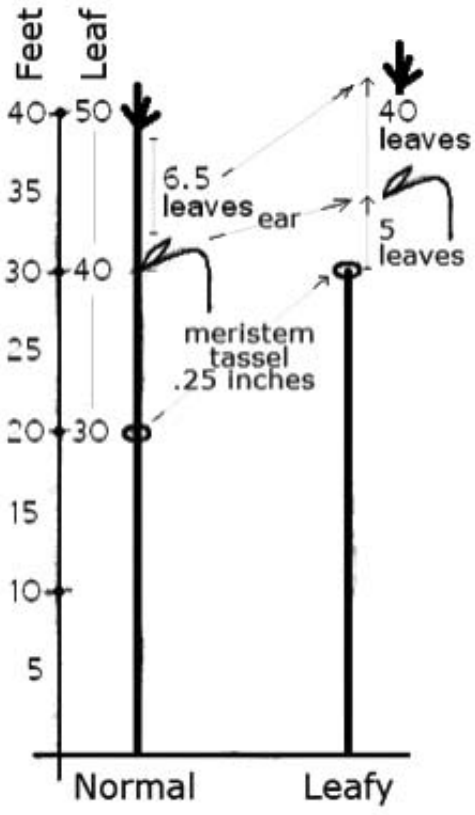

Figure 1. Organ location in the tallest maize of the subspecies Chiapas 234 + Leafy.

BERGAMO, ITALY

Consiglio per la Ricerca e la sperimentazione in Agricoltura
CRA-MAC, Unità di Ricerca per la Maiscoltura

Resistance to *Fusarium verticillioides* and total antioxidant capacity in Italian maize varieties

— Alfieri, M; Torri, A; Balconi, C; Lanzanova, C; Locatelli, S; Valoti, P; Redaelli, R

The market of maize-based foods has recently faced a quick expansion, mainly due to the need to prevent or reduce food al-

lergies like coeliac disease. In this context, it is important to have a qualitative description of the genotypes to be exploited as raw materials, in terms of chemical composition of the grains, presence and amount of molecules with a functional role, and safety characteristics. Therefore, several research projects have been recently devoted to the screening of maize germplasm, particularly local populations. CRA-MAC maintains more than 700 Italian varieties, collected in different regions in the 1950s and representing a large part of the numerous ecotypes differentiated over the centuries in our country.

A main threat for the safety of maize kernel is the presence of fungal pathogens, in particular *Fusarium verticillioides*, which is the most toxigenic fungus for maize worldwide. It produces mycotoxins (fumonisins) that accumulate in the grain and can be found in the finished products for human food and animal feed. Plants respond to pathogenic attack with a complex network of responses including the activation of antioxidant molecules (Boutigny et al., Eur J Plant Pathol 121:411-413, 2008).

The aims of this research are: i) evaluation of the resistance or susceptibility to fungal pathogens (*F. verticillioides*) in a set of Italian varieties; and ii) evaluation of their grain nutritional quality, with a particular focus on their antioxidant activity.

Twenty-seven maize varieties (Table 1) were tested in open-pollinated field trials during 2011 and evaluated in terms of resistance or susceptibility to *F. verticillioides* by: i) artificial field inoculation of two toxigenic strains by KIA method (Kernel Inoculation Assay; Ferrari and Balconi, Dal Seme 1:38-40, 2008); ii) ear visual rating (number of infected kernels at the inoculation point, NCK); and iii) quantification of the fumonisin content in the grain (ELISA). Non-inoculated or sterile water-inoculated ears were evaluated as controls. Varieties were grouped into three classes of infection based on NCK values: i) 0-30 kernels (low); ii) 31-60 (medium); and iii) over 60 (high).

Artificial inoculation determined, in susceptible materials, a higher NCK and a wider kernel contamination as compared to controls, confirming to be a useful tool to identify genotypes with differential responses to *F. verticillioides* attack.

The number of contaminated kernels (NCK) after Fusarium inoculation ranged from 11 to 82, with an average value around 40. A similar trend, in terms of susceptibility or resistance, was observed for some genotypes also for fumonisin accumulation (*data not shown*).

Total antioxidant capacity (TEAC), expressed as mmol/kg Trolox equivalent (TE) on a dry matter basis, was also evaluated (Serpen et al., J Cereal Sci 48:816-830, 2008). Among the genotypes, a large variability was observed for total antioxidant capacity: in particular, for materials inoculated with *F. verticillioides* TEAC values ranged from 13.28 to 24.40 mmol TE/Kg d.m. In most varieties, the ears inoculated with the fungal pathogen showed TEAC values higher than the relative controls; this increase of production of antioxidant molecules could be considered one of the possible responses to the pathogen attack.

Some of the varieties analyzed in this study were characterized by both high total antioxidant capacity and resistance to pathogen attack. Genotypes with such traits could be an interesting material to introduce in breeding programs focused on the nutritional quality and safety of maize kernel. These preliminary results will be completed with the analysis of the materials replicated in 2012.

Table 1. Maize varieties analyzed and their origin.

Variety	Origin
VA56	Marano vicentino
VA62	Nostrano dell'Isola
VA63	Nostrano locale
VA65	Locale
VA66	Locale
VA67	Locale
VA68	Nostrale
VA69	Locale
VA70	Locale
VA74	Fiorentino
VA83	Bianco perla
VA89	Scagliolo frassine
VA90	Polenta rossa
VA108	Ostesa
VA109	Ostesa
VA111	Nostrano
VA112	Pignolino nostrano
VA113	Nostrano del Garda
VA114	Cinquantino bianchi
VA121	Pignoletto d'oro
VA553	Scagliolo Marne
VA572	Nostrano dell'Isola Finardi
VA904	Cinquantino 2° raccolto
VA1196	Rostrato della Valchiavenna
VA1269	Rostrato Esine
VA1304	Spinato di Gandino
VA1306	Rostrato Marinoni

This research was supported by Project Risorse Genetiche Vegetali/FAO, funded by the Italian Ministry of Agriculture (MiPAAF).

BLOOMINGTON, ILLINOIS
University of Illinois at Bloomington

On the mechanism of haploid production by RWS

— Weber, D

Haploids have become one of the most effective tools in modern genetics and breeding. When a haploid inducer line is crossed as a male parent onto a diploid female, most kernels produced contain a diploid embryo and a triploid endosperm; however, a portion of the kernels has a haploid embryo and trip-

loid endosperm. Such kernels germinate normally and grow into haploid plants. The haploid plants are maternal haploids because the female parent contributed the chromosomes.

In most cases, kernels with haploid embryos are selected using the *R1-nj* allele of the *R1* locus on chromosome 10. The kernels with haploid embryos are germinated and treated with a chromosome-doubling agent (such as colchicine), and pollen from doubled (diploid) sectors is used to self-pollinate the haploid plant. The kernels produced are doubled haploids and completely homozygous (instant inbreds).

RWS, a line developed at the University of Höenheim, Stütgart, Germany (Röber et al., Maydica 50:275-283, 2005), is widely used for producing maize haploids. The corn breeding industry uses RWS and lines derived from it extensively. However, the mechanism by which haploid inducing lines produce kernels with haploid embryos is not well understood.

Two major mechanisms have been proposed to explain how these haploid inducers might produce kernels with haploid embryos. First, an abnormal fertilization event might take place in which one sperm fertilizes the two polar nuclei and the other sperm fails to fertilize the egg of an embryo sac, producing a kernel that has a triploid endosperm and a haploid embryo. Second, the normal double fertilization events would take place, and then the chromosomes contributed by the female parent are eliminated from the embryo after fertilization.

The following experiment was performed to distinguish between these two hypotheses. RWS (which is *Bm2, Lg1, Gl1, J1,* and *Gl*) was crossed as a male parent onto a female parent (Mangelsdorf's multiple chromosome tester) that is homozygous for recessive plant-expressed mutations on five of its chromosomes (*bm2, lg1, gl1, j1,* and *g1* on chromosomes 1, 2, 7, 8, and 10, respectively). 1,200 kernels were field-planted. Of the 1,108 plants that grew, 121 expressed all five of the recessive mutations (were maternal haploids). In addition, four exceptional plants were identified that expressed only one of the mutations (one was *bm2*, one was *lg1*, one was *gl1,* and one was *g1*). Each of these four plants had a morphology that was typical of a monosomic plant. Kernels of this same cross were also grown in a sand bench planting (it is only possible to classify *gl1* and *lg1* in sandbench plantings). Of the 535 seedlings, 35 of the seedlings were *gl1* and *lg1*, and one exceptional plant was *gl1* and *Lg1*. Cytological analysis indicated that each of the five exceptional plants were monosomics (2n=19). These five exceptional plants could only be produced if chromosome loss occurs after fertilization. However, this experiment does not preclude the possibility that some of the haploids were produced by an event in which a sperm failed to fertilize the egg of an embryo sac.

Obtaining chromosome counts from mature field-grown maize plants

— Weber, D

Two procedures are typically employed to obtain tissue for determining chromosome numbers in maize plants: i) rapidly growing root-tips can be harvested from germinating kernels or from seedlings growing in pots; or ii) microsporocyte (immature tassels) samples can be removed from plants at the appropriate stage.

As I was carrying out the work in the previous report, I learned that it was also possible to obtain excellent root-tips for chromosome counts from field-grown plants that were past the pollen-shed stage (anthesis). I dug up the plants and transplanted them into potting soil in our greenhouse, and much to my surprise, I found that plants that were nearing senescence still would produce excellent root-tips. I used this procedure to obtain excellent root-tips from three of the exceptional plants described in the previous report. All three of these exceptional plants were past anthesis, and I was able to obtain accurate chromosome counts for each of these plants.

A reappraisal of Kindiger and Hamann's 1993 protocol to produce large numbers of paternal haploids for cytoplasmic conversions

— Weber, D

Kindiger and Hamann (Crop Sci 33:342-344, 1993) developed a novel system to efficiently produce paternal (androgenic) haploids in maize. They generated tertiary trisomic plants with two normal chromosome 3s plus a B-3Ld chromosome in the inbred, W23. The two normal chromosome 3s carried the *ig1* allele, and the dominant *Ig1* allele was present on the B3-Ld chromosome. The *ig1* locus in chromosome 3 is close to the breakpoint in B-3Ld, and in the inbred, W23, *ig1/ig1* plants are completely or almost completely male-sterile, and plants with a dominant allele of the *ig1* locus are male-fertile.

They indicated that theoretically transmission of the B-3Ld chromosome should be 50% through the female and only 2% through the pollen (Beckett, Can J Genet Cytol 25:346-353, 1983). Therefore, when a 3(*ig1*)/3(*ig1*) female parent is crossed by a 3(*ig1*)/3(*ig1*)/B-3Ld(*Ig1*) male parent, nearly all of the progeny would be *ig1/ig1* (and male-sterile in the inbred, W23) because the B-3Ld chromosome is rarely transmitted through the pollen. Progeny that received the B-3Ld (*Ig1*) chromosome would be male-fertile and could easily be recognized. Selfing of the male-fertile individuals 3(*ig1*)/3(*ig1*)/B-3Ld(*Ig1*) or crossing them to their male-sterile sibs 3(*ig1*)/3(*ig1*) can be used to maintain the 3(*ig1*)/3(*ig1*)/B-3Ld(*Ig1*) stock.

They also crossed 3(*ig1*)/3(*ig1*)/B-3Ld(*Ig1*) males by female parents with several different types of male-sterile cytoplasms and recovered 3(*ig1*)/3(*ig1*)/B-3Ld(*Ig1*) lines with the male-sterile cytoplasms.

Seed of selfed 3(*ig1*)/3(*ig1*)/B-3Ld(*Ig1*) plants and seed of 3(*ig1*)/3(*ig1*) plants with L, MY, ME, S, SD, VG, CA, C, and Q male-sterile cytoplasms crossed as female parents by 3(*ig1*)/3(*ig1*)/B-3Ld(*Ig1*) male parents were obtained from the Maize Genetics Stock Center and planted in field plantings in 1998. These stocks were originally provided to the Maize Genetics Stock Center by Kindiger's lab. A high frequency of distinctively smaller plants

were observed in field-plantings of each of these stocks, as shown in Table 1.

The smaller plants definitely did not have the morphology of a haploid plant; instead, they had a morphology that was typical of a monosomic-3 plant or a plant that was hypoploid for the long arm of chromosome 3. Monosomic-3 plants and plants hypoploid for 3L have a similar morphology. They are much smaller and have more upright leaves that are thicker, shinier, and narrower than those in their diploid siblings. Chromosome counts were carried out on several of the smaller plants, and they each had 20 chromosomes.

Therefore, the smaller plants produced by this system are not haploids; however, they appear to be hypoploids for the long arm of chromosome 3, and therefore appear to have one normal chromosome 3 and a 3B chromosome. Such plants would be expected if the complete B-3Ld translocation was present in the male parent.

The Maize Genetics Stock Center (personal communication) confirms that the Kindiger *ig1* maintainer stock carries the complete TB-3Ld translocation — crosses of the maintainer stock to *a1* testers segregated for colorless kernels with colored plumules.

Table 1. Frequencies of small plants in progenies from crosses of *ig1 ig1* male-sterile cytoplasms (females) with *B-3Ld Ig1* (male).

Cross	Cyto-plasm	Grew (#)	Small (#)	Small (%)
3-*ig1*/3-*ig1*/B-3Ld-*Ig1* self	N	111	22	19.8
3-*ig1*/3-*ig1* x 3-*ig1*/3-*ig1*/B-3Ld-*Ig1*	L	15	3	21.3
3-*ig1*/3-*ig1* x 3-*ig1*/3-*ig1*/B-3Ld-*Ig1*	MY	17	5	30.7
3-*ig1*/3-*ig1* x 3-*ig1*/3-*ig1*/B-3Ld-*Ig1*	ME	15	2	13.3
3-*ig1*/3-*ig1* x 3-*ig1*/3-*ig1*/B-3Ld-*Ig1*	S	79	3	3.7
3-*ig1*/3-*ig1* x 3-*ig1*/3-*ig1*/B-3Ld-*Ig1*	SD	19	8	29.6
3-*ig1*/3-*ig1* x 3-*ig1*/3-*ig1*/B-3Ld-*Ig1*	VG	26	2	7.7
3-*ig1*/3-*ig1* x 3-*ig1*/3-*ig1*/B-3Ld-*Ig1*	CA	22	6	21.4
3-*ig1*/3-*ig1* x 3-*ig1*/3-*ig1*/B-3Ld-*Ig1*	C	120	22	14.0
3-*ig1*/3-*ig1* x 3-*ig1*/3-*ig1*/B-3Ld-*Ig1*	Q	126	53	33.3
Total		550	126	22.9

BUENOS AIRES, ARGENTINA
IEGEBA-CONICET and LACyE (Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires)

Knobs number variability in Argentine Andean maize populations (races Amarillo Grande and Garrapata)

— Fourastié, MF; Realini, MF; Poggio, L; González, GE

In this work we studied six populations belonging to two races of maize native to northwestern Argentina (NOA) in order to analyze the variation respect to the number of heterochromatic blocks (knobs), using fluorescent banding techniques (DAPI banding). Cámara Hernández et al. (Razas de maíz nativas de la Argentina, Facultad de Agronomía, Universidad de Buenos Aires, 2011) identified and described 28 native maize races from NOA. Two of these races, Amarillo Grande and Garrapata, differ by their morphological maize ears and grains, and grow at different altitudes in the provinces of Jujuy and Salta (Argentina).

Zea taxa with 2n = 20 chromosomes are variable in number, size, and position of the knobs, which can be found in 34 different chromosomal positions in corn and teosinte (Kato, Mass Agric Exp Stn Bull 635:1-185, 1976; McClintock, Maize Breeding and Genetics 59-184, 1978). This variability was used for the characterization of maize races of Latin America (Grobman et al., NAS-NRC 915, 1961; Longley, J Agron Res 56:177-195, 1938; Ramírez et al., NAS-NRC 747, 1960; Wellhausen et al., NAS-NRC 511, 1957). Number and size variations of the knobs are related to intra- and interspecific differences in DNA content found in the genus *Zea* (Laurie and Bennett, Heredity 55:307-313, 1985; Poggio et al., Ann Bot 82:115-117, 1998; Rosato et al., Am J Bot 85:168-174, 1998; Tito et al., Theor Appl Genet 83:58-64, 1991).

In this work, we studied the number of knobs using DAPI chromosome banding (Sumner, Chromosome banding, Unwin Hyman, 1990) on interphase nuclei and mitotic metaphases. The studied materials, collected in the province of Jujuy, Argentina, were provided by the Vavilov Lab of Universidad de Buenos Aires (UBA) and cultivated in the greenhouse of the Facultad de Agronomía, UBA.

We analyzed the number of knobs on populations cultivated at three different altitudes above sea level of Amarillo Grande race (populations: VAV 6669: 2000 m, VAV 6644: 2020 m, and VAV 6636: 2755 m) and Garrapata race (populations: VAV 6661: 2192 m, VAV 6662: 2780 m, and VAV 6626: 2795 m). In each population 25 individuals were studied (5 individuals per maize ear), and the results were averaged from at least 20 cells per individual.

The results were analyzed by analysis of variance (ANOVA) with full nesting for general linear mixed models. Factors considered were race (fixed factor), population growing at different altitudes (random factor) nested on race, and maize ear (random factor) nested in the population growing at different altitudes. Additionally, we calculated the variance components to population, maize ear, and error factors (variability in the number of knobs between individuals and measurement errors); $p < 0.05$ values were considered significant. All statistical analyzes were performed using the statistical program Infostat, FCA, National University of Córdoba (Di Rienzo et al., InfoStat version 2012) with the R program interface (R Core Team, R Foundation for Statistical Computing, 2012) for this unbalanced nested design.

No significant differences between races Amarillo Grande and Garrapata in relation to the number of knobs were detected ($F1;2 = 0.47$; $p = 0.53$). However, we found a significant contribution to the variability between populations of the same race cultivated at different altitudes above sea level (CI: 0.462; 4.892). We also found a significant contribution to the variability among

maize ears of the same population (CI: 0.552; 1.672). Notwithstanding, differences among populations belonging to each race cultivated at different altitudes were detected. Of the total variability, 45% is due to the variability among populations and 36% is attributed to variability between the maize ears (Table 1).

Figure 1 shows a significant decrease in the knobs number in populations cultivated at higher altitudes, in concordance with the results found in other NOA populations (Rosato et al., Am J Bot 85:168-174, 1998).

Table 1. Estimated variance components. (CI: Confidence intervals.)

Factors	Estimated variance	CI for variance	Variability relative to total (%)
Population	$\sigma^2_{ALT}=1.50^2=2.25$	$(0.46^2; 4.89^2)$	45.0
Maize ear	$\sigma^2_{Esp}=1.35^2=1.82$	$(0.68^2; 2.71^2)$	36.5
Error (individuals)	$\sigma^2_{Esp}=0.96^2=0.92$	$(0.55^2; 1.67^2)$	18.5

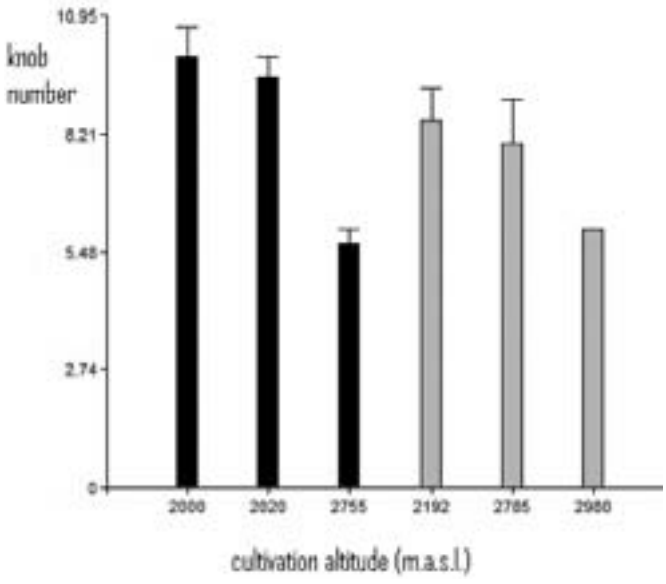

Figure 1. Knobs number of populations of Amarillo Grande (black) and Garrapata (gray). Mean and standard deviation (m.a.s.l.: meters above sea level).

Meiotic analysis of F1 hybrids among teosintes

— González, GE; Fourastié, MF; Realini, MF; Poggio, L

The association of homologous or homeologous chromosomes in the meiotic behavior of F1 hybrids reveals the relative affinity between genomes of parental species. Chromosomal rearrangements and genetic incompatibilities producing abnormal meiosis (abnormal spindle, laggard chromosomes, among others), acting as reproductive isolation mechanisms, could also be detected. In the present work the genome affinities based on the chromosome association of seven artificial hybrids among teosintes are discussed. Moreover, pollen stainability is reported.

Artificial crossings between teosintes were carried out in the greenhouse to obtain the F1 hybrid plants. The taxa using as parental differs in chromosome sizes and total DNA amounts: *Zea mays* ssp. *mexicana* (2n = 20; 2C = 6.79pg), *Zea mays* ssp. *parviglumis* (2n = 20; 2C = 5.86pg), *Zea luxurians* (2n = 20; 2C = 8.83pg), *Zea diploperennis* (2n = 20; 2C = 6.36pg), and *Zea perennis* (2n = 40; 2C = 11.36pg) (Tito et al., Theor Appl Genet 83:58-64, 1991)**.** Young panicles from each F1 hybrid were fixed in a 3:1 solution of absolute ethanol: acetic acid and squashed in 2% acetic haematoxylin. Normal (stained) and aborted (unstained) pollen grains were distinguished using Alexander's stain (Alexander, Biotech Histochem 44:117-122, 1969).

Table 1 (opposite page) shows the meiotic configurations and pollen stainability of the studied F1 hybrids.

The hybrids with 2n = 20 involving *Z. luxurians* as one parent presented: heterozygosis for heterochromatic blocks at pachytene; 5 to 10 heteromorphic bivalents; univalents with different sizes; and two asynchronous groups of 5 bivalents each at diplotene-metaphase I. At anaphase I, a maximum of 10 laggard chromosomes were observed, some of them with early separation of their chromatids. Particularly, the meiotic behavior of the F1 hybrids *Z. luxurians* x *Z. m.* ssp. *parviglumis* revealed differences in paracentric inversions among parentals, since up to 3 bridges with fragments were recorded at anaphase I. The lack of pairing and the presence of heteromorphic bivalents probably are due to partial homology among chromosomes and/or differences in DNA content and chromosome sizes between the parental species.

Z. m. ssp. *parviglumis* x *Z. diploperennis* hybrids show, at diakinesis-metaphase I, 10 bivalents as the most frequent configuration and two asynchronous groups of 5 bivalents each. Moreover, evidences of abnormal spindles at anaphase I and citomixis (cell fusion) were detected frequently. The low viability of pollen, jointly with evidence of citomixis detected, suggests the existence of genetic incompatibility between the two parent species.

The hybrids with 2n = 30, *Z. perennis* x *Z. luxurians* and *Z. perennis* x Z. *diploperennis*, showed 5III+5II+5I as the more frequent meiotic configuration, with trivalents type "fry-pan" and homomorphic bivalents.

The variation in total genomic DNA content among the progenitor species of the analyzed hybrids, which reflects differences in chromosome sizes, explains the high frequency of heteromorphic bivalents and univalents of different sizes detected, particularly when *Z. luxurians* is involved as one of the parental species. The presence of bridges and fragments in the hybrids indicate differences in structural rearrangements (paracentric inversions) between parents. The low pollen viability probably is due to structural/genic differences between parents, responsible for the postcigotic reproductive isolation detected among them.

These results show that the genomic relationships between teosintes, revealed through meiotic behavior of their F1 hybrids, are relevant in the study of genome organization and diversification of the genus *Zea*.

Table 1. Meiotic configurations of the F1 artificial hybrids. SD: Standard deviation. I: Univalents. II: Bivalents. III: Trivalents. (1): From Poggio et al., Genome 42:993-1000, 1999. (2): From Naranjo et al., Acad Nac Cs Ex Fís Nat, Buenos Aires, Monogr 5:43-53, 1990.

Hybrids	(2n)	III (X ± SD) (range)	II (X ± SD) (range)	I (X ± SD) (range)	Most frequent configurations (%)	Nº of cells	Pollen stainability (%)
Z. m. ssp. parviglumis x *Z .m. ssp. mexicana*	20	-	9.83 ± 0.38 (9-10)	0.33 ± 0.0 (0-1)	10 II (83%)	77	84%
Z. luxurians x *Z. diploperennis* (1)	20	-	8.65 ± 0.12 (7-10)	2.7 ± 0.23 (0-6)	9 II + 2 I (38%) 8 II + 4 I (45%)	84	5%
Z. luxurians x *Z. m. ssp. parviglumis*	20	-	9.16 ± 0.96 (5-10)	1.68 ± 1.59 (0-10)	10 II (45%) 9 II + 2 I (33%)	145	7%
Z. luxurians x *Z. m. ssp. mexicana*	20	-	7.5 ± 1.34 (5-10)	5 ± 2.44 (0-10)	8II + 4 I (36%)	67	7.25%
Z. m. ssp. parviglumis x *Z. diploperennis*	20	-	9.49 ± 0.56 (8-10)	1.01 ± 0.51 (0-4)	10 II (52%) 9 II + 2 I (44%)	63	6%
Z. diploperennis x *Z. perennis* (2)	30	4.91 (1-8)	5.25 (2-10)	4.73 (2-8)	5 III + 5 II + 5 I (40%)	168	2%
Z. luxurians x Z. perennis (1)	30	5.26 ± 0.19 (3-8)	4.76 ± 0.23 (2-8)	4.70 ± 0.20 (2-7)	5 III + 5 II + 5 I (37%)	46	2%

Comparative study of *Zea* karyotypes

— González, GE; Fourastié, MF; Cámara-Hernández, J; Poggio, L

The taxa of genus *Zea*, maize and teosintes, have both intra- and interspecific variation in genome size, due to differences in heterochromatin content (Poggio et al., Ann J Bot 82:115-117, 1998; Tito et al., Theor Appl Genet 83:58-64, 1991). The heterochromatin mainly forms telomeric or subtelomeric blocks called knobs (Kato, Mass Agric Exp Stn Bull 635:1-185, 1976). A comparative analysis of the karyotypes of different taxa of genus *Zea* is presented. In addition, we studied the karyotype variation in the percent and location of heterochromatic regions, and discuss the inter- and intrachromosomal asymmetry for each karyotype.

C and DAPI banding techniques were applied on mitotic metaphases chromosomes of *Zea luxurians* (cv. 2228, Guatemala), *Zea mays* ssp. *mexicana* (KG-04- 2, Mesa Central, México), *Zea mays* ssp. *parviglumis* (cv. 8391, Balsas Valley, Guerrero, México), *Zea diploperennis* (cv. 2232, San Miguel, Jalisco, México) and the Argentinean race of maize Orgullo Cuarentón (VAV 6482, Lab de Recursos Genéticos Vegetales I. N. Vavilov, Facultad de Agronomía, UBA). Chromosomal parameters were measured with MicroMeasure 3.3 (available at http://www.colostate.edu/depts/biology/micromeasure). Chromosomes were ordered from largest to smallest, as usual for maize, classified according to the nomenclature of Levan et al. (Hereditas 52:201-220, 1964), in order to facilitate comparison with related grasses. Intra- and interchromosomal asymmetry indexes (A1 and A2) were calculated according to Romero-Zarco (Taxon 35:526-530, 1986).

Table 1 presents the karyotypic formulae, the intra- and interchromosomal asymmetry indexes, the percent of heterochromatin, and the DNA content (2C). Figure 1 (next page) shows the representative mitotic metaphases, the idiograms, and the karyotypic formulae of each analyzed taxa.

DAPI banding showed that *Z. luxurians* is the species with the highest number and size of heterochromatic knobs, which is related to the higher percent of heterochromatin and chromosomal size observed in this species. Moreover, karyotype formulae variation was detected between the studied taxa, mainly due to the differences in the number and position of the heterochromatic knobs.

In relation to the symmetry of karyotypes it was noted that *Z. diploperennis* has the most asymmetrical karyotype with high levels of intra- and interchromosomal asymmetry, while *Z. luxurians* has elevated intrachromosomal asymmetry but low interchromosomal asymmetry. Moreover, *Z. m.* ssp. *mexicana*, *Z. m.* ssp. *parviglumis* and maize Orgullo Cuarentón race vary in both asymmetry indices (A1 and A2). This variation is not directly

Table 1. Formulae and karyotypic parameters. A1: interchromosomal asymmetry index. A2: intrachromosomal asymmetry index. Ref.: m: metacentric. sm: submetracentric. st: subtelocentric. pg: picograms. n/d: no data. (1): From Tito et al. (1991). (2): Poggio and Guillín, personal communication. (3): From Rosato et al. (1998).

	Karyotype formulae	A1	A2	% heterochromatin	ADN amount (2C)
Z. luxurians	5m+4sm +1sm-st	0.39	0.15	21.16	8.83 pg[1]
Z. m. ssp. parviglumis	9m+1st	0.24	0.24	18.75	5.86 pg[2]
Z .m. ssp. mexicana	n/d	0.38	0.23	n/d	7.09 pg[1]
Z. diploperennis	5m+4sm+1st	0.44	0.37	8.5	6.36 pg[1]
Maize (Orgullo Cuarentón)	9m+1sm	0.21	0.23	7.84	6.15 pg[3]

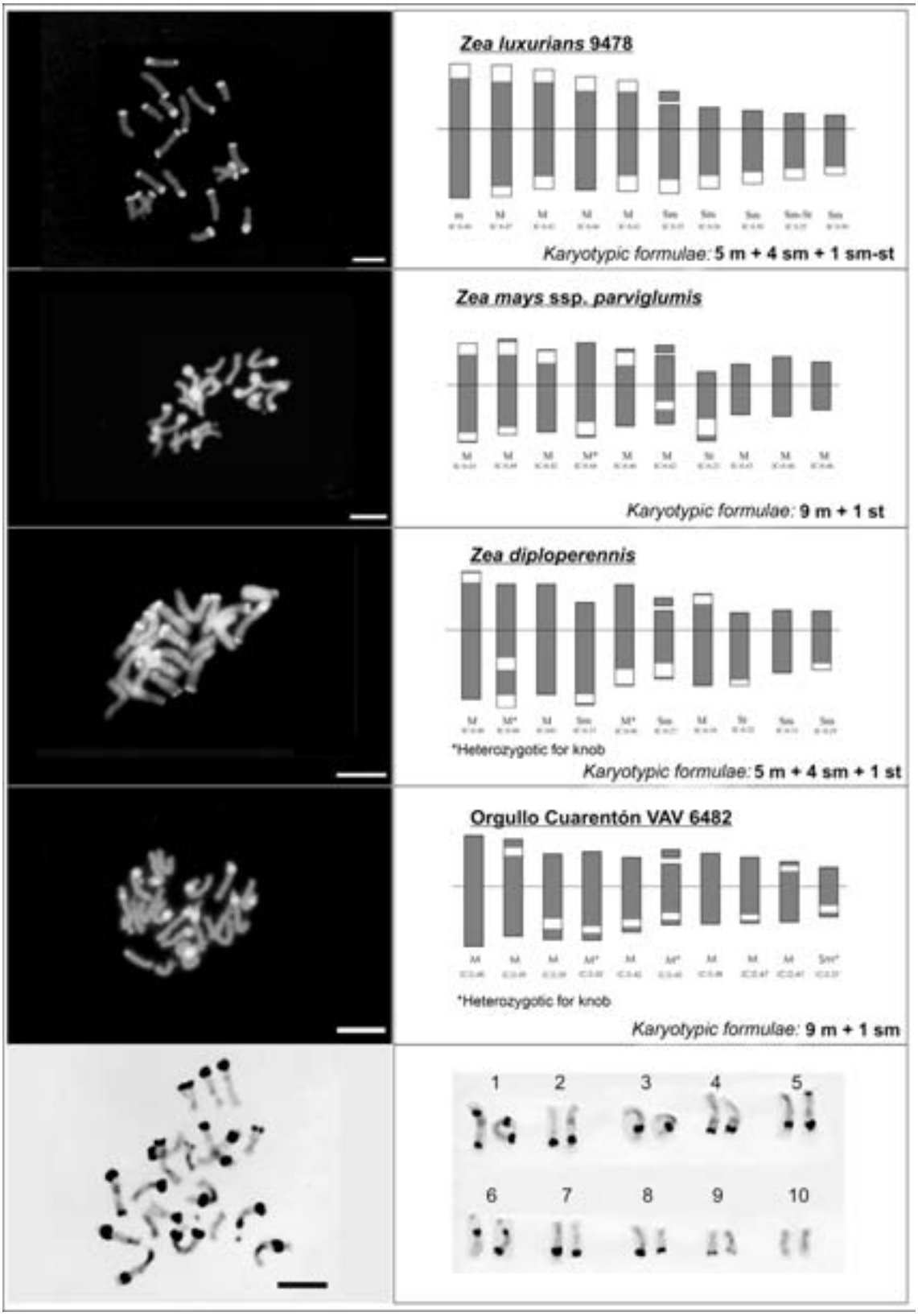

Figure 1. Mitotic metaphases, idiograms, and karyotypic formulae of each Zea taxa analyzed. Bar: 10 µm.

related to the DNA content or the percent of heterochromatin, but depends on the heterochromatin distribution. Therefore, the greatest asymmetry observed in *Z. diploperennis* karyotype is due to the heterochromatin distribution mostly on the long arms of chromosomes.

These results indicate that in genus *Zea*, the formulae and symmetry of the karyotypes depends on the number, size, and position of the heterochromatic regions.

Karyotypical studies of two maize races from Northeast Argentine (NEA): DAPI-banding and Fluorescent *In Situ* Hybridization (FISH)

— Realini, MF; Poggio, L; González, GE

Knobs heterochromatic blocks occur in all *Zea* species with 2n = 20, varying in size and number across maize races and their wild relatives (González and Poggio, Genome 54:26-32, 2011; Kato, Mass Agric Exp Stn Bull 635:1-185, 1976; McClintock et al., Colegio de Postgraduados, Chapingo, México, 1981; Poggio et al., Cytogenet Genome Res 109:259-267, 2005). Variation in DNA content has been proposed to be due principally to differences in the amount of heterochromatin, which is mainly located in distal knobs (Laurie and Bennett, Heredity 55:307-313, 1985; Poggio et al., Ann Bot 82:115-117, 1998). These structures can be observed as subtelomeric bands by DAPI-banding, and using Fluorescent In Situ Hybridization (FISH) (González et al., Chrom Res 14:629-635, 2006). Knobs have been described as highly repeated tandem arrays of 180-bp and TR-1 (350 bp) sequences, both repeated in different proportions constituting different *knobs* (Ananiev et al., Proc Natl Acad Sci 95:10785-10790, 1998; Dennis and Peacock, J Mol Evol 20:341-350, 1984).

In this study we present the karyotypic formulae, asymmetry indexes and the position and composition of the knobs of two races of maize from Argentine Northeast (NEA), Tupi Amarillo (VAV 6563) and Rosado (VAV 6565). DAPI-banding and FISH techniques were applied. The plant material was provided by the Vavilov Lab, Universidad de Buenos Aires (UBA), and cultivated in the greenhouse of the Facultad de Agronomía, UBA.

DAPI-banding was performed according to Sumner (Chromosome banding, Unwin Hyman, 1990). DAPI fluorochrome (4'-6-diamidino-2-phenylindole) preferentially stains AT-rich heterochromatin in plants (Guerra, Genet Mol Biol 23:1029-1041, 2000).

The 180-bp and TR-1 knobs sequence of maize was obtained from GenBank (http://www.ncbi.nlm.nih.gov/). These sequences were isolated and amplified from total genomic DNA of maize by PCR methods. The sequences obtained were labeled with biotin and digoxigenin by PCR as well by enzymatic methods. For the latter we used enzymatic kits: BioNick Labeling System (Invitrogen) to label with biotin and Dig High Prime (Roche) to label with digoxigenin. FISH was performed according to González et al. (Chrom Res 14:629-635, 2006). FISH slides were observed with a Zeiss AxioPhot epifluorescence microscope (Carl Zeiss, Germany), and microphotographs were taken with a Leica CCD digital camera.

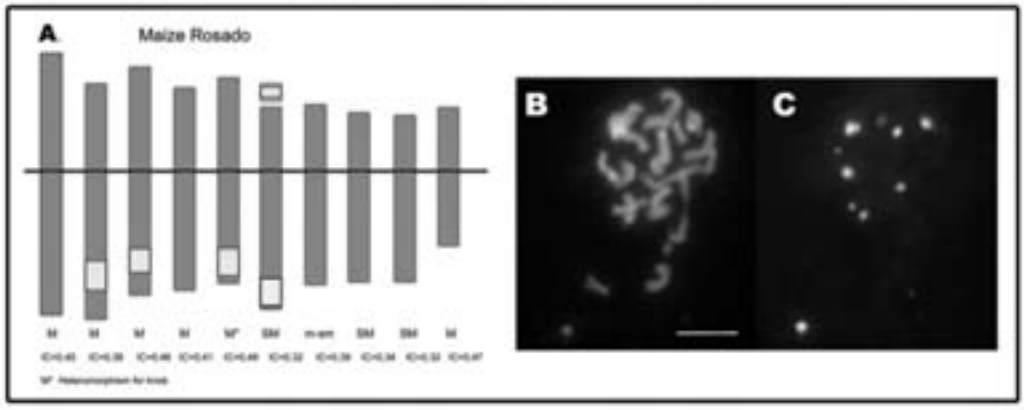

Figure 1: A. Idiogram of maize Rosado. The white blocks represent the coincident DAPI-positive band and 180-bp and TR-1 FISH signals. B. DAPI-banding. C. FISH using 180-pb as probe on mitotic metaphase chromosomes. Ref.: M: metacentric. SM: submetacentric. IC: centromeric index. Bar 10 µm.

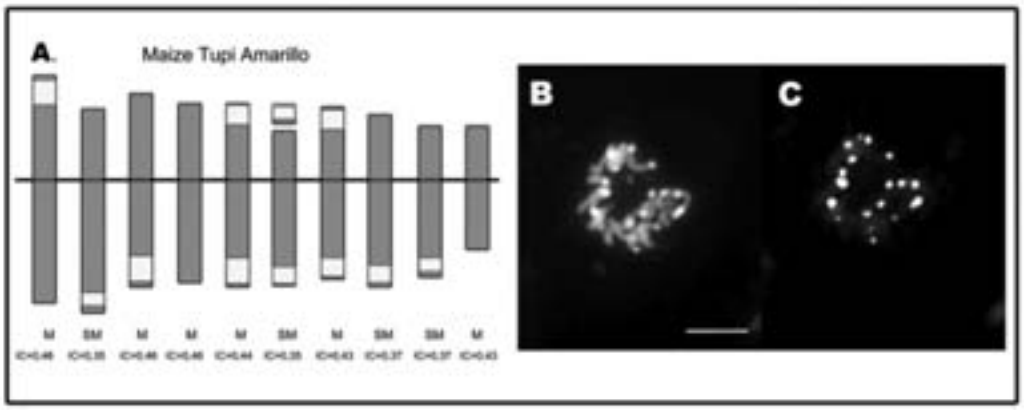

Figure 2: A. Idiogram of maize Tupi Amarillo. The white blocks represent the coincident DAPI-positive band and 180-bp and TR-1 FISH signals. B. DAPI-banding. C. FISH using TR-1 as probe on mitotic metaphase chromosomes. Ref.: M: metacentric. SM: submetacentric. IC: centromeric index. Bar 10 µm.

Chromosomal parameters were measured using the freeware program MicroMeasure 3.3 (http://www.colostate.edu/depts/biology/micromeasure). The relative chromosome length, arm ratio, and centromeric indexes were calculated to determine the karyotypes. The chromosomes were ordered from the largest to the smallest, as usual for maize, and chromosome morphology was described according to Levan et al. (Hereditas 52:201-220, 1964). To estimate the karyotype asymmetry, two numerical parameters were used, according Romero Zarco (Taxon 35:526-530, 1986): A1 (intrachromosomal asymmetry index) and A2 (interchromosomal asymmetry index).

In each race a maximum of 25 individuals were studied (3-5 individuals per maize ear) and at least 10 cells per individual were analyzed.

The karyotype parameters analysis let us to elaborate the idiograms from the two maize races (Figs. 1A & 2A; previous page).

DAPI-banding allowed locating the knobs as DAPI-positive bands on mitotic metaphase chromosomes (Figs. 1B & 2B). In FISH experiments, simultaneous hybridization with the 180-bp and TR-1 probes showed that these sequences co-localized with all the DAPI-positive bands in both races. Different intensities of hybridization signals with each probe suggest the DAPI-positive knobs are composed by different proportions of 180-bp and TR-1 sequences.

In Table 1 the karyotypic formulae, A1 and A2 indexes, percentage of heterochromatin, and number of knobs are presented for Tupi Amarillo and Rosado maize races.

We observed that the percentage of heterochromatin of Tupi Amarillo is about 8% higher than Rosado (Table 1); this difference between both maize races is due to the higher number of knobs in Tupi Amarillo. This race showed higher intrachromosomal asymmetry (A1) but lower interchromosomal asymmetry (A2) compared with Rosado. This is due to differences in the size and the distribution of the knobs on the both chromosomal arms (Figs. 1A & 2A).

All results obtained in this work allowed us to identify cytogenetically the maize races studied. Then, the variations of the patterns for number, position, and sequence composition of the heterochromatic knobs are useful markers for a proper cytogenetic characterization of maize races.

The cytogenetic characterization of different Argentinean races of maize will contribute to the knowledge of the genetic variability within native materials, useful for its integration in future breeding plans and biodiversity conservation.

Table 1. Karyotypic parameters of Tupi Amarillo and Rosado maize races. Ref.: A1: intrachromosomal asymmetry index. A2: interchromosomal asymmetry index. m: metacentric. sm: submetacentric.

	Tupi Amarillo	Rosado
Karyotypic formulae	6m + 4sm	6m + 1m-sm + 3sm
% of heterochromatin	19.64	11.7
Range of number of *Knobs*	16-22	10-11
A1	0.35	0.27
A2	0.18	0.26

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New sources of tassel-less mutation

— Micu, AA

A large diversity of genes have been described and located in maize, including genes that determine the structure and functioning of generative system. One of these systems, based on CMS and fertility-restoring genes, is used in hybrid seed production. An alternative to the androsterility type is the tassel-less mutation, first described more than four decades ago (Micu, Genetica, v.6:2, 21-27, 1970).

In our research on possible sources of tassel-less mutation during 2010-2012, we made observations on 293 lines that descend from 80 distinct sources and that represent a wide diversity of biological and morphological traits. All observed material consists of inbred lines at S_7-S_9. In previous generations no similar mutations were noticed.

The results are summarized in Table 1. In 2011 the tassel-less mutation was observed in source HM 77. In 2012 the tassel-less mutation was observed in four other sources. In order to determine the genetical character of tassel-less mutation, the mutants from each source were pollinated with normal plants from their source. The tassel-less mutation from source HM 77, observed in 2011 and 2012, proves to be hereditary. For further research of this mutation we have obtained 59 inbred crosses *tls X normal*. Morphological expression of this character is gradual: from partial

Table 1. Sources of tassel-less mutation.

No.	Source	Number of patterns	Number of plants (total / tassel-less)
1	HM 13 (S9)	3	44/11
2	HM 47 (S9)	3	52/16
3	HM 75 (S9)	1	15/11
4	HM 77 (S7-S9)	2	118/37
5	Descendent from B73 line (S7)	1	25/16

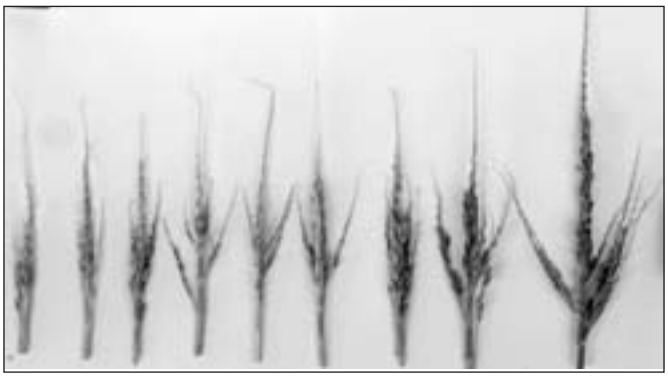

Figure 1. Gradual expression of tassel-less mutation (source HM 77).

Figure 2. Tassel-less mutation (Source HM 77).

reduction to total absence of tassels (Figs. 1 & 2). Revealing mutant plants in S_7-S_9 (and not earlier) indicates a possible polygenic hereditary base of tassel-less mutation in studied sources.

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The genetic of kernel set in one of the maize hybrids

— Mikhailov, ME

The double haploid lines derived from MK01 x A619 maize hybrid show the very large spectrum of kernel set. About 10 lines from 45 are characterized by full ears (as MK01 parent), 1-2 lines have empty or near empty (few kernels on ear) cobs, and the other lines are intermediate (see Fig. 1). This phenomenon is not due to meiotic aberrations because artificial pollination produces normal full ears for all these lines. It appears this is due to difficult outlet of silk from ear. This feature is clearly inherited from A619 parent line, which shows the mean kernel set 70-90% at different years (if we take a full ear as 100%). The hybrid MK01 x A619 is characterized by full ears, as the parent MK01.

To investigate the genetic control of this phenomenon, we

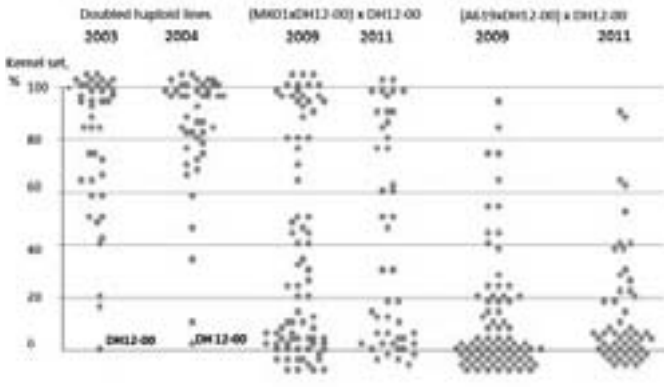

Figure 1. The genetic distribution of some populations for kernel set. Comment. Some of the points with coordinates 0 and 100 are located below 0 and above 100.

have chosen the worst double haploid line, DH12-00, and derived from it the analyzing crosses (MK01 x DH12-00) x DH12-00 and (A619 x DH12-00) x DH12-00. In 2009 the 79 plants of first cross and the 79 plants of second cross were tested, and in 2011 so were 47 and 50, respectively. In the first cross the clear bimodal distribution for kernel set was observed. This suggests the small number of genetic factors that control this trait and differ in MK01 and DH12-00 lines. The A619 and DH12-00 lines should be distinguished by larger number of genes.

To evaluate and eliminate the environmental variation, the selfed progeny of these crosses were tested (Table 2). The genetic variance obtained was used for estimation of number of genetic factors influencing kernel set. Number of factors was estimated by the formula Castle-Wright modified to our case:

$$n = \frac{(2+\beta)^2}{64\,\sigma_g^2}$$

Mean degree of dominance β was calculated as

$$\beta = \frac{KS(PxDH12-00) - (KS(P) + KS(DH12-00))/2}{KS(P) - KS(DH12-00)}$$

where KS is mean kernel set of genotype given in brackets and P is MK01 or A619.

These results suggest that variation between double haploid

Table 1. Mean kernel set in 2011.

Genotype	Mean kernel set (%)
MK01	99.8 ± 0.2
A619	82.5 ± 4.5
MK01 x A619	99.9 ± 0.1
DH12-00	0.8 ± 0.3
MK01 x DH12-00	88.7 ± 3.7
A619 x DH12-00	60.4 ± 7.0
(MK01 x DH12-00) x DH12-00	39.7 ± 5.6
[(MK01 x DH12-00) x DH12-00]⊗	33.6 ± 4.6
(A619 x DH12-00) x DH12-00	17.9 ± 4.8
[(A619 x DH12-00) x DH12-00]⊗	11.6 ± 1.7

Table 2. Variances of kernel set and estimation of number of genetic factors.

Parameter	[(MK01 x DH12-00) x DH12-00]⊗	[(A619 x DH12-00) x DH12-00]⊗
Number of families	27	46
σ^2 between families	661.58	127.65
σ^2 environmental	58.33	22.11
σ^2 genetic	603.26	105.54
Mean degree of dominance	0.78	0.46
Number of genetic factors	2.0 ± 0.6	6.0 ± 1.3

lines for kernel set likely is caused by two recessive mutations of A619 line preventing outlet of silk from ear. In the A619 line these mutations do not act in full as they are compensated by several (5-7) semi dominant suppressors. In a recombinant progeny this compensatory gene complex breaks down.-

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More about curious mottling in highly paramutant R1 kernels

— Coe, E

In a previous note I asked, "Mottling expression is curious, and so is blotching — what is responsible?" This note does not answer the question but gives more information.

Typical mottling of r/r/R kernels shows colored aleurone cells in irregular, scattered distributions that are inconsistent with the morphogenesis of the aleurone layer. Tantalizing clues appear with highly paramutant *R1-iv* or *R1-v* (four or five times paramutagenized), in which pigmented cells are greatly reduced in frequency. The distributions suggest some systematic process is at play. Photographs at high magnification have been added to the database (Figs. 1A-G).

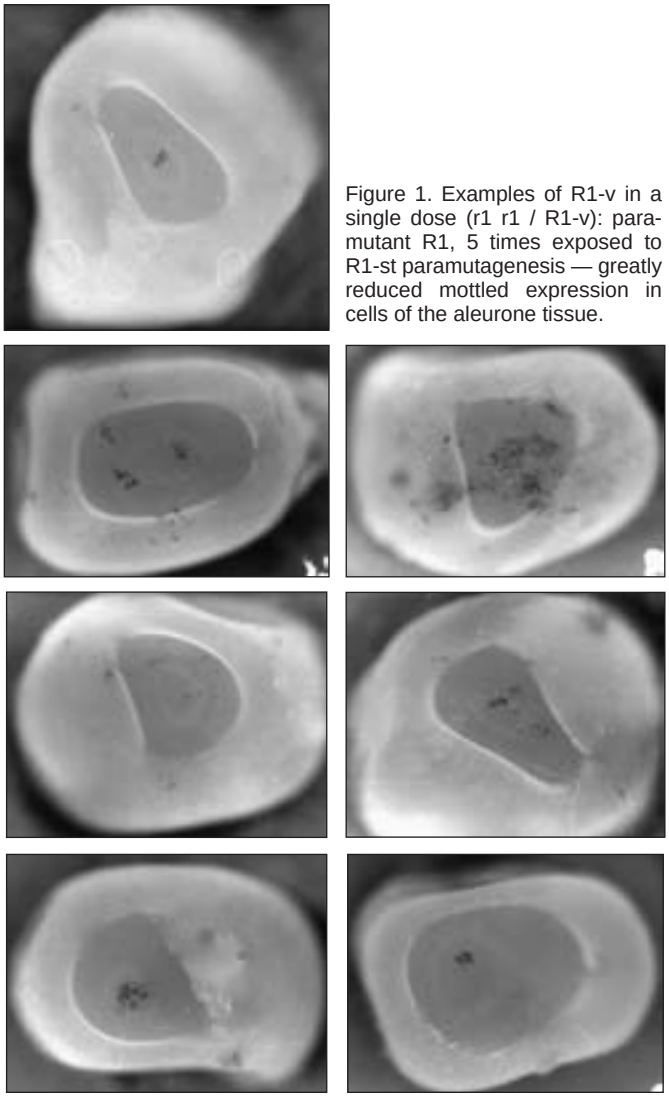

Figure 1. Examples of R1-v in a single dose (r1 r1 / R1-v): paramutant R1, 5 times exposed to R1-st paramutagenesis — greatly reduced mottled expression in cells of the aleurone tissue.

In highly paramutant genotypes there may be as few as 10-20 colored cells in an entire aleurone tissue of some 160,000 cells. If each cell makes an independent decision to be "on" or "off," a binomial or Poisson distribution would result in a random display of single colored cells. However, colored cells occur not in single, independently pigmented cells, but in irregular, very localized clusters, as seen in the images. _

What do the clusters suggest?

Procedures to improve Stock 6

— Chang, MT; Coe, E

Stock 6 was named by Coe in his genetic collection. Ed recognized its high haploid induction rate in 1952. He then converted Stock 6 in 1960 to carry homozygous *A B Pl C R-nj* anthocyanin genes, expressing purple plant color, purple plumule and purple seed crown for easy identification of haploid seeds. The induction rate of Stock 6 is about 2% to 3% with poor agronomic traits such as poor stalk, poor roots, easy to lodge and ear rot. These poor traits were improved by Chang with advice from Coe according to the following procedures, and the rate of haploid induction was increased by selection.

A green plant, yellow seed unfixed material (*AA cc rr bb plpl*) that had shown it was producing haploids in the field was used as female and crossed by purple plant, purple seed crown and purple plumule Stock 6 (*AA CC R-njR-nj BB PlPl*) pollen.

20 F1 seeds (*A/a C/c R-nj/r B/b Pl/pl*) were planted and selfed for F2 seeds.

The F2 seeds segregated nine colored crown and plumule seeds vs. seven colorless seeds. Selected and planted 200 F2 seeds of the most dark-colored crown and plumule to enhance the probability of homozygous *AA CC R-njR-nj* plants.

Selfed F2 plants with dark purple leaves. Ears should either segregate purple crown and colorless seeds or all homozygous colored seeds. Selected the homozygous ears that showed seed color fixation (*AA CC R-njR-nj*).

Planted 50 F3 seeds from each ear with seed color fixed. Identified the most dark purple leaf, sheath, and tassel plants and selfed. These plants were supposed to be homozygous for *AA BB PlPl* genes.

Planted 20 ear-to-row F4 seeds from each selfed F3 ear. Selected the rows showing all purple plants to confirm plant color fixation, and selfed all ears.

The genetic make-up of F5 seeds was fixed for *AA CC R-njR-nj BB PlPl*. The phenotypic expression was all purple plants and purple crown and plumule. Planted 200 F5 seeds and also 500 hybrid seeds as tester.

Selfed each F5 plant and carried pollen to cross onto two hybrid ears. Harvested all selfed F5 ears with ear number -1, -2, -3 and so forth. Also harvested all crossed ears with pollen source X-1,

X-2, X-3 and so forth.

Screened all crossed ears for haploid seeds production. For example, there should be two ears crossed by -1 F5 plants or X-1. If one ear showed 6% haploids and the other ear showed 4% haploids, then the -1 F5 plant had a 5% induction rate. If one ear showed 9% and the other ear 4%, the induction rate was too far apart, so the lower 4% was assigned to define the F5 plant induction rate. Screened all the ears and sorted induction rate from low to high. A range of induction rate from almost 0% to 8% was obtained.

Selected the highest rate F5 ears and planted 20 F6 seeds ear-to-row from 10 ears to form a 200-seed population. Also planted 500 hybrid seeds as tester, using the above procedures again to determine induction rate of each plant. The induction rate was increased in the range of 1% to 9% in response to selection.

Selected the highest induction rate F6 ears and planted 20 F7 seeds ear-to-row from 10 ears to form a 200-seed population. Also planted 500 hybrid seeds as tester. Repeated the above procedures. Also planted 200 F7 seeds with highest induction rate, selfed the haploid plants to form a new pure Stock 6 line with higher induction rate and better phenotypic traits.

Repeated the above procedures for a couple of more generations. The F10 seeds from higher induction F9 ears were sibbed to retain plant vigor and progeny were sibbed again and again. From the progenies, several high induction lines were produced to form new Stock 6 lines. The average haploid induction rate is 6% to 8%.

Seeds of this improved Stock 6 are being sent to the Maize Genetics Cooperation Stock Center.

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L-proline amount in callus tissues of Lancaster maize inbred lines under chloride load

— Derkach, KV[1]; Abraimova, OE[1]; Dzubetskij, BV[1]; Cherchel, VJu[1]; Sitalo, MJu[2]; Konoschuk, JuV[2]; Satarova, TM[1]

Salinity of soil and soil waters are actual problems of land utilization. Chloride salinity is the most common kind; in Ukraine it is dominated by sulphate and carbonate forms. Chloride salinity has a super-negative effect on the maize plant. Growth inhibition is observed already at 0.1% salt content in soil, but the salt level of 0.3-0.4% provokes the wilting and plant death. Numerous metabolic cell abnormalities occur under the salt influence: inhibition of enzyme activities, photosynthesis, protein synthesis, and disorders of respiratory processes (Dolgyh, Ph.D. Dissertation, 2005).

Protective plant response to the negative effect of abiotic factors is induced by a lot of cell systems. One of the responses to the stress factors (salinity, drought, and low temperatures) is the accumulation of free L-proline in the cells. The precursor of proline synthesis is glutamate or ornithine. Under the stress proline content increases due to the regulation of two opposite processes: the intensification of its biosynthesis and the inhibition of its catabolism.

Proline is an organic compound of low molecular weight that lightly resolves in water and forms colloidal polymer structures. Free proline and proline in the protein molecules are the required components of any plant cell. This aminoacid is a component of the antioxidant protective system; it stabilizes the subcellular structures and macromolecules, regulates redox potential, and participates in the modification of functions of mitochondria. Proline is a part of the signal transmission systems that control gene expression in response to stress (Anjum et al., Afr J Agric Res 6(9):2026-2032, 2011).

Ions Na^+ and Cl^- from the nutrient medium overcome the cell wall and enter the cell through anionic and cationic channels, penetrate through protein hydrate coverage and affect the noncovalent bonds that maintain the structure of the protein molecules. Proline does not penetrate through hydrate coverage and does not enter into direct contact with the proteins, but creates obstacles for the hydrate coverage destruction and the protein denaturation by ions (Alyohina et al., Physiology of plant: 636, 2006). Proline is an important cell osmoprotector. It protects proteins from denaturation and forwards their native conformation, interacting with them during stress. Additionally, it helps to achieve the osmotic balance of cytosol with vacuoles and other cell organelles.

The subject of our work includes the determination of proline amount in maize callus tissues under chloride load and, after its removal, the characterization of influence of sodium chloride on the regeneration potential of callus tissues. Research material was represented by five inbreds of maize commercially valuable Lancaster germplasm (DK633/266, DK633/325, DK236, DK3070, DK6080) and one inbred of Polish germplasm (PLS61). Primary explants for induction of callus tissues were immature embryos, 1.5 mm in length. Callus tissue was initiated within 30 days on N6 medium (Chu et al., Sci Sinica 18:659-668, 1975) modified with 690 mg/l L-proline, 100 mg/l inositol, 100 mg/l casein hydrolyzate, 1 mg/l 2,4-D, 0.1 mg/l abscisic acid and two levels of sucrose - 30 g/l or 60 g/l. Chloride load in vitro was simulated by adding into the medium for subcultivation sodium chloride in concentrations of 6, 30, and 60 g/l. The content of L-proline was determined for 330-day stabilized maize callus tissues obtained in two different ways. In the first version the callus tissue was subcultivated on the N6 medium with 0 (control), 6, 30, or 60 g/l sodium chloride for 300 days right after the induction period. In the second version the callus tissue was subcultivated on the N6 medium with 0 (control), 6, 30, and 60 g/l sodium chloride during 210 days right after the induction period and maintained during the following 90 days on hormone-free regeneration medium MS (Murashige et al., Physiol Plant 15:473-497, 1962) without sodium chloride.

Determination of the proline amount was performed by a modified method (Bates et al., Plant soil 39:205-207, 1963). Callus tissue sample (approximately 1 g) was poured by boiling distilled water (10 ml) and placed for 10 minutes in a boiling water bath. 2 ml of glacial acetic acid and 2 ml of ninhidryn reagent was placed into the clean test tube. Then 2 ml of extract was flowed to the same

test tube. Samples were incubated for one hour in a boiling water bath separating and then rapidly cooled in ice. 10 ml of toluene for extraction of the proline was added to the obtained samples. The mixture was separated in a separatory funnel. The color intensity of proline fraction was measured spectrophotometrically at the wavelength of 520 nm. Proline amount was calculated according to calibration curve constructed with crystalline proline. For the determination of proline amount the average sample from pieces of 3-5 typical calli was composed. Proline content was evaluated in 1 g of callus tissue. Data processing was carried out according to (Atramentova et al., Statistic methods in biology: 288, 2007). Data in tables are presented as $\bar{x} \pm mt_{0.05}$, where $\bar{x}$ is the average value of the index, m is the error of average value, and $t_{0.05}$ is student criterion for significance level of 0.05.

Proline content in the maize callus tissues under the sodium chloride in the medium for subcultivation was raised compared to control and was being increased simultaneously with the increasing of sodium chloride concentration (Table 1). Proline content in control depended on genotype and fluctuated between 483.16 to 1,509.23 mg proline/g callus tissue.

Ninety days after the removal of chloride load proline content variations decreased for callus tissues cultivated on different levels of sodium chloride (Table 2). After removal of chloride load in callus tissues of inbred DK3070, levels of proline were higher for callusogenic medium with 30 g/l sucrose than with 60 g/l sucrose. Regenerants were recovered only for inbred PLS61 on the subcultivation medium with 6 g/l sodium chloride. Proline amount in callus tissues of PLS61 after the removal of chloride load was rather high compared to nonregenerable inbred DK3070.

The experimental data allow concluding that the response of maize callus tissues to chloride load leads to the accumulation of proline. The content of proline in callus tissues depends on the concentration of sodium chloride in the nutrient medium and increases with its magnification. Concentrations of sodium chloride in the nutrient medium of 30 and 60 g/l completely suppress regenerative potential of maize callus tissues, while 6 g/l sodium chloride permits the plant regeneration of certain genotypes.

Table 1. Content of L-proline in maize calllus tissues under chloride load.

Sucrose content in the medium for callus induction, *g/l*	Sodium chloride content in the medium for callus subcultivation, *g/l*	L-proline content in callus tissues, *mg L-proline/g callus tissue*
DK633/266		
60	0	483.16 ± 17.13
60	6	764.98 ± 18.45
60	30	1790.87 ± 19.88
60	60	2633.71 ± 19.24
DK633/325		
60	0	1431.33 ± 41.62
60	6	2573.36 ± 39.40
60	30	2698.25 ± 44.30
60	60	3621.32 ± 43.63
DK236		
30	0	1509.23 ± 22.20
30	30	2100.29 ± 16.40
30	60	4059.92 ± 27.69

Table 2. Content of L-proline in maize callus tissues after removal of chloride load.

Sucrose content in the medium for callus induction, *g/l*	Sodium chloride content in the medium for callus subcultivation, *g/l*	L-proline content in callus tissues, *mg L-proline/g callus tissue*
DK3070		
30	0	250.18 ± 14.97
30	6	319.72 ± 18.17
30	30	390.46 ± 34.21
30	60	627.00 ± 44.58
60	0	192.57 ± 13.69
60	6	272.75 ± 23.90
60	30	259.11 ± 12.39
60	60	334.94 ± 38.22
DK6080		
30	30	1833.14 ± 54.71
30	60	5529.57 ± 53.06
PLS61		
60	6	2082.63 ± 21.25
60	30	2257.69 ± 46.52
60	60	2304.97 ± 40.18

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Responses to aggregate trait selection for Chilo partellus (Swinhoe) resistance in maize (Zea Mays L.) population

— Mutinda, CJM; Ajala, SO; Ayiecho, PO

The primary objective of the study was to improve maize population ICZ3 for resistance to the spotted stem borer, *Chilo partellus*, while improving grain yield. ICZ3(IC-90-W1) is a white-grained, early-to-medium population. Data presented herein uses rank summation index (RSI, Mulamba NN and Mock JJ, Egypt J of Genet and Gytol 7:40-51, 1978), a facile method that we show here is feasible for selecting resistance to *Chilo partellus*, while improving grain yield.

A total of 144 S_2 lines and 140 S_2 test cross progenies derived from ICZ3 were evaluated for damage parameters (leaf feeding, dead heart, and stem tunneling) caused by *Chilo partellus* and several agronomic traits. The experimental locations were Mbita Point Field Station (MPFS) and Ungoye, which are ICIPE testing sites. Both locations are on the shores of Lake Victoria in

Western Kenya (latitude 0^0 25'-0^0 30' South, longitude 34^0 15 East and altitude 1240), and have bimodal rainfall distribution with two distinct peaks. The early season ("long rains") starts from late March and ends in late September or early October, and the late season ("short rains") starts from late September or early October to December. Ungoye is 35 km from Mbita Point.

Genotypes were grown in a randomized complete block design with single row plots. Each row was 5 m long separated into two 2.25 m halves with a space of 0.5 m in the middle. Spacing was 0.75 m between rows and 0.25 m between hills. Each hill was planted with two plants but later thinned to one three weeks after germination to give a maximum of 10 plants/2.25 m row and a density of approximately 53,333 plants/ha. All plants in half of the row were artificially infested with 30 first instar *C.partellus* larvae reared on artificial diet (Ochieng et al., Insect Sci and Application 6:425-428, 1985) three weeks after emergence. Two replicate plantings of each experiment were made at each site. Appropriate culture practices, such as fertilizer application, weeding, bird or monkey scaring were carried out as deemed necessary during the season.

Data on foliar lesions and dead heart were taken at four weeks after infestation. Foliar lesions were scored on a 1-9 scale (1 = resistant and 9 = susceptible), while dead heart was assessed as the proportion of plants in a plot showing the symptom. Extent of stem tunneling by the larvae was estimated at harvest as the percentage of the plant height. Other agronomic data recorded were plant height, stand at harvest, number of ears harvested, mean length of five ears per plot, moisture content at harvest, and grain yield. Grain yield was obtained as grain weight adjusted to 13% moisture content. Yield reduction was calculated as the difference between the yield of the uninfested control and the infested.

Dead heart and stem tunneling data for each location were transformed into arc-sine values before subjecting to analysis of variance (ANOVA). On this transformed scale, error variances were highly homogenous according to Barlett's test (Barlett, J Royal Society Supp. 4:137-183, 1939). Combined ANOVA was therefore carried out. Two RSIs were constructed to determine the ranking of each line within the population for suitable response. The first index (RSI-1) was obtained by ranking the means of each leaf feeding (LF), dead heart (DH), and stem tunneling (ST) for each line, summing the ranking of the line to obtain its aggregate performance compared with other lines within the same population. A second (RSI-2) was obtained using the three traits and grain yield. Rank Summation Index was summarized as:

- RSI = ΣRi's, where Ri is the rank of the mean of each of the desired traits.
- RS1-1 = Aggregate performance of a genotype using the ranking of leaf feeding, dead heart, and stem tunneling.
- RSI-2 = Aggregate performance of a genotype based on ranked means of leaf feeding, dead heart, stem tunneling, and grain yield.

Thus the lowest possible values for the two indices would be three and four, respectively. An entry with the least damage for foliar feeding, dead heart, and stem tunneling and highest grain yield will rank first for the four traits.

Expectations of mean squares (EMS) from analysis of variance were used to estimate genotypic (σ^2g), genotype x environment (σ^2ge) interaction, error (σ^2), and phenotypic (σ^2ph) components of variance, while expectations of mean cross products (EMCP) from analysis of covariance were used to estimate genotype correlations. Standard errors (S.E.) for each of the variances (σ^2i), except phenotypic variance, were calculated per Hallauer 1972 Iowa State J of Sci 45:575-593:

S.E. $\sigma^2i = [2/C^2\{msi^2/(dfi=2)\}]^{½}$

Standard errors for phenotypic variance were computed as:

S.E. σ^2 ph = $[(1/re^2)\{msg/(dfi+2)\}]^{½}$

Abbreviations: msi, dfi, and C^2 are mean squares, degree of freedom, and coefficient of the component in the EMS for trait I, respectively; msg is the mean square for genotype, r = number of replicates, and e = number of environments or locations. Habitability (σh^2) estimates were calculated as proportions of total variance due to genetic causes with S.E., also calculated as proportions of S.E. of σ^2g to σ^2ph. Entry means across locations and replicates were used to calculate simple correlations and stepwise multiple regressions. Predicted responses (ΔG) for single trait selection were calculated as:

$(\Delta G)=k.\ ph.\ h^2$

where k is the standard selection differential (k = 1.76 for selection intensity of 10%), ph is the phenotypic standardized deviation, and h^2 represents heritability for the trait.

RSI values were subjected to both analysis of variance and covariance, and the information obtained from EMS and EMCP was used to estimate variance components and heritability. Predicted response to selection for RSI was then calculated using the above formula. This was then compared with the formula of Mock and Eberhart (1972) for calculating gains from aggregate selection as follows:

$\Delta H = ai\Delta gi$

Where ai is the economic weight for the ith trait (character) and Δgi, which was calculated using the formula of Pesek and Baker (1969a), is the predicted response for trait (character) due to index selection.

Economic weights were -1,-1,-1, and 1 for foliar, dead heart, stem tunneling damages, and grain yield, respectively. Coefficient (b values) used in the estimation were obtained by solving the equation $bi = (Xij)^{-1}(gij)(ai)$, where Xij and gij are variance covariance matrices of phenotypic and genotypic values, respectively, for the four traits in each of the progeny types.

Correlated responses due to single and aggregate trait selection created by RSI were calculated as:

$CR_{y(x)} = i_x.h_x.h_y.r_{gx.y}$ phy (Falconer, 1960)

Where ix = selection intensity applied to trait x, $.h_x$ and $.h_y$ are square roots of heritability estimates for traits x and y, respectively, $r_{gx.y}$ is the genetic correlation between the two traits, and phy is the square root of phenotypic variance for trait y.

Estimates of heritability components of variance for traits and RSI are presented in Table 1. Heritability estimates in most cases were moderate for the S_2 families, thus suggesting that si-

Table 1. Genotypic (σ^2g), genotype X environment (σ^2ge) interaction, environment (σ^2e), phenotypic (σ^2ph) variances and heritability (h^2) estimates of traits and Rank Summation Index (RSI) in each of the two progenies.

Progeny	Trait	σ^2g	σ^2ge	σ^2e	σ^2ph*	h^2
Test	Leaf feeding	0.01±0.02	-0.07±0.05	0.95±0.08	0.21±0.05	0.05±0.09
crosses	Dead heart %	0.01±0.01	0.01±0.02	0.27±0.02	0.09±0.02	0.11±0.11
	Stem tunneling	0.45±0.43	0.90±0.74	10.86±0.82	3.62±0.86	0.12±0.11
	Grain yield (t/ha)	0.10±0.12	0.15±0.22	3.51±0.29	1.05±0.25	0.10±0.11
	Plant height (cm)	314.03±214.53	424.40±354.79	5151.24±430.77	1814.04±304.60	0.17±0.12
	Rsi-1	40.61±16.54	-38.62±40.27	3956.47±332.02	1010.42±229.15	0.40±0.02
	Rsi-2	6.55±2.12	-4.90±5.24	5071.93±425.63	1272.08±300.68	0.01±0.002
S_2 lines	Leaf feeding	0.03±0.03	0.06±0.04	0.64±0.06	0.11±0.05	0.27±0.27
	Dead heart %	0.01±0.01	-0.01±0.02	0.45±0.04	0.11±0.03	0.09±0.09
	Stem tunneling	1.12±0.99	-0.56±1.24	21.47±1.86	6.21±1.47	0.18±0.16
	Grain yield (t/ha)	0.20±0.11	-0.11±0.14	2.51±0.22	0.78±0.18	0.26±0.14
	Plant height (cm)	118.860±33.33	23.25±29.99	444.54±38.55	241.37±1.84	0.49±0.14
	Rsi-1	18.05±15.40	-17.98±30.80	3604.90±309.12	910.29±218.87	0.02±0.16
	Rsi-2	405.30±223.71	-790.60±447.43	5173.87±443.66	1303.47±361.54	0.31±0.17

multaneous improvement of these traits should be possible, along with the use of selection indices. However, for the test cross hybrids, the heritability estimates were low for the majority of the traits. For most traits, genetic (σ^2g), environmental (σ^2e), and phenotypic (σ^2ph) variances exceeded twice their standard errors. Except for a few cases the estimates of genotype by environment variances (σ^2ge) were either negative or smaller than their respective standard errors.

Table 2. Simple linear correlations of *Chilo partellus* parameters on mature plant traits and grain yield.

Trait	Progeny type	Leaf feeding	Dead heart	Stem tunnel-ing	RSI-1	RSI-2
Plant height (cm)	(i)	0.11	-0.07	0.29**	-0.13	0.00
	(ii)	0.05	-0.03	0.22**	-0.13	-0.02
Stand count	(i)	-0.09	0.19*	-0.11	0.31**	-0.04
	(ii)	-0.08	0.30**	-0.08	0.48**	0.08
Ear length	(i)	-0.07	0.40**	-0.09	0.95**	-0.13
	(ii)	-0.14	0.32**	-0.15	0.99**	0.21**
Ear number	(i)	0.01	0.26**	0.09	0.34**	-0.14
	(ii)	-0.01	0.25**	-0.03	0.45**	0.12
Moisture (%)	(i)	-0.09	0.27**	-0.14	0.49**	-0.14
	(ii)	0.04	0.24**	-0.14	0.61**	-0.02
Grain yield (t/ha)	(i)	-0.03	-0.04	-0.05	-0.01	-0.08
	(ii)	-0.01	-0.03	-0.04	-0.02	0.04

*,** significant at P < 0.05 and 0.01, respectively. (i) = testcrosses. (ii) = progenies.

C. partellus leaf feeding and stem tunneling were generally negatively correlated with mature plant characteristics, including grain yield (Table 2). Dead heart showed highly significant correlations with stand count, ear length, ear number, and moisture % at harvest for the two progeny types. Rank summation index RSI-1 showed highly significant (P < 0.01) correlations with the four agronomic traits as opposed to those involving RS-2, which were generally negative apart from a few cases.

The possible contribution of each of the insect damage parameters to grain yield reduction was examined using step-wise multiple regressions (Table 3). In the two progeny types, stem tunneling had the greatest contribution towards grain yield reduction (R^2 being 0.36 for S_2 lines and 0.45 for the testcross). Other groups similarly find low association of yield loss with foliar feeding, but with mostly with either dead heart (Mohyuddin and Attique (PANS 24:111-113, 1978; Pathak and Othieno (Maydica 35:247-252, 1990), or to stem tunneling (Ajala and Saxena, Appl Entomol Zool 29(4):469-476, 1994; using step-wise multiple regressions, and a path coefficient analyses).

Predicted direct responses to selection for grain yield, and insect parameters were much lower than when single trait se-

Table 3. Step-wise multiple regression of grain yield and insect damage parameters. Abbreviations. b-values: unstandardized partial regression coefficients; R^2: coefficients of determination (R^2); ΔR^2: R^2 change.

Family type	Trait	b-value	R^2	ΔR^2
Test crosses	Leaf feeding	-0.01	-0.01	0.01
	Dead heart %	1.40	0.01	0.00
	Stem tunneling	-0.14	0.45	0.44
S_2 lines	Leaf feeding	0.002	0.20	0.20
	Dead heart %	-0.09	0.27	0.07
	Stem tunneling	0.02	0.36	0.09

Table 4. Predicted direct response (ΔG/CYCLE) to single trait selection, the aggregate trait RSI and to index selection in each of the two progeny types.

Family type	Gains from selection				
	Single trait selection				
	Grain yield (t/ha)	Leaf feeding	Dead heart (%)	Stem tunneling (%)	RSI
Test crosses	0.32	-0.08	-0.12	-0.22	4.48
S_2 lines	0.40	-0.16	-0.05	-0.79	10.75
	Index selection				
	Grain yield (t/ha)	Leaf feeding	Dead heart (%)	Stem tunneling (%)	aiΔgi
Test crosses	0.12	-0.09	-0.06	-0.08	4.20
S_2 lines	0.10	-0.08	-0.04	-0.63	3.55

lection analysis was carried out for each of the traits (Table 4). Response due to index selection (ΔH) was higher for test cross hybrids than that of the S_2 progenies while for Rank Summation Index (RSI), more progress was achieved in S_2 progenies than in the test cross hybrids where RSI gave more than double the progress of the aggregate trait selection in S_2 progenies.

Predicted correlated responses in grain yield based from selection based on insect parameters and the rank summation indices are presented in Table 5. Gains expressed as percentage of the means in the two progeny types were lower than expected from direct selection for grain yield in all cases except for RSI-2 in S_2 families.

Table 5. Predicted correlated responses (per cycle) in grain yield (t/ha).

Selection criteria	Test cross hybrids	S2 families
Leaf feeding	-0.02	-0.05
	(-0.84)	(-2.04)
Dead heart (%)	(-3.45)	-0.23
Stem tunneling (%)	-0.25	(-9.39)
	(-4.32)	-0.32
RSI-1	-0.002	(-13.06)
	(-0.03)	-0.001
RSI-2	-0.64	0.20
	(-11.50)	(8.16)
()= Correlated responses expressed as % of the overall mean yield of the respective progeny type		

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Effect of the medium used in the growth of *Agrobacterium* strain on subsequent *gus* expression of the infected immature zygotic embryos of the tropical maize line

— Ombori, O; Muoma, JO; Machuka, J

The generation of stably transformed transgenic plants to assess gene function is a lengthy process. As an alternative, assessment of the transfer of transgenes into plant cells or tissues is often performed by use of transient gene expression assays. Transient *gus* expression systems are valuable tools for understanding the functions of genes in specific organs of plants.

Transient *gus* expression was carried out in the present study to test whether the constitution of the medium for growth of *Agrobacterium* had significant effect on the subsequent *gus* expression in the immature zygotic embryos of H627 maize line. Two different media were tested, namely, YEP (Yeast Peptone extract) and LB Luria-Bertani medium (LB) agar. EHA101(pTF102) *Agrobacterium* strain grown on these media was used to infect immature zygotic embryos of H627 maize line. The infection comprised N6 salts, vitamins, 1.5 mg l^{-1} 2,4-D, 0.7 mg l^{-1} L-proline, 68.4 g l^{-1} sucrose, 36 g l^{-1} glucose, and 200 µM Acetosyringone. The T-DNA region of the pTF102 vector contained *gus* reporter gene. The infected embryos were transferred onto the co-cultivation medium (N6 salt, vitamins, 1.5 mg l^{-1} 2,4-D, 0.7 mg l^{-1} L-proline, 30 g l^{-1} sucrose, 0.85 mg l^{-1} silver nitrate, 200 µM AS, 400 mg l^{-1} cystene, and 3 g l^{-1} gerlite) and incubated in the dark at 20°C for three days. Transient *Gus* activity studies were carried on immature embryos on the third day of co-cultivation. One hundred embryos were stained for *gus* activity with 1 mM 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (XGluc) and 50 mM NaH_2PO_4 (pH 7.0) solution and incubated in the dark at 37°C for 24 hours. The tissues were

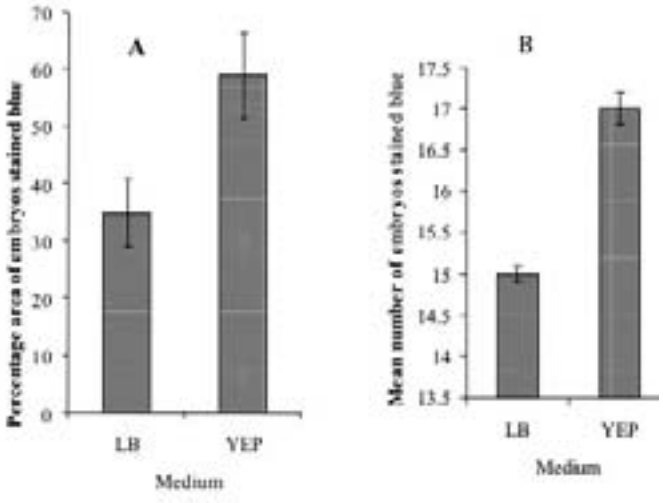

Figure 1. Immature embryos of H627 maize genotypes that showed *gus* activity when infected with EHA101(PTF102) pre-cultured in two different media before infection. A: Percentage areas of embryos stained blue. B: Mean number of embryos stained blue.

cleared using 70% (w/v) ethanol for 1-2 hours.

The gus expression was observed mostly at the edges of the infected immature zygotic embryos. The number and percentage area of the embryos that were stained blue due to transient *gus* expression was significantly ($p < 0.05$) higher when EHA101(pTF102) *Agrobacterium* strain was grown on YEP medium compared to LB agar prior to the infection of immature embryos of H627 (Fig. 1). This indicates that the type of medium used for the growth of *Agrobacterium* strains prior to transformation influenced the transfer of the transgenes into the infected embryos.

Agrobacterium mediated transformation of leaf-derived callus of tropical maize (*Zea mays L.*) inbred lines

— Ombori, O; Muoma, JO; Machuka, J

Genetic transformation of maize has been a challenge. In this study embryogenic callus was initiated from young leaves of six tropical maize inbred lines (CML216, CML331, TL09, TL18, TL27, MU25). Embryogenic callus were immersed in the infection medium containing N6 macro and micro salts and modified KT vitamins supplemented with 2 mg l^{-1} glycine, 100 mg l^{-1} myo-inositol, 2.88 g l^{-1} proline, 100 mg l^{-1} casein hydrolysate and 2.2 mg l^{-1} 2,4-D, 68.4 g l^{-1} sucrose, 36 g l^{-1} glucose, and 200 µM AS (acetosyringone). The callus was then immersed in EHA101(pTF102) *A. tumefaciens* suspension supplemented with 200 µM AS and then incubated in the dark for 60 minutes. The infected callus were transferred onto the co-cultivation medium containing N6 macro and micro salts and modified KT vitamins supplemented with 2 mg l^{-1} glycine, 100 mg l^{-1} myo-inositol, 2.88 mg l^{-1} proline, 100 mg l^{-1} casein hydrolysate, 2.2 mg l^{-1} 2,4-D, 800 mg l^{-1} silver nitrate and 20 g l^{-1} sucrose, 200 µM AS (acetosyringone) and 0.3 % gerlite and incubated in the dark at 20°C for three days. Callus was transferred onto selection medium I containing N6 macro and micro salts and modified KT vitamins supplemented with 2 mg l^{-1} glycine, 100 mg l^{-1} myo-inositol, 2.88 mg l^{-1} proline, 100 mg l^{-1} casein hydrolysate, 2.2 mg l^{-1} 2,4-D, 800 mg l^{-1} silver nitrate and 20 g l^{-1} sucrose, 200 µM AS (acetosyringone), 1.5 mg l^{-1} bialaphos and 0.3 % gerlite. Surviving callus was transferred onto selection medium II containing the same composition as selection medium I except 1.5 mg l^{-1} bialaphos was replaced with 3 mg l^{-1} bialaphos. Actively growing callus on selection medium II was transferred onto the regeneration medium containing N6 macro and micro elements and modified KT vitamins supplemented with 2 mg l^{-1} glycine, 100 mg l^{-1} myo-inositol, 2.88 g l^{-1} proline, 100 mg l^{-1} casein hydrolysate, 2% sucrose, 0.3% gerlite and 0.5 mg l^{-1} benzylaminopurine (BAP).

Table 1. Selection of a leaf-derived callus on bialaphos-containing culture medium.

Genotype	Number of callus inoculated	Number of callus surviving	Transformation frequency (%)	Number of putative transformed plants regenerated
CML216	59	1	1.7	1
CML331	18	0	0	0
TL09	47	0	0	0
TL18	227	4	1.8	3
TL27	15	0	0	0
MU25	204	2	0.9	0

All the non-infected callus (control) turned brown, necrotic, and died when they were grown on selection medium II after four weeks of culture. Actively growing resistant embryogenic callus were observed in CML216, TL18, and MU25 maize callus lines on the fourth week on selection medium II. Infected callus of CML331, TL09, and TL27 did not survive on bialaphos (Table 1). The frequency of callus that survived on selection medium containing bialaphos was very low (Table 1). The difference between the maize lines on the percentage of callus that survived was statistically significant ($p < 0.05$). Transformation frequency of the resistant surviving callus selected on bialaphos (3 mg l^{-1}) containing medium ranged between 0 to 1.8 % (Table 1). Plants were regenerated on a medium containing 0.5 mg l^{-1} BAP from putative transformed callus (surviving callus) of CML216 and TL18 and none from CML331, TL09, TL27, and MU25 (Table 1). The number of plants regenerated was low, ranging from 1 to 3 among the genotypes.

Transient *gus* activity was used as an initial step to assess if the transfer of the transgene had taken place. Transient *gus* expression was confirmed by histochemical β-glucuronidase (*GUS*) activity. Transient g*us* expression was observed on the third day of co-cultivation of the leaf-derived embryogenic callus after infection with EHA101(pTF102) *A. tumefaciens*. Transient *gus* expression was detected in the infected leaf-derived callus of CML216, CML331,

Table 2. Transient *gus* expression of a leaf-derived callus of six tropical maize inbred lines infected with EHA101(pTF102) *A. tumefaciens.*

Genotype	Total number of callus tested	Number of callus showing *gus* activity	Frequency of callus showing *gus* activity	Mean percentage area of callus showing *gus* activity (%)
CML216	5	1	20	4.0 ± 1.0a[+]
CML331	5	3	60	8.00 ± 4.00a
TL09	20	0	0	0a
TL18	52	21	40.3	13.00 ± 2.00b
TL27	50	0	0	0a
MU25	46	0	0	0a

[+]Means followed by the same letters within columns are not significantly different according to Tukey's Honest Significant Difference at 5% level.

and TL18 and maize lines tested. Transient *gus* activity was not detected in non-transformed callus (control) and infected callus of TL09, TL27, and MU25 maize lines (Table 2). The frequency of callus expressing *gus* activity ranged from 0% to 60%. Significant differences ($p < 0.05$) were detected among the maize lines tested on percentage area of callus expressing the *gus* activity. TL18 had the highest mean percentage area of callus showing *gus* activity (13%) followed by CML331 (8%), CML216 (4%), and TL27 and MU25 (0%). Transformation frequency of CML331 embryogenic callus on the selection medium did not correspond to the transient *gus* expression. This could be due to transient expression when the transgene is transferred into the cytoplasm of the plant cell but stable integration into the maize genome does not occur. Transient *gus* expression assays have proved to be a useful tool that is routinely used as an initial step to demonstrate *gus* expression in cells and tissues of *Agrobacterium*-mediated transformed plants prior to stable integration of the transgene.

Results from this study show that the transfer of the transgene into the infected callus was genotype dependent. There is need to assess if there is stable integration and inheritance of the transgene in the plants that were regenerated in this study.

PIRACICABA, SP, BRAZIL
ESALQ, Universidade de São Paulo

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, Ann Bot 82:569-576, 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 and 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, it was further demonstrated the presence of telomeric sequences on the termini of this chromosome (unpublished). Another type of 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 R_1 progenies resulting from regenerated plants derived from a callus culture designed 12F, obtained from line 13342/5. Figure 1 shows C-banded mitotic prophases of regenerated plants, respectively homozygous for the normal K7L (Fig. 1A) and heterozygous for the presence of the amplified K7L (Fig. 1B). Fourteen heterozygous plants were self-fecundated, and in the progeny 19 amplified K7L homozygotes, 58 heterozygotes, and 39 normal K7L homozygotes were obtained. The homozygous plants for the K7L amplification survived. We interpreted that the amplification of K7L would not have been derived from a BFB event as it was the case of 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

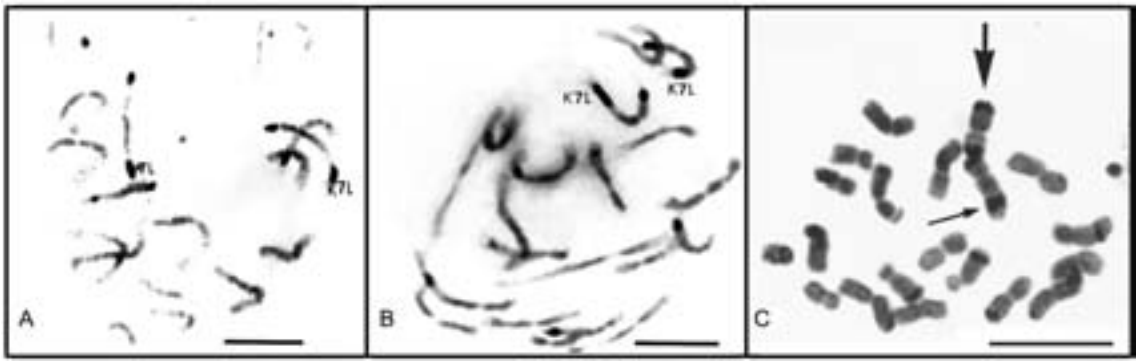

Figure 1. C-banded mitotic prophases of plants regenerated from a two-year-old callus culture designated 12F (A, B) and C-banded mitotic metaphase of a 2-month-old callus culture derived from hybrid 13342/5 x 13342/1 (C). Note the normal size of K7L in both homologues in A and a K7L amplification in B. The small arrow in C points to chromosome 7 showing an asymmetric band on the long arm, and the large arrow indicates a normal band for long arm of chromosome 7. Bar scale = 10µm.

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, 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, Mexico, 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., 1996).

PISCATAWAY, NEW JERSEY[1]
Waksman Institute, Rutgers University
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A sequence-indexed single gene knockout resource for maize

— Li, Y[1]; Huang, J[1]; He, L[1]; Wang, Q[1]; Xiong, W[2]; Segal, G[1]; Du, C[2]; Dooner, HK[1]

The purpose of this note is to apprise the maize genetics community of progress in our NSF-funded project to develop a single-gene-knockout reverse genetics resource based on the transposon *Ds*. We have three objectives: (i) to construct a set of 120 roughly equidistant transgenic *Ds* launching platforms that will allow simple visual selection of element transposition from any region of the genome and, thus, enable researchers to generate regional gene knockout collections; (ii) to isolate several thousand *Ds* insertion sites from model platforms and sequence-index them using a combination of next-generation sequencing (NGS) technology and computational tools that should make the method generalizable to any collection of insertions produced in a common background; and (iii) to develop a web-searchable database of insertion site sequences cross-referenced to stocks available from the Maize Genetics Stock Center.

The transposons *Ac* and *Ds* tend to insert in or close to genes and are, therefore, excellent gene-searching engines in the highly repetitive maize genome (Cowperthwaite et al., Plant Cell 14:713-726, 2002; Vollbrecht et al., Plant Cell 22:1667-1685, 2010). Both elements show a distinct preference to transpose to linked sites: about one-third of all transpositions are within 7 cM on either side of the donor site (Cowperthwaite et al., Plant Cell 14:713-726, 2002; Dooner and Belachew, Genetics, 122:447-457, 1989; Greenblatt, Genetics 108:471-485, 1984). Therefore, *Ac/Ds* elements are excellent gene tagging tools for localized transposon mutagenesis and complement *Mutator* elements, which show a more random pattern of insertion across the genome.

In our project, we transform the *c1* HiII hybrid with *Agrobacterium* using a standard binary vector system (Frame et al., Plant Physiology 129:13-22, 2002). Our engineered construct is based on the *c1-m2* mutable allele originally described by McClintock (Carnegie Inst Wash Yrbk 47, 155-169, 1948), in which a *Ds* element is inserted in the third exon of the *c1* gene (Cone et al., Proc Natl Acad Sci USA 83: 9631-9635, 1986). In the presence of *Ac*, *c1-m2* shows a spotted aleurone phenotype. We have modified the *Ds* element to include a *GFP* marker expressed behind a 22-kD zein promoter that allows us to trace the movement of the element in the genome (Fig. 1).

As outlined in Figure 2, when test-crossed with a colorless *Ac* donor line, most transgenotes showed kernel spotting. The c-m spotted phenotype resembles that of the native *c1-m2* allele and segregates 1:1, suggesting transgene integration at a single locus.

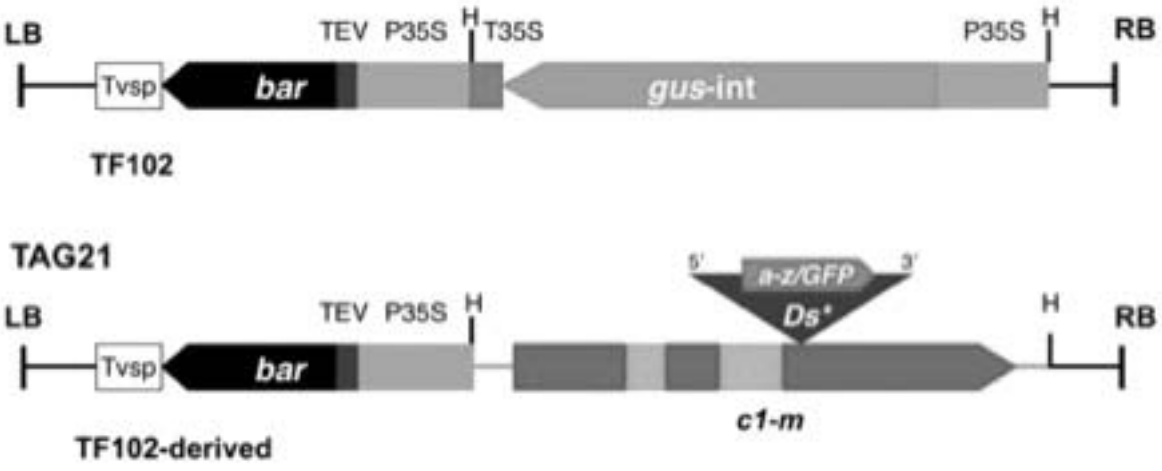

Figure 1. T-DNA construct used in *Agrobacterium* transformation of Hi-II (*c1*) embryos. The *gus* HindIII (H) fragment of pTF102 was replaced with the *c1-m Ds** excision reporter shown in TAG 21 (LB, left border; RB, right border).

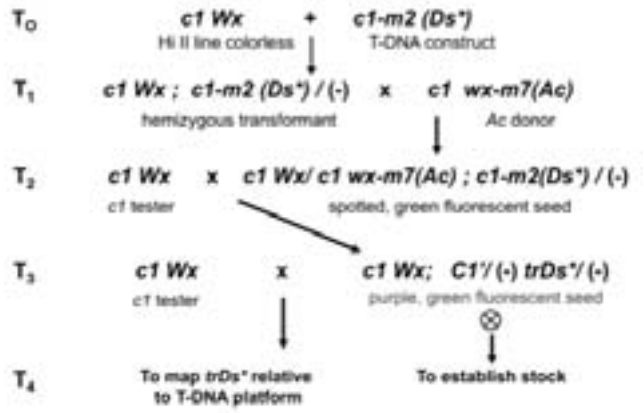

Figure 2. Genetic scheme to generate transgenic *c1-m* lines and isolate *C'* revertants carrying a *trDs** element.

Furthermore, all spotted kernels are green fluorescent, confirming that the spots are due to transpositions of *Ds:GFP* in response to *Ac*. Southern-blot and progeny analyses confirmed the integration, expression, and inheritance of the transgenes in the T1 and T2 generations. Sequences adjacent to the T-DNA launching platforms were isolated by inverse PCR, sequenced, and mapped to the B73 reference genome. So far, we have generated 160 active transgenic lines and mapped 82 platforms to the maize genome. Their location and those of any new mapped platforms can be found in our project website, http://www.acdsinsertions.org. These lines are being deposited in the Maize Stock Center and will serve as starting materials for the generation of gene knockouts by community researchers.

We have measured the reversion frequency of each platform by crossing to a colorless *c1* tester and selecting for C' revertants. The average reversion frequency was 2.97 x 10^{-2} on the male side and 1.64 x 10^{-2} on the female side, a bit higher than that of the native *c1-m2* allele (2.0 x 10^{-2} as male, and 0.7 x 10^{-2} as female). The higher reversion frequency makes these lines more efficient for generating mutations.

To date, more than 12,000 purple C' revertants have been selected from nine platforms, and those from three platforms have been further characterized genetically. The *GFP* marker in the *c1-m2 (Ds)* allele serves to track *Ds** reinsertion after excision from the *c1* gene. The C' revertant kernels carrying a *trDs** are readily distinguished from those without *Ds* under blue light illumination in a fluorescence dissecting microscope. The average reinsertion frequency is close to 50%, similar to what has been observed in nontransgenic systems.

We use the *c1-m2* transgenic lines as pollen donors because about 93% of C' selections from the male side are concordant (C' endosperm and embryo), whereas only 1/3 of C' selections from the female side are concordant, i.e., heritable. The genetic distance between the *trDs** and the *C'* allele in the T-DNA can be obtained readily by scoring the fraction of green fluorescent colorless kernels in the testcross progeny. As expected, the majority (74.1%) of the genetically linked reinsertions are tightly linked (within 7 cM) to the original T-DNA platform. 80% of the tightly linked sites fall between 0 and 3.5 cM.

The *trDs** elements are mapped to the reference genome by isolating and sequencing the reinsertion sites. To do so, we adopted the splinkerette-PCR method (Uren et al., Nature Protocols 4:789-798, 2009) and constructed libraries for next generation sequencing (NGS) of pooled *trDs** insertion sites. In brief, genomic DNA of seedling tissue from *C' GFP* revertants arranged in 3-D pools is sheared to 2-3 kb and ligated with a double stranded splinkerette oligonucleotide. The ligation products are amplified by nested PCR, in which the first reaction is carried out with a *GFP* primer and an adaptor primer that can only anneal to a template synthesized by linear amplification with the *GFP* primer. The nested reaction is carried out with a primer from the end of *Ds* and a barcoded adaptor primer. The amplified insertion junctions are sequenced in-house by a SOLiD 5500xl system.

At present, we have used the above protocol on two sets of 960 C' revertants arranged in 3-D pools of 10 plates x 8 rows x 12 columns. A new pipeline, *InsertionMapper*, was specifically developed for the project. Using this pipeline, we have been able to assign *trDs** junction sequences to 1320 individual C' revertants and anchor them to the reference genome. Among them, 1183 are inserted in single copy DNA, and 809 are in genes. The results of the physical mapping generally are consistent with those of the genetic mapping. All the above information is provided in our website, http://www.acdsinsertions.org, and updated periodically. The website offers BLAST search capabilities for researchers to identify the stock(s) of interest. The stocks will be available from the Maize Genetics Stock Center, upon advance APHIS notification of interstate movement.

Acknowledgment. This project is supported by NSF-PGRP grant DBI 0929350.

SHOKAN, NEW YORK

SSC's doubled haploid research at Ames and DeKalb

— Chase, SS

This note comes after much encouragement from Dave Weber for information about my work on "doubled haploids." While developing the system my aims were always practical — produce better parents. I was lucky to have Sprague's Stiff Stalk Synthetic available, plus some fine stocks I had brought with me from Cornell (taken from accompanying letter from SSC to Ed Coe August 2012).

The amount of haploid work accomplished at Ames and DeKalb was substantial. At both places the work was carried out in competition with other activities: at Ames, organizing lectures and teaching plant cytogenetics at the leading edge to graduate students, and at DeKalb in competition with a company-wide reorganization of corn breeding, development of hybrids for the north of France, development of a breeding program for "Colorado" type hybrids for Argentina, and much more.

I am pleased the "doubled haploid" technique is in substantial use today. In Botanical Review, 1969, I ventured that "Agro-

nomically, other than in the commercial breeding program for which I was personally responsible, little use has been made of the [doubled haploid] method in the development of homozygous diploids. Increased interest in and use of such radical techniques is likely in the future as the challenges of intensified commercial maize culture and of the highly competitive hybrid seedcorn market necessitate an increasingly high degree of responsiveness on the part of the maize breeder."

The practicality of the method today certainly owes much to Coe's "stock 6" and its derivatives, to improvements and techniques for genome doubling, and to the higher technical skills of maize breeders.

Firsts (achieved by SSC):

1) First haploid to doubled haploid. The first homozygous diploid derived from a haploid was out of sweet corn (Golden Cross Bantam).

2) First substantial confirmation of different rates of parthenogenesis among female parents. (Stadler obtained a frequency of about 1:100 in a diploid multiple recessive tester; most prior information suggested rates of about 1:1000.)

3) First recognition that rates of parthenogenesis were influenced by the male parent.

4) First haploids in quantity.

5) First observation of high rates of somatic chromosome complement doubling in haploids.

6) First doubled haploids in quantity.

7) First to use "embryo markers" for dry seed haploid selection (Pu, etc.).

(A major disadvantage of Pu, purple embryo marker, is — was — that it occurs widely in Corn Belt maize; Stadler told me that 15% of the then available inbreds in use had the purple plumule phenotype, hence Pu was not a "clean" marker for my purposes.)

8) First doubled haploid line(s) in successful commercial hybrid(s). (Example: DeKalb 640)

9) First "second generation" doubled haploid lines in commercial hybrid (H2386 and H2398, both ex H73xH225).

10) First cloning of haploid through reproductive process (W23 haploid).

11) First, with Sam Goodsell, to demonstrate cytoplasmic transfer through androgenesis.

12) First to demonstrate (in W22) the possibility of "fixing" high-performing substrains of long established inbreds through haploidy.

SIOUX FALLS, SD

Quantitative trait loci for leaf angle, leaf width, leaf length, and plant height in IBM-94

— Wassom, J

Modern maize varieties are more productive than varieties of a few decades ago, partly due to higher population densities and adaptations that permit vigorous growth at high densities. Plant forms that enable efficient light interception at high population densities will increase yield production under modern field conditions. Leaf angle has been shown to affect yield especially at high densities. To determine the QTL affecting leaf traits and plant height in maize I experimented with IBM-94, a B73 x Mo17 recombinant inbred line (RIL) population developed by other researchers for genetic studies (Coe et al., Plant Physiol 128:9-12, 2002; Cone et al., Plant Physiol 130:1598-1605, 2002; Lee et al., Plant Mol Biol 48:453-461, 2002).

Seed for the 93 RIL constituting IBM-94 was obtained from the Maize Genetics Cooperative Stock Center (http://maizecoop.cropsci.uiuc.edu). In year 2006 the original seed was grown and plants were self-pollinated to produce enough RIL seed to plant replicated experimental plots at Sioux Falls, South Dakota, USA. In years 2007, 2008, and 2009 the 93 RIL were grown in randomized complete blocks with three replicates each year. Space was limited, so each plot included four plants spaced 279 mm apart in rows 76 mm apart. There were no extra spaces between plots in rows. At anthesis or soon after, the total plant height to the tassel tip and the leaf at the uppermost ear shoot were measured on the two center plants in each plot. Leaf measurements included the leaf angle from vertical, maximum leaf width, and distance from the ligule to the tip of the straightened leaf.

Statistical analysis, including analysis of variance and heritability was performed with PLABSTAT (University of Hohenheim, Germany, https://plant-breeding.uni-hohenheim.de/~ipspwww/soft.html). Genetic map distances of markers and molecular marker genotypes of each RIL were obtained from the Maize Genetics and Genomics Database (MaizeGDB) (http://www.maizegdb.org). Phenotype data for the RILs was combined with marker genotypic information and map distances from the MaizeGDB IBM2 map to analyze for QTL using PLABMQTL (University of Hohenheim, Germany, https://plant-breeding.uni-hohenheim.de/~ipspwww/soft.html).

There was significant variation among genotypes (Table 1; opposite page) and 1 to 3 QTL identified for each of the measured traits (Table 2). Larger plots and the larger IBM-302 population might have improved precision and enabled detection of more QTL. The total area taken up by nursery rows and experimental plots was about 50 by 75 feet, illustrating that even with limited resources the IBM-94 population and MaizeGDB data can be used for QTL mapping.

ST. PAUL, MINNESOTA

Adapted *Zea diploperennis:* Zea diploperennis-maize hybrid adapted to the U.S. corn belt

— Carlson, LA; Price, SC

Experiment 1. 1979, a cross between *Zea diploperennis* No. 1190 as female parent and Minhybrid 8201 (A641 x W182B) as male was made in St. Paul (materials were furnished by John Doebley of the University of Wisconsin and Jon Geadelmann of the University of Minnesota). Approximately 6,000 F2 seeds were collected from an isolation plot of the F1 plants. In 1982

Table 1. Characterization of IBM-94 RIL grown at Sioux Falls, SD, in 2007, 2008, and 2009.

	Leaf Angle		Leaf Width		Leaf Length		Plant Height	
	Degrees		mm					
RIL Mean †	26 ± 2.6		91.0 ± 2.95		764.6 ± 20.76		2231.3 ± 101.24	
Range among RILs ‡	9 to 53		71.2 to 114.5		641.7 to 907.0		1323.2 to 2796.8	
	ANOVA							
Source of Variation	MS, F							
Genotypes	623.8	10.49**	576.6	7.24**	26374	6.80**	362149	3.93**
Years	1026.0	9.54*	990.6	3.31	582080	35.41**	18780568	71.16**
Genotype × Year	59.5	1.68**	79.6	1.52**	3878	1.86**	92242	4.27**
Replications in Years	107.5	3.04**	299.2	5.72**	16436	7.87**	263924	12.21**
Error	35.3		52.3		2089		21617	
	Variance Components and H^2							
σ^{2g}	62.7 ± 10.13		55.2 ± 9.40		2500 ± 429.8		29990 ± 5965.4	
σ^{2e}	3.3 ± 2.61		2.5 ± 2.57		2027 ± 1475.5		66368 ± 47600.4	
σ^{2ge}	8.1 ± 2.19		9.1 ± 2.96		596 ± 141.2		23542 ± 3226.4	
$\sigma^{2rep(e)}$	0.8 ± 0.58		2.7 ± 1.61		154 ± 88.4		2605 ± 1419.0	
σ^{2error}	35.3 ± 2.14		52.3 ± 3.16		2089 ± 126.2		1617 ± 1304.8	
H^2, %	90.47		86.20		85.30		74.53	
H^2, 90% confidence int.	86.24, 93.26		80.08, 90.23		78.78, 89.59		63.25, 81.97	

*,** Significant at α = 0.05, 0.01, respectively.

† ± Standard error

Table 2. Regression models with QTL for leaf angle, leaf width, leaf length, and plant height in IBM94 RILs. The QTL were detected and included in regression models if LOD values in scans were greater than the LOD threshold corresponding to á = 0.05, by permutation test. Effects that are positive in sign are favored by the Mo17 allele.

Bin†	Marker interval	Chrom. and position	CV‡	Support Interval§	LOD	Partial R^2	Effect¶	R^2_{adj} #	ˆp ††
Leaf Angle									
1.05–1.05	umc1603–uaz273	1/480	59.8%	465–495	4.27	16.4%	-3.802	27.1%	32.5%
5.04–5.05	csu308–umc1482	5/375	44.6	345–390	4.80	13.9	3.084		
9.01–9.01	umc1867–lim343	9/30	62.9	15–45	4.76	10.4	-2.760		
Leaf Width									
2.04–2.04	umc2088–umc2250	2/320	86.5	300–340	5.20	21.6	4.076	25.0	31.2
8.03–8.03	umc1735–php20714	8/280	85.9	260–300	5.28	15.2	-3.080		
Leaf Length									
2.09–2.09	bnlg1893–AY110389	2/660	54.4	640–680	4.08	11.1	-21.531	9.1	11.9
Plant Height									
4.06–4.06	umc2027–AY110310	4/350	40.3	330–360	4.01	10.4	-26.64	8.4	32.1

*, ** α = 0.05 or 0.01, respectively, for the probability that this QTL affected the trait independently of other QTL.
† Bins where the flanking markers are located and positions are the coordinate values on the MaizeGDB IBM2 map (http://www.maizegdb.org).
‡ Frequency of detection within a 1-LOD support interval in 1000 CV runs with families randomly divided for detection and validation.
§ Interval with LOD scores within 1 LOD of the QTL peak
¶ Effects were determined in a simultaneous multiple regression that included factors with LODs ≥ the α = 0.05 threshold.
# R^2 adjusted for the number of terms in the multiple regression models.
††The proportion of genotypic variance explained by all QTL in the models.

380 seeds of the 1980 F2's were planted. Only two plants flowered at the normal (110-day maturity) time. These were observed and crossed with each other, and a satisfactory quantity of good seed was saved. Each following year the resulting seed (17LD) has been planted and has bloomed and produced seed. A minority (less than 5% of the plants) continue to bloom too late in Minnesota to mature seed.

In summation: The plants have a very pronounced teosinte growth habit, with 3 to 30 tillers; 10 to 200 silking locations; 10 to 100 viable seeds per ear; numerous brace roots extending from the 1st to the 7th node; some plants regenerate from planted nodes with brace roots. Seed is available on a limited basis from 1988 by contacting LAC.

Experiment 2. During 1985 in St. Paul, B73 was crossed with *Zea diploperennis* originating from Laventana, Jalisco, Mexico. The female parent was B73. A large amount of F1 seed was obtained, of which eight plants were grown in 1986 in St. Paul. The F1 plants were planted in May, and were "short-dayed" at the three leaf stage by covering them with 30 gallon trash barrels from 6 p.m. until 8 a.m. The short-day treatment was discontinued after 24 days. Three of the plants tasseled 11 days later. The eight F1 plants were grown in isolation to obtain as much F2 seed as possible. More than 600 seeds were obtained. In 1987 597 F2 plants were observed at the University of Minnesota. Seven plants flowered without the short-day treatment during the period July 28 through Aug. 15. Sibs were made between these plants. The balance of the plants did not mature. In 1988 about 35 F3 plants were grown in isolation without the short-day treatment in St. Paul. Open pollination with no selection was used, and a reasonable amount of seed was saved. All of the plants set seed with a three-week range in the time of maturity.

In summation: The plants have a very pronounced teosinte growth habit, with 1 to 10 tillers; 4 to 30 silking locations; 10 to 150 viable seeds per ear; numerous brace roots extending from the 1st to the 10th node; some plants regenerate from planted nodes with brace roots; new plant regeneration takes place when a tiller is held to the ground with the new roots growing from the node.

Experiment 3. The above seeds from 1979 and 1985 were combined and backcrossed two times to *Zea diploperennis* in the greenhouse. These plants continued to bloom in the long days of the Northern Corn belt. They continue to have multiple tillers and multiple ears but the ears returned to, for the most part, *Zea dip.*-like morphology. Most seeds were enclosed in a cupule. The progeny have been replicated several times (4) over the years in isolation. Seed from these plants, which I named "87½ *Zea dip.*," is available from the author (LAC) or the North Central Regional Plant introduction Station at Ames, Iowa.

In 2011 a single plant among 186 seemed to develop a cob-like structure. 51 plants from this selection were grown in isolation in 2012. All plants were harvested, but 20 plants were harvested individually. Of these, 13 plants yielded 1,032 grams of seed. 311 grams remain as ear or ear segments as of June 1, 2013. 721 grams are disarticulated. No attempt was made to preserve the ear structure. Without counting or weighing, at least 600 grams of these seeds had a pronounced extruded endosperm. The other seven plants, without any articulated ears, yielded 1,179 grams of seed. All seeds from these seven plants were enclosed by the cupule.

Figure 1. Adapted *Zea diploperennis*, example ear.

URBANA, ILLINOIS
USDA/ARS/MWA
Maize Genetics Cooperation • Stock Center

2012/2013 allele tests at the Maize Genetics Stock Center

— Stinard, PS; Sachs, MM

During the past year, we obtained positive allele tests for the following previously uncharacterized mutants: *te*-87-2490-22* is allelic to *te1* and is now called *te1-87-2490-22. stb*-N938C* is allelic to *oro1* and is now called *oro1-N938C. vp*-UFMu-03777* is allelic to *vp5* and is now called *vp5-UFMu-03777*.

We obtained negative allele tests for the following mutants: *clpp1-ys* is not allelic to *oro1. l3*, a poorly characterized pale luteus seedling mutant on 6L, is not allelic to *l10*. Previous tests showed that *l3* is not allelic to *l12* or *l15*. *l3* still remains to be tested against the 6L mutants *w1* and *w15*, which also have a pale luteus phenotype.

zebra7 and *luteus17* are allelic to *lemon white1*

— Stinard, PS and Sachs, MM

Based on map location, function, and predicted phenotype, Stinard (MNL 86:29-31, 2013) hypothesized that the maize *lemon white1 (lw1)* locus encodes the plastidial (MEP pathway) isopren-

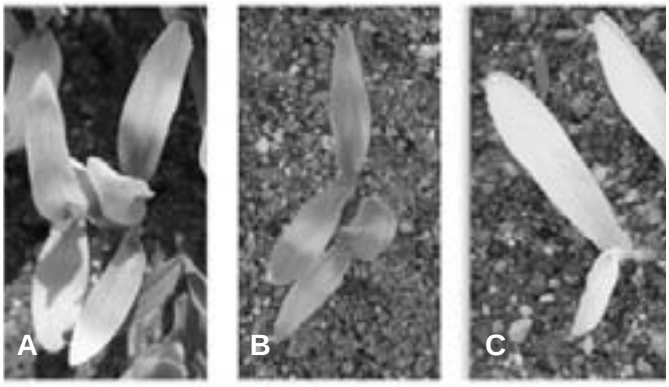

Figure 1. Seedlings from the cross of a *zb7-N101* heterozygote by a *lw1* heterozygote, alongside homozygous *zb7-N101* and *lw1* controls. A. *zb7-N101/lw1* double heterozygotes. B. *zb7-N101* homozygotes. C. *lw1* homozygotes.

oid biosynthetic enzyme HMBPP reductase (HDR), associated with gene model GRMZM2G027059 on chromosome 1L. Using a map-based cloning approach, Lu et al. (Molecular Plant 5:1100-1112, 2012) associated HDR with the *zebra7 (zb7)* locus, also on 1L. Presumably due to their divergent mutant phenotypes, no allele tests between *lw1* and *zb7* have been reported. In order to resolve the discrepancy between the loci associated with HDR, we conducted tests of allelism between *lw1* and *zb7*, and also included the 1L mutants *l17* and *w18* in our tests due to their map locations in proximity of *lw1*. It should be noted that *l17* mutants are also associated with lemon endosperm, but *zb7* and *w18* mutants are not.

Plants heterozygous for *lw1*, *l17-N544*, and *w18-N495A* were crossed to plants heterozygous for *zb7-N101*. The progeny kernels from these crosses were planted in sand benches and the resulting seedlings scored for mutant phenotypes. Crosses of *lw1* and *l17-N544* heterozygotes to *zb7-N101* heterozygotes segregated for zebra seedlings (Figs. 1 & 2). Crosses of *w18-N495A* heterozygotes to *zb7-N101* heterozygotes resulted only in green nonmutant seedlings (data not shown).

In order to rule out the possibility of nonallelic noncomplementation between *zb7-N101* and *lw1*, zebra plants from the allelism test cross between these two mutants were grown to maturity in the field and self-pollinated. These doubly heterozygous *zb7-N101/lw1* plants were also crossed to plants heterozygous for *l17-N544* in order to provide additional phenotypic data. Seeds from these selfs and crosses were planted in the sand bench, and the resulting seedlings were scored for mutant phenotypes. All seedlings from the selfs of doubly heterozygous *zb7-N101/lw1* plants were either zebra or albino — no green seedlings resulted (Fig. 3). We conclude that *zb7-N101* and *lw1* are mutants at the same locus. Crosses of the doubly heterozygous *zb7-N101/lw1* plants to *l17-N544* heterozygotes resulted in ears giving rise to seedlings segregating for luteus (presumable *l17-N544/lw1* heterozygotes), zebra (presumable *l17-N544/zb7-N101* heterozygotes), and green (heterozygotes for the nonmutant *L17* allele; Fig. 4). These data provide additional confirmation of the allelism of all three mutants. Since the *lw1* locus name has precedence in the literature (Tulpule, Am J Bot 41:294-301, 1954) vs. *zb7* and *l17* (Neuffer

Figure 2. Seedlings from the cross of a *zb7-N101* heterozygote by a *l17-N544* heterozygote, alongside *l17-N544* control. A. *zb7-N101/l17-N544* double heterozygotes. B. *l17-N544* homozygotes.

Figure 3. Seedlings from the self pollination of a *zb7-N101/lw1* double heterozygote. Zebra seedlings on the left were grown from yellow kernels. Albino seedlings on the right were grown from lemon kernels.

Figure 4. Seedlings from the cross of a *l17-N544* heterozygote by a *zb7-N101/lw1* double heterozygote. Green (presumed *L17* heterozygote) and zebra (presumed *l17-N544/zb7-N101* double heterozygote) seedlings on the left were grown from yellow kernels. Luteus (presumed *l17-N544/lw1* heterozygote) seedlings on the right were grown from lemon kernels.

and Beckett, MNL 61:50, 1987), we propose that the *lw1* locus name be retained, and *zb7* and *l17* mutant alleles be renamed as alleles of *lw1*.

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Maize mutants a boon to society in past and in future what…?

— Tufchi, M; Singh, NK; Yadav, A; Tiwari, G; Verma, SS; Jaiswal, JP; Shrotria, PK; Gaur, AK; Kumar, A

This report is based on BC_2F_1 populations derived from crosses between Pant 10k1375 x CML161 and backcrossed with recurrent parent (Pant 10k1375). The Pant10K1375, a normal maize inbred line, developed in Maize Breeding Programme at Pantnagar. The CIMMYT Maize Line 161 (CML-161), a QPM inbred line, was developed by CIMMYT. The F_1 of initial cross were backcrossed with recurrent parent, and BC_1F_1 and BC_2F_1 plants were selected for heterozygous *opaque2* allele (o2) using SSR markers for conversion of normal maize line (Pant 10k1375) into QPM. Progeny populations of all the 12 plants found positive based on the *phi057 and umc1066* SSR markers for *o2* allele were planted on September 9, 2012. The selected plants were self pollinated to generate the BC_2F_2 populations for conversion programme of normal maize to QPM. Progenies of 12 plants, initially selected for heterozygosity at the *o2* locus, were found to have altered phenotypes.

The most susceptible population was $BC_2F_1/11$, where frequencies of altered phenotypes were 42.42%, compared to the minimum of 2.08% altered phenotypes in $BC_2F_1/2$. (Fig. 1). Seven mutant phenotypes were observed, with terminal tassel replaced by ear being most frequent (Fig. 2).

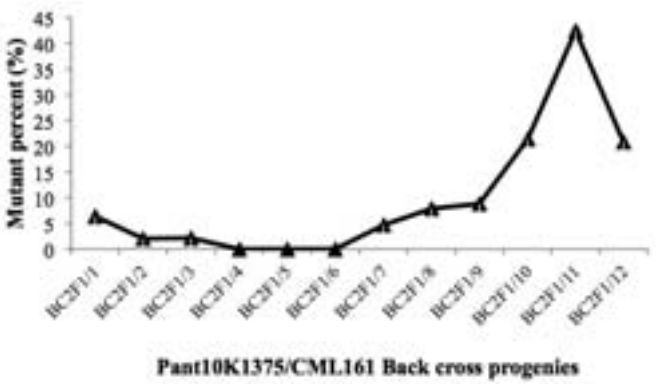

Figure 1. Frequency of mutants in Backcross progenies in maize.

Dwarf mutant. These mutants varied in height between 29-77 cm and had presence of tassel and ear. The ears had normal silk, but the tassel was small in the form of cluster (abnormal). Seed development was observed in these cobs since other plants having normal tassel development were present in the vicinity of the plant. Intermodal distance between the nodes is minimized with fewer leaves. This may be due to suboptimal synthesis of endogenous gibberellic acid (Fig. 3A).

Tassels with both anthers and ears. Some plants were found to have both sexual expressions in the tassel. Generally, the main rachis of the tassel was converted into a small ear that set seed, whereas the remaining tassel branches developed anthers with pollen grains along with few seeds on tassel spikelets (Fig. 3B). This type of mutant showed similarity with tasselseed mutants (Nickerson and Dale, Ann Mo Bot Gard 42:195-212, 1955).

Cob at top without tassel. In 25 plants, the terminal tassels were entirely modified into small single ears with silk (Fig. 3C). These ears were found to have seed set but were smaller both in size and numbers of kernels than normal ears.

Earless mutant. We found one plant having no ear but a nor-

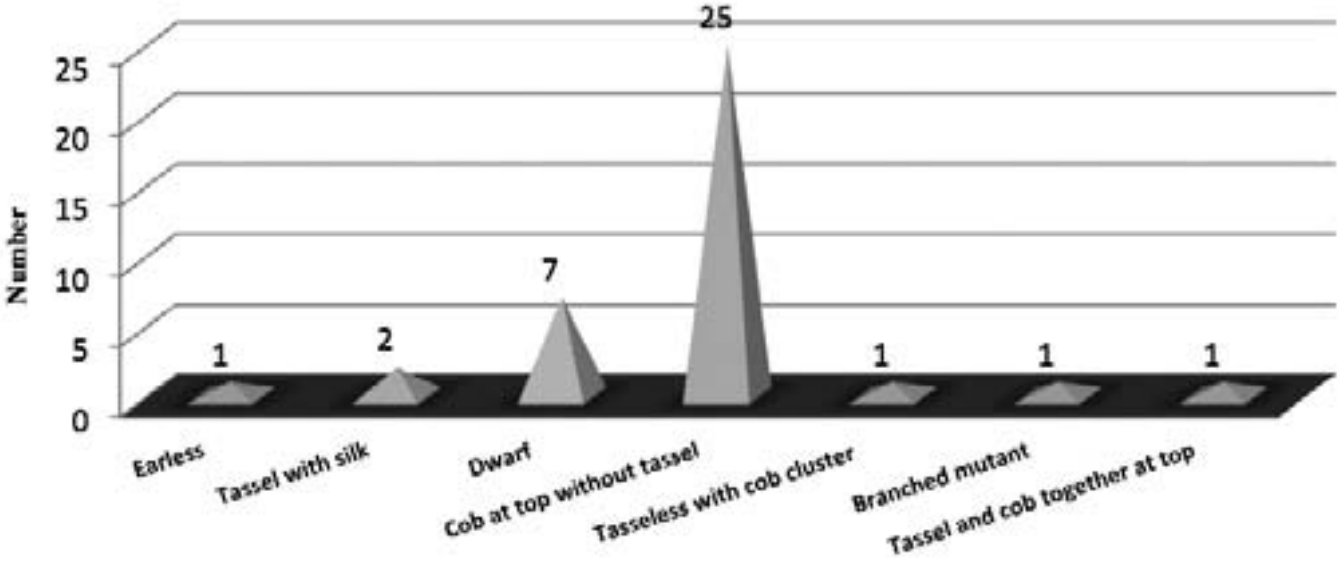

Figure 2. Frequency of mutant types.

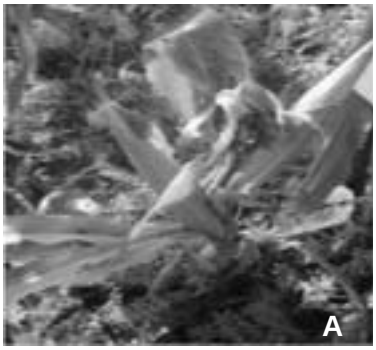

Figure 3A. Dwarf mutant.
Figure 3B. Cob and tassel together at top.

Figure 3C. Cob at top without tassel.

Figure 3D. Earless.
Figure 3E. Branched.

Figure 3F. Tassel with silk.
Figure 3G. Tassel-less with cob cluster.

mal tassel in BC_2F_1-1 plant population (Fig. 3D). Known earless mutants include *barren stalk1 (ba1),* which also has a defective tassel (first report Hofmeyer, Ph.D. Dissertation, Cornell University, Ithaca, New York, USA, 1930)

Branched mutant. Lateral and basal branching was observed in this type of mutant, terminating with a cob either singley or in clusters. The height of these plants was lower than normal and they completely lacked tassels (Fig. 3E).

Tassel with silk. In two plants, a rudimentary tassel with profuse silk, but without ear was observed. Such plants were also of short height with less intermodal distance (Fig. 3F).

Tassel-less with cob in cluster. A single plant was found to have many ears lacking silks, on separate nodes and without tassel formation. Ears remained barren or set very few seeds. This plant also branched laterally and was shorter than normal plants (Fig. 3G).

The unusual expressions in ear, tassel, plant height, and branching may be due to environmental factors as the pre-flowering, flowering, post-flowering, and grain filling stages coincide with the low temperature along with short day length. There is also probability of involvement of genetic factors or interaction of genetic and environmental factors. However, progeny analysis or other genetical studies are required to be carried out to confirm the basis of plant phneotypes alteration. Such analysis is also essential to assess the worth of such phenotypically modified plants in maize improvement programme. Richey and Sprague (Amer Nat LXVI:433-443, 1932) reported the role of environment, i.e., shorter daylight periods and lower temperatures, and heredity in the development of silks in the tassels. Heslop-Harrison (Biology Reviews 32:38-90, 1957) also shared the viewpoint that low temperatures, particularly when experienced through the dark period of the daily photoperiodic cycle, promote female sexual expression and depress male. In case of widespread occurrence of unusual plant phenotypes, the quality as well as the quantity of the maize grain or green cob will certainly suffer.

Acknowledgement. Director Experiment Station, GBPUA&T Pantnagar, is duly acknowledged for providing facilities to carry out the experiment.

1989 interview by Sherret Spaulding Chase (SSC) with Dr. Chin Chum Li, population geneticist (1912-2003)

The interview took place October 24, 1989, in the office of Dr. C. C. Li, professor in the Graduate School of Public Health, Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA 15261; population geneticist; Cornell graduate; and contemporary of Dr. C. H. Li. Recorded and transcribed with the assistance of my daughter, Alice R. Chase Robeson.

The purpose of this meeting with Dr. C. C. Li was not to interview him on his own experience but to seek his advice on how best to advance my nomination of Dr. C. H. Li (also known as Li Jing Xiong, 1913-1997) for the World Food Prize. Both C. C. Li and C. H. Li had their careers interrupted by Lysenkoism, and I was concerned that I not create further problems for C. H. Li by my recommendation. C. H. Li directly enabled the feeding of about 100 million Chinese by pioneering the breeding and production of China-adapted hybrid maize. His accomplishments are today well known in China; a statue has been raised to him at the Agricultural University in Beijing (Figure 1). He opposed Lysenkoism at a crucial national meeting (see also Master of Arts thesis, Alice R. Chase (Robeson), "The influence of Lysenkoism on China's genetics: the importance of the Qingdao symposium," University of Maryland Baltimore County, 1993). He is a hero of the current generation of crop breeders. For more concerning C. H. Li's accomplishments, see Crop Science 39:1-3, 1999.

CCL: I'm seventy-seven; I retired in 1982, so this is almost seven full years. This coming January will be my eighth year of retirement, and they would not let me have the office much longer. Occasionally I publish small papers; I am still reading some things and writing some things. It is hard to change my life — a whole life's habits. When I think — sometimes I like to write a small paper. My wife is here, and I have only one son and one daughter.

The best single paper on Chinese Lysenkoism is this paper [Genetics in China: The Qingdao Symposium of 1956]. This is by a woman, P. Li (Li Pei shan). She is working at the Institute of Natural Sciences History of China. She is about seventy years old; I never met her. When I was in Beijing, she was a graduate student in biochemistry, and later she joined this institute. According to this description, she was also at Qingdao — she knows the conference very much in detail.

The most important point she makes is this: In 1956, Chairman Mao declared a new policy: "let one hundred flowers bloom; let one hundred schools of thought contend." Now, this has a double hundred meaning. What I did not know is why he proclaimed this policy. One of the reasons behind it is genetics. He assigned a number of people to study the problem. He knew the story about me. He said C. C. Li left Beijing University simply because of his disagreement with genetics, but why? Mao appointed a number of people to investigate the problem; the people [investigating] are not geneticists, they are Party [Chinese Communist Party] people. Li Pei shan was one of these people and her boss, Yu Guang yuan, who recently was arrested, he is also in his seventies. These are all Party members, but Mao wanted to study this.

The Party members have this advantage — they have all of the Soviet literature. All of Lysenko's paper, speeches, meetings — the information was all shipped to China. Remember the timing. Lysenko enjoys the highest power in 1948. In 1948, that big meeting at the Academy of Agricultural Sciences [in Russia] — it was at that meeting Lysenko announced that "my speech was approved by the Central Committee of the Communist Party." And China was liberated in 1949. See the timing: very close. In 1949, there are a number of Soviet Lysenkoists visiting China. They begin to tell us what to do — and how to do it. They are teaching and directing — the result is not good. Such as cotton: We in China have been breeding cotton for years before Lysenko. The Lysenkoists said cotton should be dense planted. The cotton plant is quite big. We said the distance between the plants should be two feet, and between rows should be more than three feet so that you get flowers and cotton. The Lysenkoists said no, there is no competition between members of a species; they are brothers. The planting was dense; the result was a lot of leaves, no cotton. Foolish things like that!

But anyway, the Chinese Party committee studied the problem; I've heard they made a report stating that there were serious problems with Lysenko. This is hard to track down from Party people. Only the Party can convince Chairman Mao. If I explain to Chairman Mao, he would probably say, "You are an American spy. I cannot trust you!" This has to be done by Party people. The Party people, Yu (Yu Guang yuan) and Li (Li Pei shan) and all have seen these doubts about Lysenko. Although they are not geneti-

Figure 1. Statue of Dr. C.H. Li (Jing Xiong Li) at Agricultural University in Beijing (provided courtesy of Ming-ting Chang).

cists, they read Lysenko's stuff, and they read our stuff and realize there is no comparison. It is due to this that they urge Mao to let the Mendelians present their stories. This is why "the hundred schools can contend." At least, "we cannot persecute them" — as is what happened in Russia.

Now, how to implement this one hundred flowers bloom policy? They suggested one way to implement this policy is to call a conference. Let the Lysenkoists and the classical geneticists all come to the same room and debate; with no persecution under the conditions there; nobody can be called an American spy — we will just talk about genetics. Such a meeting was called at Qingdao in Shandong in eastern China in August of 1956. This meeting played an important part in the double hundred policy — a hundred flowers bloom at this meeting, a hundred schools contend at this meeting. There are a number of people who were absent; I don't know why. C. H. Li was there. But I know several other prominent geneticists who were not there. And the Lysenkoists' leader was not there, either.

This is the historical background — this article by Li Pei shan is the most complete article in English that I know of. The English literature is this article and Laurence Schneider's "Learning from Russia: Lysenkoism and the Fate of Genetics in China, 1950-1986." They are all I know. The others are mine. This is my report in 1961 at the AAAS (American Association for the Advancement of Science) — they wanted me to review this. The National Science Foundation and several other universities at that time began to exchange scientific literature with China. Finally, they accumulated several tons of literature, mostly in Chinese. They do not know what to do. Their solution is to distribute it to various people for them to review and to hold a symposium. About genetics — they found me. They sent me about this much [hand gesture indicating an immense stack]. I could not find this stack now — this was about thirty years ago. This is the only report in English about the activities of the Lysenkoists in China. And I wrote a review of the Qingdao conference proceedings; that is, I wrote a book review of this, Schneider's booklet. And this is my book review in the Journal of Heredity. I outline and review the major outline of the debate at the meeting — a straightforward review of the several topics, and how useful the Marxism and Leninism philosophy is to natural science. They say that you can get guidance — if you study Leninism, you can find the right experimental material and select the right method. This paper [held in hand] came out last week. This is a University of Pittsburgh staff writer, David A. Petechuk, who wrote about my life and my difficulty with Lysenkoism in China.

And now we come to this question — you know the Nobel Peace Prize was awarded to a monk leader in Tibet, the Dalai Lama. To this information, the Beijing government reacted very strongly. First they protested — they called the Norwegian ambassador in Beijing to their Ministry of Foreign Affairs and protested — "what are you people doing?" The ambassador explained to them that the government cannot control the Nobel Prize committee; that committee is an independent committee. It is not a government agency; the government has no control over it. If the Nobel Prize committee decides the award goes to Mister A, it goes to Mister A. The government has no say about it. This the Communist Party did not believe; in China, everything is decided by the party; what do you mean — that what your people did, your government cannot control? Later on, the Chinese communists protested again. They said, since the award was announced, nothing could be done about that now — but in December when there is the ceremony of giving the award, government officials, especially the king of Norway, cannot attend. This the Nobel Prize committee cannot accept. They said, from the very first year, during the awarding ceremony, the government officials and the king attend. So, the Chinese do not know how to solve this — their way of thinking is extremely anti-foreign.

Do you know the name of that rich man in New York? He is a Jewish person from Eastern Europe. He donated one million dollars per year — not just one million dollars — to subsidize some publications and some research work in the social sciences. Now, every Chinese scholar can come to the United States; and they can do their research in China. This money supports the research, supports the publication, so forth. Those who received the grant from this organization have all been arrested! The government says, you are all CIA spies! Why, this money is not from the CIA. I forgot the name [George Soros]; he threatened to sue the Chinese government. He said, that is my money! He not only gave to China; he gave money to Hungary, Poland, to every country where they do not support social research. Anybody who receives help from the United States will be investigated; not necessarily put in jail — but the government will watch; they will interfere.

Of this I am afraid; if we nominate C. H. Li this year, at this time, it is more likely to cause him trouble than honor.

[SSC indicates here that the initiating nomination of C. H. Li has already been made by the president of the Chinese Academy of Agricultural Sciences and has been seconded by several key Chinese individuals.]

CCL: I did not know that; this then is all right! He was nominated in China! We are just helping, with a seconding nomination. This is OK — I thought that this was without the consent of the Chinese authority.

CCL: Since this is initiated by the Chinese side, we can second; yes, yes — we can second. I wouldn't dare initiate him, since their reactions might be negative. We mean to honor this man — the communists might take it otherwise. Yes, this is OK, I think; yes, this is OK.

SSC: This is still a question — nominations for the World Food Prize are to be made by organizations. In addition to the Academy of Agriculture nomination from China, there are organizations/companies which would or will nominate him. Do you think it would be better for the U.S. nominators to simply reinforce the Chinese nomination? Separately, I am listed by the Academy of Agriculture as a seconding individual nominator.

CCL: Yes — a seconder, a supporter; that's fine. That's fine so far. In addition to that, you want organizations — in this country? On the phone you mentioned an association of corn geneticists?

SSC: Well, the company that I worked for is where this started. … Now, Pioneer has had a number of scientists in their top administration. William Brown became the president and later chairman of the board. His junior is Don Duvick, and Duvick

is now, my guess, a candidate to be president in the next round. I will be in touch with him next week, if possible. He was out of the country. When I get back myself, I will approach him about the possibility — it would be interesting if the Pioneer organization would endorse this nomination also. They already have an association with Dr. C. H. Li. Pioneer hybrids are already being tested in China. Again, this political business worries me. For example, would it be better for a U.S. nominating organization to be a primary nominator or should they endorse — second — the nomination of the Academy? Of course, a U.S. group might not even know, at this stage, that there will be an initiating nomination from the Academy.

CCL: Everything in China is political — there is no such thing as the individual.

SSC: I was wondering if you could give me some idea of the sorts of difficulties that C. H. Li faced during his career. I know he has faced many difficulties. I have a picture of him as a young man, carrying a little pot of millet, of Setaria [actually a wheat-rye hybrid, Triticale!]. Apparently, he was moving in advance of the Japanese. He wanted to save his research material — for further cytogenetic study. That was the period of the Japanese takeover. And later, just as he went back home after his period of graduate studies in the United States, the communists took over. There was that period of turmoil; then Lysenkoism came in. Then there have been a number of other changes, then the Red Guards and the period where intellectuals were "sent down" to work in the country — "to learn from the peasants." He too went down to the country to work. He worked rather well, he was used to working with his own hands on the land with the peasants and was able to continue his corn breeding work successfully, but he still was forced "to go down." I was just wondering if you could list the sort of challenges which he must have had to overcome in order to function.

CCL: The details, of course, I have no way of knowing, but I know his general attitude. His general attitude reflected very well at the Qingdao meeting [the decisive Qingdao conference in 1956]. C. C. Tan spoke several times at the conference. Every time he emphasized that the two schools of geneticists should get together and compromise; "we should learn from each other." First, he said, "The Lysenko School and the Morgan School are both very young, so the two young schools should get together and learn from each other." He spoke along this line three times, and finally, according to the meeting report, two conferees — one is C. H. Li and one is Wu (whom I also know) — Wu Zhongjian — were against this philosophy and against C. C. Tan's suggestion. They said there could be no compromise. C. H. Li said, "What precisely do you mean by compromise? You accept half of Lysenko and let them accept half of a gene? Precisely what do you mean by compromise? In all natural sciences, there is only one language. And there is only one truth. I don't want to fight, but I don't understand what you mean by compromise." So, I know C. H. Li's attitude is tough, very tough. Both C. H. Li and Wu, an animal geneticist — here I marked it off for you — Wu and C. H. Li stood their ground. We were together in the same department for a short period because, when the communists came, three agricultural colleges — Beijing University, the Agricultural Institute of Tsing Hua, and the Communist Agricultural College — all three merged into one. They became the Independent Beijing Agricultural University. At that time, Li and I were in the same department. This was in 1949 and 1950. Since I was under attack, we couldn't get together and talk. That was forbidden. If you are under investigation, you are socially isolated. You see nobody, and nobody sees you. So I couldn't see him.

You see, this is a communist tragedy. It is very interesting. When the communists want to attack something, they always pick a target. They are not against all geneticists because there are too many of them. They concentrate on one, and if they can liquidate this one, then all the others would be quiet. And the one they choose is me — because I was the department head of Agronomy. I was teaching genetics and biometry. I was also the director of the Agricultural Experimental Station. Li was not — and the other geneticists were in the Academy of Sciences. They had nothing to do with agriculture, so they chose me. During that period, all these people have to stay away from me. And at that, I have a choice — I can turn over, I can change my color, I can say Lysenko's genetics is new, progressive — it is the ultimate truth; all Morgan stuff is reactionary. I could have said that. If I said that, I could have kept my position. I would probably have other good jobs, too. But I just could not say that. To say something against your own — what you understand to be true — it is very difficult. Without knowing it, I got myself in an argument — once, twice, we argued all night, and the communists say, "Li is hopeless. Now we have to get rid of him; he is resistant to learning." That means I do not learn new things!

One old Party member by the name of Chang [he died several years ago] was an associate professor and also a Party member. He was sent to see me after supper. I knew there was something — after supper we talked over a pot of tea. We talked until midnight; his suggestion is "Dr. Li, you just recant." Recant means I criticize myself, say I was wrong in everything; from now on I will be a new man and learn new things. He said, you simply recant and your problem is over. We will not get you anymore if you recant; on the other hand … As a Party member and as a colleague, he was an associate professor in Veterinary Sciences — he knew genetics. That's why they sent him over to see me. He said, if you don't, you will force the Party to do something. I will feel sorry for you. And his final question to me: "You tell me why you are so confident of this Mendelian ratio business?" I said, "Take the human group — if the mother is of a certain group and the father of a certain group, we know what are the possible groups for the children, and the impossible groups. If the child belongs to an impossible group, I can say — your wife has another man. This is a very serious accusation, but I have no other choice. I can say this, because I have this much confidence in Mendelian Law." Chang's color changed and he said, "You say that, that's almost hopeless." He stayed almost all night, because that was his mission. Probably the best thing for me to do was recant, but that was the last straw. After that, I knew I had to get out. Yes, yes — no more compromise.

SSC: How did you get out?

CCL: I really couldn't say goodbye. I made up my own mind. I didn't even tell my wife during my planning stage. I just sat in my office and thought it out myself. I had no underground — we had no connection with an underground. I did everything myself. This was taking a big chance. First, I had to wait until a time when

I had an excuse to travel. My family home was in Shanghai, and in the winter time, when the semester is over, we had about three weeks winter vacation. That covers Christmas and Chinese New Year — between the end of the first semester and start of the second semester. My mother was in the hospital. I wrote a letter to the dean saying, "Since this was the three-week vacation, I want to go to Shanghai to see my mother." They didn't suspect anything. In China, when your mother is sick and you want to see your mother, the reason is very strong. This was the first thing: to choose the proper time. The second thing: willingness to sacrifice my home. I did not pack. If there had been any packing, they would say, "This is only a short train trip, why bother to pack?" Only two days before leaving did I tell my wife. I said, "Don't take anything." We sacrificed everything. "Just you and me — we want to save the people, not the things." I suggested, "I will put up a picture of Mao on the wall," but she disapproved of that — said that was overdoing it. Finally, I did not pack anything — the curtains, the carpeting; the most important thing we left was the rice. I had a big bag of rice. All was left there — my bags, my books, the rice — everything as if nothing had been touched. [SSC: The bag of rice has special importance. To reduce peasant fear of starvation, the communist government required that each household store sufficient grain for one year.]

Early in the morning we went. I had bought the tickets. So, early in the morning we called a rickshaw and we went to the train station. I didn't say goodbye to anyone. I told only two people. One was Lin — I knew he wouldn't sell me out. That was at one o'clock in the morning. When I knocked on their door, they came out in pajamas, very annoyed: "Do you know what time it is?" I said, "I know we are intruding, but I have only one thing to say: I'm not going to see you anymore. Yes, I'm leaving tomorrow."

It is this way I got off. I got to Shanghai. My mother was very happy to see me at the hospital. I didn't lie. Everything I told them was true. My mother is in the hospital. I went to the hospital. The hospital people told me that I could take my mother back home. There was nothing they could do for her — she may die in a few days; she may not die. So, the next day, I took my mother to my father's home in Shanghai. Then, I said to my mother, "I am leaving tomorrow." My mother disliked that very much: "You haven't been home for several years; you just got home, and you want to leave tomorrow!" I said, "I have my reasons." I told them, people will come into your house to search for me. The next day, against my mother's wishes, I bought tickets for Canton, and my wife and I then crossed to Hong Kong. My mother and father didn't believe that. After a week, communist agents came — "where is Li Ching Chun?" My mother said he went to Hong Kong. "Left? No, he couldn't be there; he must be here. We know the day he arrived. He couldn't just go away so soon!" "No," she said, "he really left." They searched the house. My Shanghai house had four stories — basement, first floor, second floor, attic — the attic is a full attic with a bedroom. They searched all the way from basement to the attic — they found my old books with my name. "See, all his things are here, so he must be here!" My mother tried to convince him: "He left and went to Hong Kong empty-handed. He didn't bring anything." They couldn't find me. They staked agents around the house and just watched the house — for two full weeks — and finally they were convinced I was no longer there. Then, they were convinced I was in Hong Kong, they knew somehow — they knew my brother's address in Hong Kong. They wrote to me in Hong Kong and said, "Come back — what you did will not be damaging. You will come back and work as usual — as if nothing happened." Those letters! I didn't answer. They are still trying to get me back. You see — I had no underground connections. I'm just a professor. I told my wife they could have got me anytime. If they had suspected, that first day there would have been agents at the Shanghai Railway Station. It was that easy — they did not think of that. They thought, "They will come back before school opens again." So, one could escape through an underground organization — or on one's own.

There are many prominent people who came out through protection. There were underground organizations — but I didn't have that connection. I just knew their psychology. If I just do this and this and stay 24 hours ahead of them. When their agent got to Hong Kong, I was already there.

This kind of xenophobia; this is a disease. This anti-foreign feeling is definitely a disease. They don't quite realize what western people did for China; they don't appreciate it. They say, you come here, all you want is a fast buck, that's all. But more than that — the thinking that you do.

SSC: How did C. H. Li do inbreeding during the Lysenkoist period, if Lysenko said "inbreeding" is bad? [Lysenko on inbreeding: "The product of inbreeding is less vigorous than the parent, 'degenerate,' therefore, 'evil,' not a 'progressive step'; therefore, prohibited."]

CCL: Concerning inbreeding, during the Lysenko period — maybe during those years 1949-1951, maybe he didn't do inbreeding, and maybe he just didn't call it inbreeding.

SSC: He did continue inbreeding of corn — for development of inbred lines.

CCL: Yes, he called it something else. Fortunately, he wasn't the target — he was on the side.

SSC: How could he avoid being a target?

CCL: The target according to my information from the Party, the first was me. The second, after me, was Wu, the animal geneticist; he is also anti-Lysenko. He became the number two target. Since I left, everything got quiet, so the movement just stopped. No particular person was chosen as a target anymore. Because of this, they automatically discontinued their course, no teaching and so forth. After I left, there were many, many things, too.

SSC: I know; there was a period during which C. H. Li and I wrote letters to each other. Then, it became difficult because of Lysenkoism and political sensitivities for him to write anyone in the United States, and it was also difficult for me because of the effects of McCarthyism in the United States. I was at Iowa State on the staff then — a pretty conservative place politically. So, we didn't communicate. We lost touch with each other. It wasn't until 1971, when my youngest daughter, Alice, went to China, that I had a chance to find out whether he was living. [Alice met C. H. Li. Her group was able to interview him, and she was invited to the village where his research was based to have lunch with him — at which time he showed her a photo he had carried since his

Cornell days when he and I were office mates. This photo was of my eldest daughter who was just a baby when C. H. Li returned to China — and clearly was a heartfelt indication of our warm and enduring friendship.]

Since then we have seen each other a number of times. He arranged for Kenny (my wife) and me, as individuals, to be invited over in 1975.

CCL: In 1975? You visited Beijing?

SSC: Well, yes, the corn belt. We were in Beijing and country districts nearby where corn was being grown. We were also in Shansi Province and up in the north, in and near Kirin and Chang Chun. We traveled by train and car; truck and place. It was a short trip — two very busy weeks, packed full. We did a great deal, but mostly centered on corn — corn breeding, seed production, corn growing, agricultural organizations. [We also became aware that the two "schools" — Lysenkoism and western genetics — were both being supported at that time — were still "contending." It is interesting that the U.S. Plant Studies Delegation of the National Academy of Sciences in the report of their four-week survey made in August-September 1974 made no mention of the continuing role of Lysenkoism in China. I think the Chinese being considerate hosts, the itinerary was carefully set up to avoid Lysenkoist contacts.]

CCL: Very good — did you visit after that?

SSC: I was back later in Beijing in 1985. I was not able to travel beyond the metropolitan district. I went for a biotechnology conference.

CCL: I don't think there will be any trouble with C. H. Li's nomination.

SSC: I was reassured by this, also. The fact that Liu Xu, the vice president of the Academy of Agricultural Sciences, was enthusiastic early on was one of the main reasons that I have been encouraged to pursue this. I don't know just what — I'm going to have to talk to Edward Williams, coordinator of the Prize, for advice from him as to where to go now. The information that I showed you, though apparently enough for the Chinese nomination, would not be sufficient in this country to qualify a nomination. More background information will be needed. And the Lysenkoist thing is — could be very important to the thinking here — but it is difficult to deal with at the Chinese end.

CCL: We don't mention the Lysenko affair. Yes, you know the recommendation: We just mention his work on corn. We don't mention Lysenko.

SSC: Part of his strength is that he did resist.

CCL: That goes without saying.

SSC: In this country, it does not go without saying.

CCL: In this country, you are right. But if you say so, that will embarrass the Chinese authority. My idea is we shouldn't mention Lysenko in our recommendation.

SSC: We could mention the period of difficulty.

CCL: We could take it the other way. He continued to work — on his corn genetics and breeding — that indirectly implies he resisted Lysenkoism. This is what the Soviet corn geneticists did. In 1956 — no — in 1955, before the Chinese have the Qingdao meeting, the Soviet Union has a corn meeting too in 1955. They didn't attack Lysenko; they merely talked about corn genetics and hybrid corn, and it was legitimate, because Khrushchev had visited Iowa and brought hybrid corn back. They said, this is Khrushchev's interest — hybrid corn. So, they used that at the corn genetics meeting, without mentioning Lysenko. It was obviously anti-Lysenko — without saying so.

SSC: How about the period of anti-intellectualism? Intellectuals were sent to the country to work with the peasants. Can that be mentioned?

CCL: Yes, that can be mentioned. That was a general policy whether he was a geneticist or not. Yes, that can be mentioned. And their published things can be mentioned, such as his resistance to compromise at the meeting at Qingdao, that you can mention. That's published record. Communists know that, too. Yes, but beyond what the communists publish, we don't mention. At the Qingdao conference — he stood on his own ground. There was no compromise. That, I think, is strong enough.

SSC: More recently, during the anti-intellectual period, C. H. Li was "sent down" to Shansi Province, near DaZhai, and, as far as I can interpret the record, what he did was to continue his corn breeding and develop or work with a group — an experiment station. There he did some of his key breeding work on developing corn inbreds and hybrids resistant to the primary corn diseases of China. There he was working — as he was — with the peasants. This was very appropriate, a creative way. There is no problem in mentioning this, and there could be no problem in going back to the Japanese period, when at great risk and difficulty he saved key breeding material for study [the wheat-rye hybrid].

SSC: You have been very helpful to me today.

CCL: As for me, I was worried that if we didn't do it right, we might get C. H. Li in trouble.

SSC: You know the thing cuts both ways. One of the reasons he is such an attractive candidate this year is that Americans would like to honor a Chinese who is not political, who has done something very important for mankind. And one of the reasons for the timeliness of this nomination is that we are opposed to what the Chinese government has just done to so many of its young leaders.

CCL: Too bad nobody has written a history of Lysenkoism in China. We have several books about Lysenkoism in the Soviet Union.

SSC: I have felt this lack, and that was the reason that last summer I wanted to interview C. H. Li about his experiences. It is very important that his experience be documented and those of others. What Schneider has written is incomplete.

CCL: Very incomplete.

SSC: It is a start.

CCL: At Cornell, there is a geneticist by this name, William Provine. This man studied genetics; now he is also in the history department. He is the one who wrote the biography of Sewall Wright. He is awfully interested in the history of genetics. He also wrote this book, this collection of Sewall Wright's reprints. He is part of the time in genetics and part of the time in history. He has a Chinese graduate student who wants to write a history of Lysenkoism in China. Provine encouraged him. He said there is no such history yet, so it is a good thing for him to work on it. This student

worked on this for almost two years. He collected many things. He even came to Pittsburgh to see me. But since this change of atmosphere, he changed his object[ive]. He said, I cannot pursue this — if I do, I cannot go back to China anymore, so he changed. I wrote him a nasty letter. What kind of scientist are you? Provine even promised to help him get published by the University of Chicago. Provine has several editor friends. He said, if you devote a few years to writing up Lysenkoism in China, I will help you to get it published. In spite of this promise, he chickened out. Yes!

SSC: You have been very gracious to give us so much of your time.

[Alice R. Chase Robeson: "An invitation from China's Prime Minister Chou En-Lai was extended to Carmelita Chase Hinton to bring 16 young people whom she knew well and were interested in China. Carmelita Hinton said that I could be one of the young people! The invitation was for three months — it stretched to four months; then four of the young people, including me, stayed for nine months. Carmelita Hinton stayed for about 11 months. My 18th birthday was Sept. 16, 1971."]

IV. MAIZE GENE REVIEW

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 Maize[G]DB, prior to publication in hard copy. The purpose is to capture community expertise to add information to MaizeGDB about gene functions and new papers. The first reviews were published in the vol 83 of this Newsletter. We thank all submitters for being very generous with their time in participating in this 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*), Paula McSteen (*bif2*) Erik Vollbrecht and Sarah Hake (*ra2*), Rachel Wang et al (*afd1, sgo1*).

March 2014 we implemented a wiki format, which is hosted at MaizeGDB. It is accessible via the current site, and also from the MaizeGDB homepage, and by links from each gene page at MaizeGDB. As with the Neuffer et al 1997 book, 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. Curators at MaizeGDB may add updates from time to time, similar to the process for the Online Mendelian Inheritance of Man. We are in the process of moving all early reviews to the wiki format.

We invite all cooperators to submit short reviews, and to update current pages. Obtain an account from the Maize Gene Review (online) to start. You may then edit previous reviews, and/or add your own. On request, MaizeGDB curators will provide review template specific to a gene to facilitate adding content. When you add new articles, please name the article using the gene name stored at MaizeGDB. If your gene is not at MaizeGDB, inform the MaizeGDB staff. This will facilitate auto-linking the MaizeGDB gene pages to the wiki pages. Please let us know when you add a new page, so we can speedily give you credit.

Anticipated contents should include, with reference citations:

- a short summary of the Gene
    - phenotypes, biological function, gene products, how cloned
- *an unpublished high quality image*, with caption that includes name of the photographer or lab head
- *pubished images,* if provided with permission from the publisher
- the reference(s) that first reports and defines this locus
- key alleles used to characterize the gene, with phenotypes, information about viability or special conditions
- regulation of/by other genes or genetic elements
- expression – tissue/conditions/inhibitors/inducers
- map location and how mapped
- any information you think is important, e.g. paralogous loci
- useful links to references, and other genomics resources

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This year we thank Gerry Neuffer for providing a lengthy review about chromosome breaking *Ds* stocks, most of which are available from the Maize Genetics Cooperation Stock Center, and where all images have been added to the MaizeGDB.

Chromosome Breaking *Ds* Sites in Maize, Revisited (Part II, Images and Descriptions)

M.G. Neuffer, Professor Emeritus, University of Missouri, Columbia, MO 65211, U.S.A.

This report is also posted at the maize gene review wiki (http://maizegenereview.maizegdb.org/doku.php?id=chromosome_breaking_ds_sites), which links to greatly enhanced images that have been supplied to MaizeGDB.

Loss of the normal allele in a heterozygote to produce a hemizygote with the recessive phenotype can be very useful in genetic studies. This may be produced in a number of ways, including x-radiation, marked ring chromosomes, and B-A chromosome translocations. The chromosome-breaking *Ds* elements are especially useful in studying the expression of lethal mutant tissue in chimeras produced by loss of the normal allele in a heterozygote. Our purpose in this project was to generate chromosome breakage in heterozygous, lethal embryo, defective kernel (*dek*) whole kernel maize mutants on all chromosome arms. Plant chimeras of hemizygous mutant whole kernel and seedling *-/dek* tissue, sustained by adjoining normal *+/dek* tissue, could thus be observed and compared. By taking advantage of its chromosome breaking properties, we were able to produce *Ds* markers on many genetically marked chromosome arms. We provide here a general review of the uses and expression of these interesting genetic tools.

The tests that we used in these experiments included stocks with marker genes on 15 chromosome arms (Table 1). At the time, stocks with usable markers on 6S, 7S, 8S and 10S were not available. We made no effort to work with chromosome 9S, where Dr. McClintock had done her pioneering work. We found 120 kernel cases and 15 seedling cases, of which 56 cases were confirmed. (There were 79 non-transmitting cases; see Part I MNL 84:11-14.) Proven cases are described below.

Table 1. The chromosome breaking *Ds* stocks.

Ds Stock	**Position**
(Marker Dek1)	
1S1	distal
1S2	probably distal
1S3	distal
1S4	proximal
Ds-1L (Marker *Bz2)*	
1L1	proximal
1L2	at the *Bz2* (*bz2-m*) locus
1L3	at the *Bz2* (*bz2-m3*) locus
1L6	at the *Bz2* (*bz2-m*) locus
Ds-2S (Marker *B1:Peru*	
2S1	distal
2S2	unknown
2S3	at the *B1:Peru* (*b1-m1*) locus
2S4	at the *B1:Peru* (*b1-md2*) locus
Ds-2L1 (Marker *W3*	unknown
Ds-3L (Marker *A1 Sh2*	
3L1	proximal
3L2	proximal
Ds-4S (Marker *Bt2*	
4S1	unknown
4S2	unknown

Ds-4L (Marker *C2*
4L1 distal
4L3 at the *C2* locus
4L4 distal
4L5 distal
4L6 distal
4L7 distal

Ds-5, (Marker *A2*
5S1 proximal
5S2 proximal

Ds-5L, (Marker *Bt1* distal to *Bt1* and proximal to *Pr1*

Ds-7L (Marker *O5*
7L1 distal
7L2 proximal?

Ds-8L1 (Marker *Pro1* unknown

Ds-9S1 (Marker *C1-I* proximal?

Ds-9L1 (Marker *Dek13* unknown

Ds-10L (Marker *R1-Sc*
10L2 proximal
10L4 proximal
10L5 *Ac* at the *R1* locus
10L6 *Ac* at the *R1* locus

In description of each of the *Ds* cases, we include the marker gene and the *Ds* component in the chromosome order. Thus, the first three of four cases affecting *Dek1* which was located on the short arm of chromosome 1 and where *Ds* was on the distal side of *Dek1* locus from the centromere, was designated as *Ds-1S1 Dek1*, *Ds-1S2 Dek1,* and *Ds-1S3 Dek1.* The fourth of the Ds-1S cases was located on the proximal side of the centromere from the marker locus and was designated as *Dek1 Ds-1S4*. In another case, involving the marker *Bz2* on the long arm of chromosome 1, *Ds* was found to be at the *Bz2* locus and also suppressed the *Bz2* gene to give a recessive mutable allele (bz background with dots or reversions to *Bz* on its surface). This case was designated as *bz1-m Ds-1L3*.

The *Ac* used was the one associated with *P1-vv* which is referred to hereafter as *Ac*. In many of the photographs of the Ds cases described below *P1-vv* can be clearly seen as red streaks on the colorless kernels. This is an expression of *P1-vv* in the maternal parent and is caused when Ac is inserted in or near the red pericarp locus. The streaks are caused whenever Ac moves away from the locus. *Ac* has most of the same properties as *Ds* and can act an autonomous element. In this paper *Ac* will be understood to be *P1-vv* unless otherwise described. In our pedigrees a dash (-) will be used to indicate the absence of *Ac* in the alternative chromosome segment.

Within the descriptions of the markers we use the terms twin spots; chains of dots, and recovery spots (these terms are further defined in Part I, MNL 84:1-11; see also the maize gene review wiki, http://maizegenereview.maizegdb.org/doku.php).

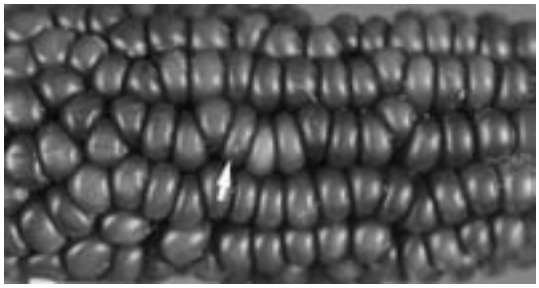
Chromosome 1S (Marker *Dek1*): The mutant *dek1* (defective kernel) has colorless kernels due to lack of anthocyanin and carotenoid pigments, because the aleurone layer is missing. Kernels have a soft opaque endosperm (termed floury), and are generally defective in that the embryo is small and round.

Embryo lethality may be forestalled when immature embryos (12-16 days) are excised before lethality and placed on a standard culture medium. If cultured this way they often produce roots, but no differentiation or growth of leaf tissue. Plant chimeras of hemizygous mutant -

/dek tissue, sustained by adjoining normal *+/dek* tissue, were observed and compared.

***Ds-1S1*. Maize Genetics Coop Stock Number: T3312A**

Chromosome breaking; position distal (*Ds-1S1 Dek1)*. With *Dek1* and *Ac* against *dek1* as a tester, *Ds-1S1* expresses as frequent small to medium colorless patches in colored kernels, and as frequent narrow, morphologically distorted, chlorophyll-deficient sectors on normal green plants. **Origination**: a single intensely purple kernel with numerous small and medium-sized colorless spots on a full purple ear, from the cross of *Dek1/dek1* x *Ac Dek1/- Dek1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot. The mosaic kernel produced a vigorous plant which had a few tiny elongated sectors of morphologically distorted tissue. The selfed ear, which had variegated pericarp (*Ac/-*), produced purple, purple with colorless dots, and colorless floury defective kernels. Subsequent plantings of mosaic kernels produced plants that had fair vigor and tiny sectors.



Ds-1S1 (Image 1). original ear. Section of a fully colored M1 ear from the cross of *Dek1/dek1* x *Ac Dek1/- Dek1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* from an open-pollinated detasseled plot. Careful examination of the ear shows a single kernel (arrow) with many tiny to small colorless patches, indicating chimeral loss of *Dek1* in the 3N *dek1/dek1/Ac Dek1* endosperm, among fully normal colored sibling Ac *Dek1* kernels. The embryo of this kernel was confirmed as *Ac Dek1/- dek1* with an associated *Ds* breaker nearby.

Ds-1S1 (Image 2). Enlargement of original *Ds-1S1* kernel from Image 1 above.

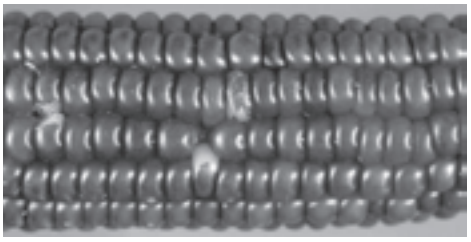
Ds-1S1 (Image 3). Selfed ear from the original *Ac Ds-1S1/- dek1* plant showing 3 to 1 segregation for intensely purple kernels, including many with small colorless spots,and for colorless floury partially collapsed *dek1* kernels. The kernels with etched-like crevices on the surface are unexpected but they occur at a frequency that may be represented as a variant expression of this *Ds* system.

***Ds-1S2*. Maize Genetics Coop Stock Number: T3312B**

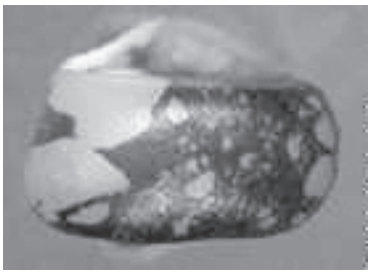
Chromosome breaking; position probably distal (*Ds-1S2 Dek1)*. With *Dek1* and *Ac* against *dek1* as a tester, expresses as colored-colorless mosaic kernels with many small, medium and large colorless areas. Heterozygous plant has frequent narrow, morphologically distorted, chlorophyll defective sectors. **Origination**: a single purple and colorless mosaic kernel on a full purple *Dek1/dek1* x *Ac Dek1/- Dek1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* ear from an open-pollinated detasseled plot. The kernel had several large patches of colorless tissue and many smaller colorless spots in the colored areas. The plant grown from this mosaic kernel was fairly vigorous and had many tiny (pencil-lead-sized) long sectors of morphologically distorted leaf tissue, like *Ds-1S1* but with greater frequency. These sectors were indented on the top of the leaf surface and the corresponding undersurface of the leaf protruded. This pattern was consistent for all smaller sectors but not for the rare larger sectors, where flexibility of the leaf surface tended to obscure the distortion. These larger sectors were also clearly lacking in chlorophyll and were albino. Thus, *dek1* has a plant phenotype of a peculiar morphological distortion of the leaf surface and also the absence of chlorophyll. Earlier work by Cone et al. (1989) using the same insertion showed that the colorless sectors were due to a failure of aleurone tissue development, not a failure to produce anthocyanin pigments.

These plants were selfed and produced ears with *P1-vv* (*Ac*) having purple, mosaic, and colorless floury kernels. The mosaic kernels were not like the original kernel, but had mostly small and medium-sized colorless sectors and

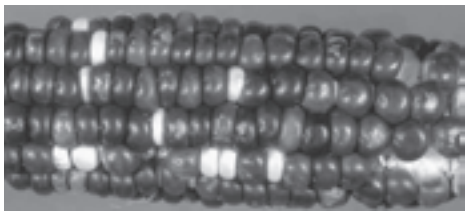
no clusters of recovery spots. The original kernel had all the characteristics of proximal location; namely, frequent large sectors with occasional recovery spots, but subsequent tests showed the characteristics of a distal location; that is, small and medium size sectors with no recovery spots. The presence of mosaics on 1/3 or more of all colored kernels indicates that almost all kernels with *Dek1 dek1 dek1* (from a self) are mosaic and therefore verifies that *P1-vv* (*Ac*) and *dek1* are closely linked. This further complicates interpretation of observations by increasing the number of variables.



Ds-1S2 (Image 4). Section of a fully colored M1 ear from the cross of *Dek1/dek1* x *Ac Dek1/-Dek1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* from an open-pollinated detasseled plot, showing two mosaic kernels (near center of ear) among the fully purple normal sibs. The bottom kernel had a normal-appearing embryo and subsequent plant, and was not analyzed further. The top kernel (see enlargement in Image 2 below), designated as *Ds-1S2*, proved to have a correspondingly affected embryo.

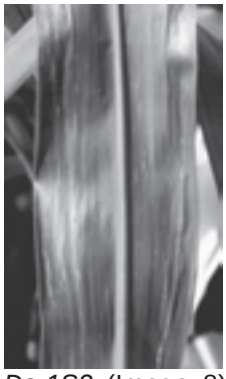


Ds-1S2 (Image 5). Origin kernel from ear shown in Image 4 above. This kernel shows two large attached colorless areas and many smaller sectors of varying size. The lace-like appearance of the colored areas represent repeated later loss of *Dek1* in a manner typical of distal location of *Ds.*



Ds-1S2 (Image 6). Self of the original *Ac Ds-1S2 Dek1/-dek1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* plant, segregating for purple, purple colorless mosaic, and colorless floury kernels, showing a 3-1 segregation for colored *Dek1* and *dek1* floury kernels. The colored *Dek1* class includes kernels with mostly tiny colorless spots and an occasional large colorless sector. The frequency of mosaic kernels is low even though *Dek Ds* and *Ac* are linked on the same arm, because 3N aleurones having two or more *Deks* would cover up each other's losses, and those with 2 or more *Acs* would delay the timing of most *Ds* losses to where they would not be seen.

Ds-1S2 (Image 7). Top leaf surface of the *Ds-1S2* plant grown from the original mosaic kernel. The surface shows small long narrow sectors of indented tissue. Some sectors are white, especially the larger one at lower left, indicating the absence of chlorophyll. This shows that an additional phenotype of *dek1* is white albino.

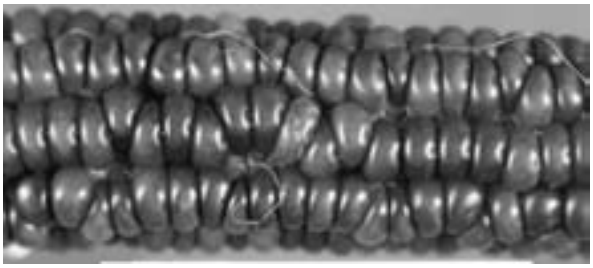


Ds-1S2 (Image 8). The underneath surface of the same leaf section as above (Image 7), showing that the sectors that are indented on

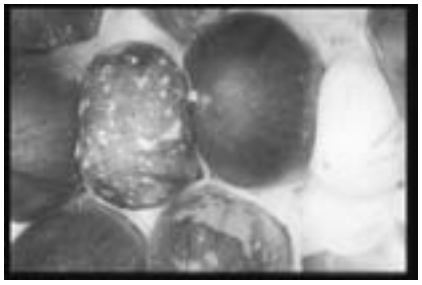
the top protrude on the undersurface. This conforms to and confirms the aberrant morphology described above. The larger sectors are also lighter in color.

***Ds-1S3*. Maize Genetics Coop Stock Number: T3312C**

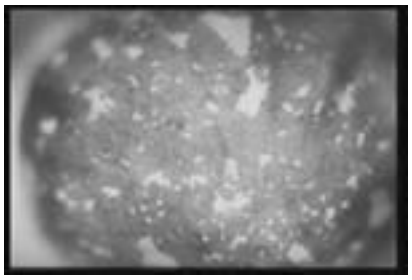
Chromosome breaking; position distal (*Ds-1S3 Dek1)*. With *Dek1* and *Ac* against dek1 as a tester, expresses as purple kernels with many small to medium sized colorless dots and as a green plant with a few tiny narrow morphological distorted sectors. **Origination:** a single purple and colorless mosaic kernel, with small to medium sized colorless sectors on a full purple ear, from the cross of *Dek1/dek1 x Ac Dek1/- Dek1, Ds-10L2 R1-sc/Ds-10L2 R1-sc.* The mosaic kernels from these ears had a slightly dilute appearance. The sectors are rarely large but there is a high frequency of tiny to medium colorless sectors. In some genotypes the background color was dilute enough to make visible a rare small intense twin spot along the borders with the colorless sectors. An occasional intense satellite dot was found in those colorless sectors that were large enough to express them. The smaller colorless sectors are so frequent that the kernel has a lacy appearance that could be mistaken for chains of satellite spots. The plant grown from the mosaic kernel was fairly vigorous and had many small morphologically distorted and some chlorophyll-deficient sectors, like *Ds-1S2*. The selfed ear from this plant segregated for purple mosaic and colorless floury defective kernels. *Ds-1S3* is more difficult to interpret because the marker gene, *Dek1*, produces intense anthocyanin pigment even in one dose. Except for occasional kernels that are dilute due to modifying genes, the background tissue is fully colored. Thus, sectors of duplicate tissue are not visible. Rarely, a dilute kernel with a few twin spots was seen. These and the characteristic small sectors suggest that *Ds-1S3* is distal to the *Dek1* locus.



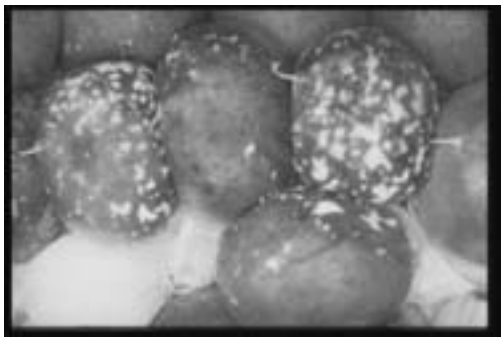
Ds-1S3 (Image 9). Ear showing *Ds-1S3* original kernel. Section of a fully colored M1 ear from the cross of *Dek1/dek1 x Ac Dek1/- Dek1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* showing a single kernel with many tiny to small colorless patches, indicating chimeral loss of *Dek1* in the 3N *dek1 dek1 Dek1* endosperm, among fully normal colored sibling *Dek1* kernels. The embryo of this kernel was expected to be *Ac Dek1/- dek1* with an associated *Ds* breaker nearby. The single purple and colorless mosaic kernel with small to medium-sized colorless sectors on a full purple ear was designated as *Ds-1S3*.



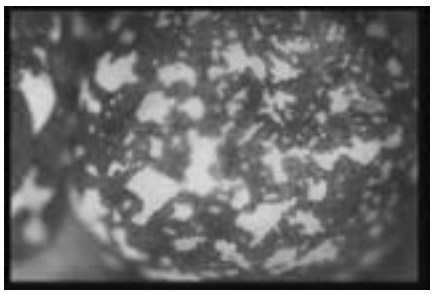
Ds-1S3 (Image 10). *Ds-1S3* kernels on the ear from the self of *Ac Ds-1S3 Dek1/dek1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* plant showing many small to medium losses of *Dek1* function caused by a distal chromosome breaking *Ds*. The diluted appearance of the middle kernel is the result of one dose of *R1-sc* in the 3N aleurone. This image shows normal full-colored kernels (1 and 3) and kernel 4 is a colorless floury defective kernel overlaid by red streaks of *P1-vv* in the pericarp.



Ds-1S3 (Image 11). Enlargement of the middle kernel from Image 10 above, showing loss of color as small as two cells in size, and also a few cases of intense few-celled dots that may be interpreted as twin spots, but are not definitive because the dilution is the result of function of one dose of the *R1* locus. Their small size and rarity makes this case subject to other interpretations. This case is similar to kernel 3 (see Images 12 and 13 below).



Ds-1S3 (Image 12). The top first and third kernels are heterozygous (*Ac Ds Dek/dek/dek* aleurone) and have large frequent losses of one dose of *Dek*. The top middle kernel (#2) is probably the reverse combination, having two doses *of Ac Ds Dek/Ac Ds Dek/dek* and shows colorless spots only where the loss of both *Deks* coincide. The dark spots arise when twin spots from the breakage-fusion-bridge cycle occur, causing an increase in the copy number of *Deks* that show on this coincidentally dilute kernel. This lighter occurrence establishes that *Ds1-S3* is located distal to the centromere. In this case, *Ac*, *Dek1* and *Ds* are all on the same chromosome strand and linked together, so there is one dose of all three of these in the pollen grain and two doses of all of these in the female gamete. The colorless kernel at the bottom lower left (#4) is homozygous *dek1*. The rounded kernel on the lower right side (#5) is lighter in color and has fewer colorless spots, and a few dark spots. The background is mottled. This kernel shows an interesting effect in that the upper right hand side of the kernel matches kernel #3 just above it, but the lower side matches kernel #2 to the left. This occurs because kernel #5 has two doses of *Ds* like the middle kernel (#2) so most of kernel 5 is like kernel #2, and a colorless patch arises only when the two of them coincide. In the upper right hand corner of kernel #5, an early event caused one of the two *Ds* cases to be lost which made the *Ds* disappear in the whole area. After the loss event, all subsequent tissue will not be colorless, it will be colored with the same kind of spotting as kernels #1 and #3 which have only one dose of *Dek Ds*.



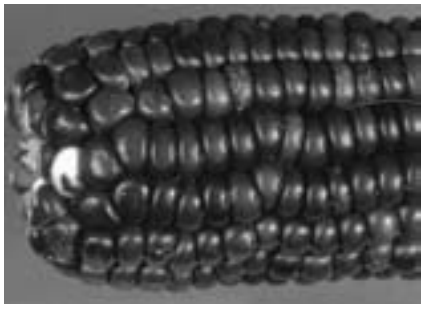
Ds-1S3 (Image 13). An enlarged view of kernel #3 from Image 12 above, showing colorless spots as small as one cell, indicating loss as late as the last cell division of the aleurone. The appearance of scattered one- to six-cell dark dots, especially along the border of the larger colorless spots, suggests twin spots but this is not definitive on such an intense background color. The fact that *Ac* is on the same chromosome but distal to *Ds* makes the interpretation of this observation difficult.

***Ds-1S4*. Maize Genetics Coop Stock Number: T3312D**

Chromosome breaking; position proximal (*Dek1 Ds-1S4)*. With *Dek1* and *Ac* against the marker *dek1* as a tester, expresses like *Ds-1S2* but more extreme. Plant has many small sectors. **Origination**: a full purple ear from the cross of *Dek1/ dek1 r1 r1* x *Ac Dek1/- Dek1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* produced a single purple and colorless mosaic kernel with a large (3/4+) colorless sector, and smaller purple areas with some colorless dots within their borders. The plant grown from the mosaic kernel was somewhat lacking in vigor and had so many small thin morphologically distorted and chlorophyll-defective sectors that the leaves had a ruffled appearance. The selfed ear segregated for purple, purple colorless mosaic, and colorless floury kernels. Outcrossing this plant on *+/dek1* ears produced 1/2 full purple, 1/4 purple mosaic, and 1/4 colorless floury kernels. The mosaic kernels (see image below) often had more colorless than purple areas, the latter appearing to be in fragmented sectors composed of chains and islands of purple tissue scattered about in the colorless background.

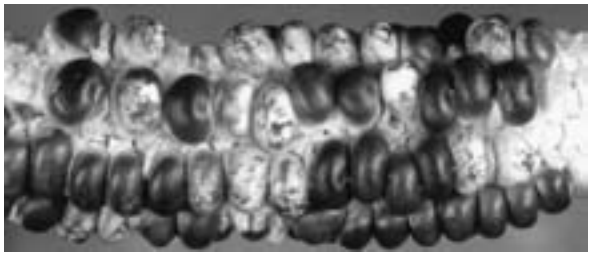
Comparison of *Ds-1S3*, which has mostly small sectors, with *Ds-1S4*, which had many larger colorless sectors on the kernel, revealed another interesting fact about *dek1* function. When the colorless sectors were small the colorless tissue was flinty, unlike the floury *dek1* phenotype. But when the sectors were large (see image below), the sector was flinty along the border marked by the purple aleurone. The

central area was often floury like typical *dek1* kernels, thus indicating a lack of cell autonomy for this characteristic. *Ds-1S4* was used in crosses on another defective kernel mutant, *Dek32*, a shrunken floury kernel mutant on chromosome 1S, and was found to uncover sectors of floury tissue that appeared only in the crown of the kernel, showing a lack of cell autonomy.



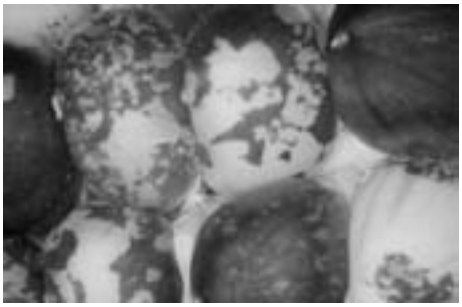
Ds-1S4 (Image 14). *Ds-1S4* original kernel. Section of a fully colored M1 ear from the cross of *Dek1/ dek1 r1 r1* x *Ac Dek1/- Dek1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* from an open-pollinated detasseled plot, showing a single kernel with a large colorless chimera covering three-fourths of the kernel surface, and a smaller colored area with irregular outline and colored surface. Note that the colorless chimera is floury and bordered by a narrow band of flinty tissue between it and the colored tissue. This is a clear indication of a lack of cell autonomy for the *Dek1* phenotype. Also note cases of pitted kernels indicating *Ac Ds* activity at an unknown locus, related to the parental stocks used.

Ds-1S4 (Image 15). The selfed ear of the original plant segregating for expected kernel phenotypes, including 1, 2 and 3 doses of the *Ac Dek Ds* chromosome segment and 3-dose *dek* kernels, as shown in the aleurone, and 1 dose of *Ac* in the overlaying pericarp. It demonstrates a wide range of expression of sectored losses of colored aleurone and associated defective endosperm and the persistence of patches, chains of dots, and islands of normal tissue. The small kernel (between the two mosaic kernels) at the center of the ear shows a collapsed phenotype as a result of the fact that in this particular case the genetic stock is weak.



Ds-1S4 (Image 16). Ear from the cross of *Dek1/dek1* x *Ac Dek1 Ds-1S4/-dek* showing both full colored normal, mosaic and small colorless and collapsed *dek* kernels. The mosaic kernels are a striking display of large patches of colorless tissue set off from smaller areas of colored cells that are often arranged in chains of dots and isolated islands of intensely colored tissue of one or more dots. These may be interpreted as recovery spots, or possibly, all colored areas may be regarded as tissue that retains an acentric fragment with a functional *Dek* locus. Also, the large colorless areas have an interior of white floury tissue bordered by a yellowish flinty tissue between the floury and the colored areas. This demonstrates the non-cell-autonomous characteristic of the *Dek1* gene product. This indicates that the substance produced by the *Dek1* gene is not confined to the cells but leaches out and goes to the border around it.

Ds-1S4 (Image 17). A selfed ear of *Ac Dek1 Ds-1S4/- dek1* plant showing 3 to 1 segregation for intensely purple kernels, including many with large colorless patches and lacy-like colored areas, and for colorless floury partially collapsed *dek1* kernels. The frequency of the colorless *dek1* kernels appears to be reduced, but this is only the consequence of the fact that some of the colorless floury kernels are collapsed and defective and are not always seen on the photograph.

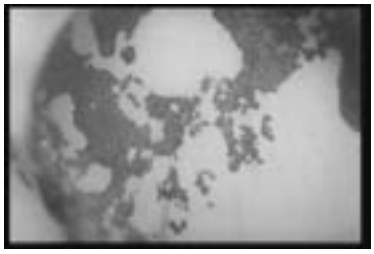


Ds-1S4 (Image 18). Enlargement of kernels from the selfed ear above (Image 4), showing *Ac Dek Ds/- dek/dek* (middle 2 kernels in top row) and *Ac Dek Ds/Ac Dek Ds/dek* (dark kernel in lower row). The middle two kernels in the top row show large colorless patches with chains of dots and isolated islands of intensely colored tissue. The bottom dark kernel shows mostly colored tissue interspersed with small lighter patches from overlapping events with a double dose of *Dek Ds-1S4*

Ds-1S4 (Image 19). Like Image 18 above, showing additional examples.

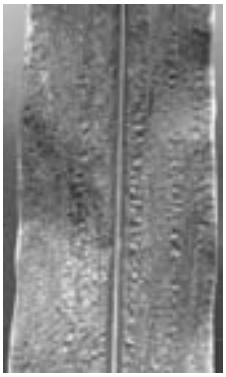
Ds-1S4 (Image 20). Kernels from a *Ac Dek Ds/dek/dek* ear showing good examples of the behavior of acentric fragments in the cell lineages of the aleurone.

Ds-1S4 (Image 21). Enlargement of the far right-hand kernel in Image 20. The kernel is *Ac Dek Ds/dek/dek* showing good examples of the behavior of acentric fragments in the cell lineages of the aleurone. Note the islands and chains of recovery spots, including one example (lower left) where the surviving fragment was carried along and maintained very well, producing a large thumb-like area of intensely pigmented tissue. Nearby are several small islands that represent lineages or replication of the acentric fragments that did not keep up with cell division, thus producing lineages from daughter cells that did not always retain a functional *Dek1*. To the right are patches of colored tissue with colorless loss areas within their borders, suggestive of fully functional lineages with periodic losses because an active *Ds* and *Dek1* gene were on a chromosome arm still attached to the centromere.



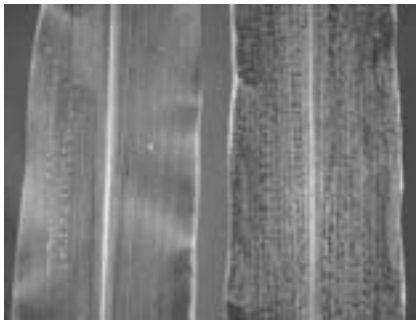
Ds-1S4 (Image 22). Like Image 21 above. Note that some of the colored areas have succeeding small colorless spots (loss of *Dek*) and some do not.

Ds-1S4 (Image 23). An *Ac Dek Ds-1S4/- dek1* plant showing wrinkles and bumps on both top and bottom leaf surfaces, apparently resulting from frequent loss of *Dek* in the *Dek/dek* leaf tissue. There are no valid visible white cells that can be seen in *(-)/dek* sectors but only a distortion of the normal green cell pattern suggesting that the mutant cells are diminished or missing (lethal). Compare this with the morphologically distorted and/or white sectors of *Dek Ds-1S2*.



Ds-1S4 (Image 24). A striking view of the defective appearance of the underside of the mutant leaf described in image 23. A clear example of protruding tissue.

Ds-1S4 (Image 25). Section of the top surface of the 8th leaf of a normal sib (left) and the *Ac Dek Ds-1S4/- dek* original plant (right) showing the consequences of the *Ac Ds* induced losses in heterozygous leaf tissue. No white streaks are apparent on the top, but the cell lineages are defective and did not survive well, producing the rough appearance on the top of the leaf. This is because the surviving cells grow into the empty spaces left by the dead cells. This is a unique characteristic of *Ds-1S4* as opposed to the other *Ds-1S* mutants. It is not known why the cells did not survive. Normally albino cells survive because surrounding cells will feed them and keep them alive. Perhaps some other gene is being lost besides *Dek1*. The segment lost must have had something vital to the development of leaf tissue. The *Ds* leaf has a wrinkled bumpy surface, darker in color, less reflective surface and irregular elongated indentations, only a few of which may be white, as compared to the smooth bright green surface of the normal leaf.



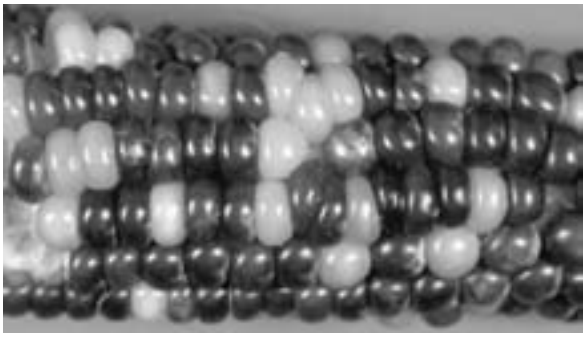
Ds-1S4 (Image 26). Section of the underneath surfaces of the leaves described in image 25 above, showing the 8th leaf of a normal sib (left) and the *Ac Dek Ds-1S4/- dek* original plant (right). The ladder-like appearance of the vein network on the undersurface of the leaf is an effect produced by the wrinkles protruding on the underneath where they are indented on the top surface.

Chromosome 1L (Marker *Bz2*): *Ds* markers found using *bz2* were of two types: 1) mosaic kernel cases where the *Ds* site was located somewhere on the long arm but not at the *bz2* locus (*Ds-1L1*); and 2) the whole kernel cases that expressed the *bronze2* phenotype (either initially stable where *Ac* is absent or mutable when *Ac* is present), in which the CB *Ds* was at the *Bz2* locus displaying its gene function suppression property. (Note: *bz2-s* = *bronze2-stable;* it does not become mutable in the presence of *Ac*.)

Ds-1L1. Maize Genetics Coop Stock Number: **T3312E**

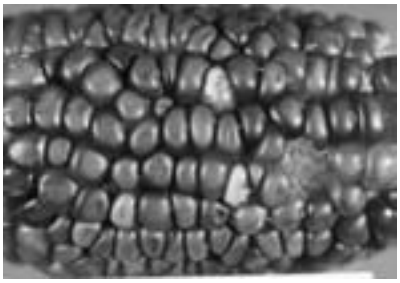
Chromosome breaking. Position proximal. With *Bz2* and *Ac* against *bz2* as a tester, expresses as mosaic kernels with intense purple background and a moderate number of variably sized bronze sectors with frequent recovery spots of *Bz* tissue in the *bz* areas. **Origination**: a single purple and dilute purple (*bz*) mosaic on a normal full purple ear, from the cross of *bz2-s* x *Ac Bz2/ Bz2, Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot. The plant grown from this kernel was normal; it produced a selfed ear segregating for *Bz, Bz bz* mosaic and *bz* kernels. Mosaic kernels from this self, and from an outcross on *bz2-s*, had a moderate number of *bz* sectors and frequent recovery spots of *Bz* in the *bz* sectors. The characterizations of colored vs. bronze sectors do not fall into simple categories like *Dek* on chromosome 1S, thus it was impossible to determine with certainty whether *Ds* was proximal or distal. This selfed plant was

outcrossed on the defective kernel mutant *dek22* on chromosome 1L. It produced kernels with sectors of defective kernel tissue, but no clear evidence of recovery spots could be seen because of the imprecision of the borders between normal and *dek22* tissue.

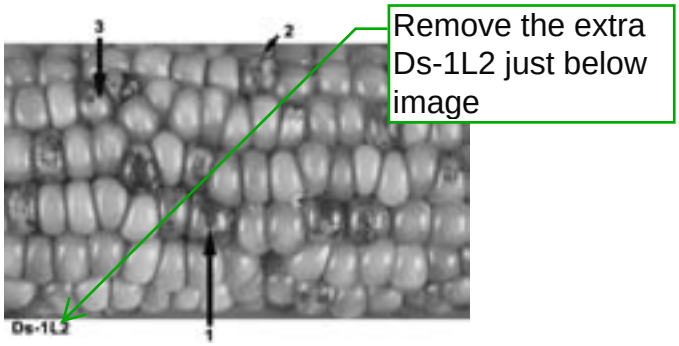


Ds-1L1 (Image 27). Ear from the cross of *+/an1-6923* x *Ac*, *Ds-1L1 Bz2/bz2* segregating one colored normal (*Ds Bz2/bz2/bz2* no *Ac*) and one colored and bronze mosaic (*Ds Bz2/bz2/bz2, Ac*) and two bronze (*bz2 bz2 bz2* with or without *Ac*) kernels. The mosaic kernels have moderate-sized losses of *Bz2* function with adjoining patches of loss, chains of dots, and frequent isolated dark colored islands (recovery spots). (Note: *an1-6923* is a short deficiency of the *Bz2* and *An1* loci and was used for a stable *bz2* allele.)

***Ds-1L2*. Stocks not available.** Position at the locus.



Ds-1L2 (Image 28). Ear from the cross of *Bz2/an-6923 R1 R1* x *Ac*, *Bz2, Ds-10L2 R1-sc/Ds-10L2 R1-sc* with full purple normal kernels and two clear purple and bronze mosaic kernels, the top one of which (*bz2-m*) has a large bronze area and areas with chains of purple dots and isolated purple recovery spots. This case was confirmed by progeny tests, proving it to be a legitimate breaking *Ds* with suppressing properties at the *Bz2* locus, and was designated as *Ds-1L2 bz-m*. The lower kernel (*Bz2 bz2*) was found to have a normal embryo and was not tested further.



Ds-1L2 (Image 29) Ear from the cross of *bz2-s bz2-m/bz2-s* x *Ac*, *Bz2 Ds-1L2/bz2-s* with one-fifth purple and bronze mosaic kernels and four-fifths bronze kernels. The mosaic kernels have large bronze areas representing losses of *Bz* and chains of dots, isolated single dark *Bz* recovery spots, and also some large colored areas with occasional apparent loss of *Bz* function. Note kernels showing (1) *Bz* revertant sectors; (2) patches of dots and chains of dots; and (3) large *bz* sectors which are stable losses of *Bz* either by excision or by stable silencing of the *Bz* locus. Substantiation of this interpretation comes from the fact that offspring from these seeds include stable revertant *Bz2* alleles; various sized sectorial losses of *Bz*; and *bz* kernels with purple dots where *Ac* is present and stable *bz* where *Ac* is absent. Therefore the most likely location for *Ds* is at the *Bz2* locus. Proof of chromosome breaking and exact location will need further tests.

***Ds-1L3*. Maize Genetics Coop Stock Number: T3312F. No image.**
Chromosome breakage and suppression; position at *Bz2* locus (also known as *bz2-m3).* With *Bz2* and *Ac* against *bz2-s* (*bronze-stable*) as a tester, expresses as bronze (dilute purple) kernels with many intense purple spots indicating *Ds* suppression of *Bz* function and *Ac*-regulated reversion to *Bz*. Also, frequent large sectors of bronze with only rare purple dots, suggesting loss of the *Bz* locus, probably due to chromosome breakage. **Origination**: Arose as a *bz* kernel with no dots or sectors, because the insertion gamete got no *Ac* as a 50% probability from the heterozygous *Ac*/- parent, from the cross of *bz2-s/bz2-s* x *Ac Bz2/Ac Bz2*, *Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot. This bronze kernel was grown out and crossed with *bz-s Ac* stock, and produced a 1-1 segregation for a type similar to

Ds-1L2 bz2-m. Subsequent crosses of pollen from a *bz2-m Ac*-carrying plant on *bz2-s* produced kernels with clear patches of loss of mutability. This indicated either chromosome breakage at the *Bz2* site, or changes of *bz2-m* to *bz2-s*, which are hard to distinguish except for the presence of recovery spots in the former.

***Ds-1L6*. Stocks not available;** position at *Bz2* locus.

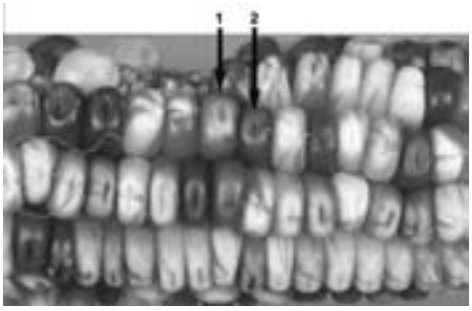
Ds-1L6 (Image 30) ear from the cross of *bz2-s/bz2-s* x *Ac*, *Bz2 Ds-1L6 bz2-m/bz2-s* with one-half purple and bronze mosaic kernels and one-half bronze kernels. The mosaic kernels have large bronze areas representing losses of *Bz* and chains of dots, and isolated single dark *Bz* recovery spots, and also some large colored areas with occasional apparent loss of *Bz* function. In this case, some of the revertant sectors are pale in color and have smaller dark full-colored dots within their borders (see kernel in middle and one on far left). This indicates that *Ds-1L6* has the property of producing intermediate alleles of the *Bz* locus, like *Ds-1L3* this probably has *Ds* activity at the *Bz2* locus.

Chromosome 2S (Marker *B1-Peru*): A special pollen stock was prepared for chromosome 2S, carrying *B1:Peru*, a dominant aleurone color allele that is a duplicate factor with the *R1* locus on chromosome 10L. The tester used was both *b1* and *r1*; since both *B1:Peru* and *R1* produce aleurone color the pollen had to contain both alleles so that changes could be seen. Because the *Ds* source was marked with the *R1-sc* allele, it was necessary to use a stock that was *Ac/-B1:Peru/B1:Peru*, *Ds-10L4 R1-sc/r*. Only those changes of *B1:Peru* that were transmitted with an *r*-carrying gamete would be seen. Changes of *R* would not be seen because they would be covered by *B1:Peru*. Individually tested male parents were used to cross on the tester because of the difficulty of preparing this stock. The carefully controlled hand pollinations produced only half correct gametes from half of the plants used.

***Ds-2S1*. Maize Genetics Coop Stock Number: T3312G**

Chromosome breaking; position distal. With the dominant aleurone color gene *B1:Peru* and *Ac* (both present in 1 dose) against *b1, r1* as a tester, expresses as a dilute purple kernel with many small colorless dots which are often associated with tiny intense colored twin spots. **Origination**: a single purple kernel with many tiny colorless dots on a dilute purple ear, from the cross of *b1, r1* by *B1:Peru*, *Ds-10L4 R1-sc/r1-g*. Since *B1:Peru* and *R1-sc* are duplicate factors, only those kernels that are *b b B, r r r* (3N endosperm) will express losses of *B1:Peru*. The plant grown from this kernel was normal and produced a selfed ear that segregated for colorless, purple, and dilute purple (some with small tiny colorless dots and associated tiny intensely colored twin spots) kernels. In subsequent crosses using progeny from dilute "twin spot" kernels to self and cross on a *b1 r1* tester, *P1-vv* was not present but the losses continued in most of the outcrosses. Therefore it was concluded that *Ac* was present but not at *P1*. Crosses on *b1 r1 P1-vv* show an *Ac* dosage response. The absence of large colorless sectors and the presence of twin spots suggest that *Ds-2S1* is distal to the *B1* locus.

Ds-2S1 (Image 31). Ear from the cross of *b b,r r* stock x *B1:Peru*, *Ds-2S1/same, r r Ac Ac* showing dilute purple *B b b, r r r* kernels with tiny colorless patches due to loss of the aleurone color *B1:Peru* allele resulting from *Ac*-induced breakage of the *B*-carrying 2S chromosome arm. Higher magnification reveals that some dark purple twin spots are associated with the colorless spots, suggesting a distal position of *Ds* in relation to *B* and the centromere.



Ds-2S1 (Image 32). Ear from the cross of (*P1-vv*) *Ac/-, b1-m Ds-2S3/b1* x (not *P1-vv*) *Ac/-, B:Peru Ds-2S1/b1* showing several genotypic variations: (1) a pale kernel (*Ds-2S3*) with many tiny colored dots (*b-m/b-m/b, Ac/-/-*); and (2) a purple kernel (to the right of #1; it is *Ds-2S1*) with a few small faintly colored dots and tiny, almost imperceptible dark dots (*B:Peru, Ds-2S1/b/b, Ac/-/-*). *Ac (P1-vv)* is clearly heterozygous in the female plant. Other expected classes are present but barely perceptible at current magnification. Since two unlinked *Ac's* (one at *P1-vv* and one elsewhere) are present, we have several doses of *Ac* in many kernels producing delayed *Ds* events in doses higher than one in the endosperm.

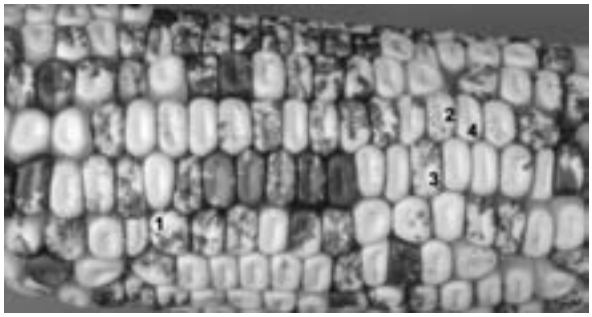
***Ds-2S2*. Stocks not available. No images.**

Chromosome breaking; position not known. **Origination**: a colored kernel with mosaic patches of colorless aleurone on a full purple ear, from the cross of *b1 b1, r1 r1* x *Ac/-B1:Peru/B1:Peru, Ds-10L4 R1-sc/r1-g*. The plant grown from this kernel was normal and produced a selfed ear that segregated: 2 purple; 6 purple colorless and mosaic; and 4 colorless kernels. Subsequent plantings from mosaic kernels produced normal green plants, which on selfing gave reduced numbers of colored and mosaic compared to the colorless kernels. Outcrosses on *b1 r1* using these plants as males produced colorless kernels with about 1% fully colored and no mosaics. This case behaves like an unstable chromosome with much reduced male transmission and moderate reduction in female transmission.

***Ds-2S3*. Maize Genetics Coop Stock Number: T3312I**

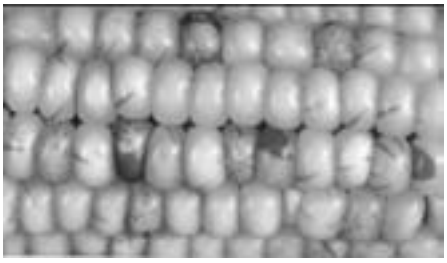
Chromosome breakage and suppression of resident locus phenotype; position at the *B1:Peru* locus. *Ds1* element at *B1:Peru* (also called *b1-m1* or *b1-m1::Ds1*, see Clark et al. 1990). With *Ac* against *b1, r1* (both in 1 dose) as a tester, expresses as colorless kernels with many small purple dots, indicating *Ds* suppression of *B1:Peru*. Occasional larger purple sectors are dilute purple and have smaller enclosed colorless spots with even smaller associated intense colored twin spots, suggesting that chromosome breakage may occur (Neuffer 1995). **Origination**: a single colorless kernel on a purple ear, from the cross of Ac/-*B1:Peru/B1:Peru, Ds-10L4 R1-sc/r1-g* x Ac/- *b1 r1-g*. Note that only changes of *B* to *b* in an *r1-g* carrying female gamete could produce a colorless kernel. This kernel when grown produced a selfed ear that was all colorless but had many kernels with small colored dots.

Subsequent tests verified that the mutability was at the *B1* locus, not the *R1* locus. A cross of this plant as pollen parent on a (no *Ac*) *r1* tester produced an ear that had colorless kernels with and without dots (revertant sectors). The dots were mostly small, but some were fairly large and dilute in color. These larger sectors had smaller colorless dots within their borders, and these colorless dots were associated with even smaller intensely colored dots (twin spots). These are an indication of loss and duplication in the daughter cell progenitors from the breakage-fusion-bridge phenomenon and suggest that *Ds-2S3* is a chromosome breaking and gene suppressing *Ds*. See image 33 below, which shows *Ds-2S3* in comparison with *Ds-10L2 R1-sc*. This case, which was designated earlier as *b1-m1* because of its gene suppressing property, has been characterized as a *Ds1*-like 400-base insertion in the middle of the *B1:Peru* gene (Clark et al., 1990).



Ds-2S3 (Image 33). Ear from the cross of *b1 r1* x *Ac/-*, *b1-m Ds-2S3/b1, Ds-10L2 R1-sc/r1* showing: 1) characteristic loss pattern of proximal *Ds-10L2 R1-sc* (which see) with large colorless patches, chains of dots and recovery spots as expressed by *R1-sc* on some kernels. 2) Typical revertant pattern of *b-m Ds-2S3*, resident site chromosome breaking and suppression as expressed by *B1:Peru* on kernels with small dots. 3) Other kernels with

both patterns; and 4) other segregants with neither, that are completely colorless.

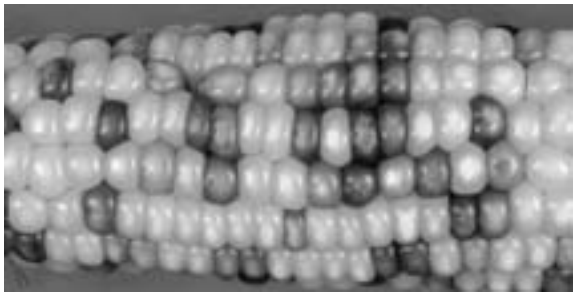


Ds-2S3 (Image 34). Ear showing *b1-m* (*Ds* loss) dots and *P1-vv* (*Ac* loss) red sectors. Ear from self of an *Ac/-, b1-m Ds-2S3/b r r* plant showing 8/44 colorless kernels with many colored dots (*b* to *B* revertants). These are probably the *b-m/b-m/b-m* and *b-m/b-m/b* kernels with one *Ac*. A few have one to three dots, probably *b-m/-/-* with two to three doses of *Ac*. The remaining kernels are colorless, either because they have no *Ac* or 3-dose *Ac* or only non-responding *b* alleles.

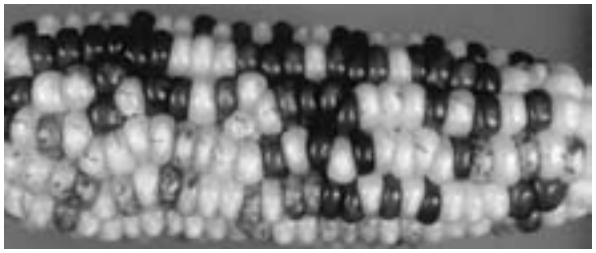
***Ds-2S4*. Maize Genetics Coop Stock Number: T3312J**

Chromosome suppression of resident locus phenotype and possibly chromosome breaking; position at the *B1:Peru* locus. (Also called *b1-md2* or *b1-md2::Ds1*, see Clark et al. 1990). With *Ac* against *b1, r1* (both in 1 dose) as a tester, expresses as dilute purple kernels with small purple dots, some of which are associated with smaller even more intense purple twin spots, suggestive of chromosome breakage (Neuffer 1995), but there are no colorless spots to indicate loss of the *B1:Peru* gene. **Origination**: a single dilute purple kernel with small dark dots on a full purple ear from the cross of *b1 b1 r1 r1 x Ac/Ac B:Peru/B:Peru Ds-10L4 R1-sc/r1*. This kernel grew to a normal green plant, and a selfed ear from that plant segregated 1/2 full purple, 1/4 dilute with full-colored dots, and 1/4 colorless kernels. Pollen from this plant was outcrossed to (no *Ac*) *b1 r1*, and produced an ear with 1/4 dilute kernels with dots, 1/4 dilute without dots, and 1/2 colorless.

The dilute mutable kernels had a uniform dilute background, with many small and a few large darker colored sectors. These darker dots were associated with smaller, even more intensely pigmented dots along or within their borders (like twin spots). No colorless areas were observed. Therefore *Ds-2S4* partially suppresses a functional *B1* locus, but does not cause chromosome breakage on chromosome 2S so as to cause loss of the *B1* locus. The reversions of dilute to stronger *B1* function that are associated with even more intense pigment in a twin spot-like effect suggest duplication of the *B1* segment, but lacks the required companion deficiency that results from the breakage-fusion-bridge cycle. This case, described earlier as *b1-md2* because of its gene suppressing properties, also has been shown to be a *Ds1*-like 400 base insertion near the start of the transcription point of the *B1:Peru* gene (Clark et al., 1990).



Ds-2S4 (Image 35). Ear from the cross of *b1 b1, r1 r1* x *b-dm Ds-2S4/b Ac/-* showing 1/4 dilute purple kernels with many purple dots and small revertant sectors, 1/4 dilute kernels with no dots, and 1/2 colorless kernels with no dots. These observations demonstrate that this case is a *Ds* insertion at the *B* locus that partially suppresses *B* function and that moves away frequently to give *B* revertants. There are no colorless dots, therefore the chromosome is not broken in such a way as to cause the loss of *B* function.



Ds-2S4 (Image 36; see also *Ds-9S1*). A double-pollinated ear of an *Ac/-, b-dm Ds-2S4/b* plant. The top half silks were pollinated by *b b r r* pollen, and show a 1:1 segregation for purple vs colorless, indicating that the 2-dose (*b-dm/b-dm/b*) kernels are full-colored and not pale. The bottom half of the ear was a test made to determine the presence of *Ac* in the female plant. *Ds* breakage and loss of *C1-I* (inhibitor of color in the aleurone) uncovers *C1* to give sectors of color, proving that *Ac* is there to cause the breaks. It was pollinated by homozygous *C1-I Ds-9S1* no *Ac* and has two classes of kernels: 1) those with a pale background composed of many tiny pale two- to

four-celled dots and fewer darker and larger dots and sectors and a scattering of intense full-colored dots. These probably are the *Ac/-/-, C/C/C-I Ds* kernels. 2) Those that are colorless have no dots because they lack *Ac*. This is a dramatic display of the activity of *Ds-9S* with *Ac* (see *Ds-9S1*). Note excellent example of chain of dots in the center of the picture.

Chromosome 2L (Marker *W3*). The phenotype of the mutant *w3* (white) is an albino seedling with a viviparous kernel.

***Ds-2L1* Stocks not available. No images.**
Chromosome breaking; position not known. With *W3* and *Ac* against *w3* as a tester, expresses as a green seedling or plant with narrow pencil-lead sized white sectors on leaf blade. **Origination:** a single green seedling with small white leaf sectors from a sandbench planting of F1 progeny of the cross *+/w3, Ch1* x Ac/*-, W3, Ds-10L2 R1-sc/Ds-10L2 R1-sc*. This seedling matured to a green plant with small pencil-lead-sized white sectors and produced a selfed ear that had both chocolate (*Ch1*) and red variegated (*Ac*) pericarp; it also segregated for viviparous (*w3 w3*) kernels. Seedlings and plants from the normal kernels produced small white sectors in 2/3 of the population, and were later confirmed to have *Ch1*. This substantiates their heterozygosity for *W3* with a chromosome breaking *Ds* on the 2L chromosome, and *w3* with *Ch1* on the other chromosome.

Chromosome 3L (Marker *A1_Sh2*). Kernels that were mosaic for both color (*A1* losses) and shrunken endosperm (*Sh2* losses) in the same sector were found in several progeny.

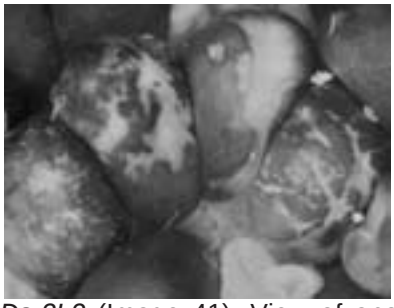
***Ds-3L1*. Maize Genetics Coop Stock Number: T3312L**
Chromosome breaking; position proximal. With *A1 Sh2* and *Ac* (all in 1 dose) against *a1-s sh2* as a tester, expresses as purple, nonshrunken kernels with large colorless sectors and many tiny colorless shrunken spots in the colored area. (Note, *a1-s* is a null allele of the *A1* locus that does not respond to *Dt*, *Ac*, or other known transposon activator-like elements.) The large colorless shrunken sectors often have smaller islands (dots) of colored nonshrunken tissue within their borders. These dots are termed recovery spots and indicate a proximal location of *Ds*. **Origination**: a single colored non-shrunken kernel with sectors of colorless shrunken tissue on a full purple ear, from the cross of *a1 sh2* x *Ac*-, *A1 Sh2*/*A1 Sh2*, *Ds-10L2 R1-sc/Ds-10L2 R1-sc*. The plant grown from this kernel was normal, and when selfed produced an ear segregating for colored nonshrunken kernels, colored nonshrunken kernels with colorless shrunken sectors, and colorless shrunken kernels. Pollen from this plant was crossed on an *a1 sh2* non-*Ac* tester and produced ears with 1/4 colored nonshrunken, 1/4 colored nonshrunken with colorless shrunken sectors, and 1/2 colorless shrunken kernels. This is expected if a chromosome breaking *Ds* that responds to an independently assorting *Ac* is present near *A1*. Crosses of these plants as pollen parents produced the above classes. The mosaic kernels had frequent large colorless sectors and many tiny colorless shrunken sectors in the colored areas. The large sectors often had dots or islands of colored nonshrunken tissue (recovery spots) within their borders, suggesting a location for this *Ds* site of proximal to *A1*. Because the single dose of the anthocyanin gene *A1* gives full purple ears, twin spots were not observable on purple areas of the ear.

Ds-3L1 (Image 38). A selfed ear of the original *Ds-3L1 A1 Sh2/a1 sh2, Ac/-* mutant plant showing 3 *Cl Sh*:1 *cl sh* kernels overlaid with *P1-vv* pericarp sectors. These purple kernels include those with various sizes of mosaics of sectors for simultaneous loss of *A1* and *Sh2*. The largest colorless *sh* areas have small islands of colored nonshrunken tissue (recovery spots) indicating proximal location of *Ds* between the centromere and the *A1* locus. The pale purple kernels are due to segregation of an aleurone dilution modifier from the inbred Mo20W, which was in the stocks used and has nothing to do with *Ac Ds* function.

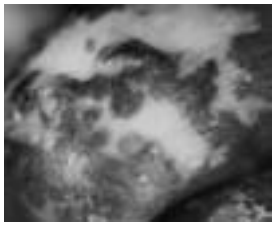
***Ds-3L2*. Maize Genetics Coop Stock Number: T3312M**
Chromosome breaking; position proximal. Like *Ds-3L1*.

Ds-3L2 (Image 39). Selfed ear of the original *Ds-3L2 A1 Sh2/a1 sh2, Ac/-* mutant plant with 3 *Cl Sh*:1 *cl sh* kernels overlaid with *Ac* pericarp sectors. These purple kernels include those with various levels of mosaics of sectors for simultaneous loss of *A1* and *Sh2*. The largest colorless *sh* areas have small islands of colored nonshrunken tissue (recovery spots) indicating proximal location of *Ds* between the centromere and the *A1* locus. The purple nonshrunken class includes those with large colorless shrunken sectors, those with many small colorless shrunken sectors, as well as tiny pits, and those with no sectors at all. Many of the large colorless shrunken class have tiny colored nonshrunken islands within their borders.

Ds-3L2 (Image 40). Enlargement of kernels on original selfed ear giving a clear view of phenotypic combinations showing: (top upper left) colorless shrunken kernel, *a sh/a sh/a sh*; (top upper right) *Ds A Sh/Ds A Sh/a sh* kernel showing small erratic sectors resulting from the simultaneous loss of both the *A Sh*-carrying chromsomes in the same cells; (middle left) *Ds A Sh/Ds A Sh/Ds A Sh* kernel showing slight dilution resulting from tiny spots that occur only with simultaneous loss of three chromosome segments. This also causes a slight rough appearance due to concurrent loss of *Sh*. (Middle right) *Ds A Sh/a sh/a sh* kernel showing a large colorless shrunken sector on the left and large colored sector with no subsequent losses within its borders.



Ds-3L2 (Image 41). View of another section of the original selfed ear (Image 1 above), with mosaic kernels showing consequence of breakage and loss in various genotypic combinations in the 3N endosperm. Whole kernels are described from left to right. (1) An *A Sh/A Sh/a sh Ac/-/-* kernel with frequent losses of both *A Sh* chromosomes which appear as colorless spots only when both losses overlap. The rough surface is due to concurrent *Sh* losses as well. (2) An *A Sh/a sh/a sh, Ac/-/-* kernel with large colorless areas from early loss with occasional smalls islands of colored *Sh* tissue. Also chains of such islands suggesting that lost fragments may recover. The bottom half of this kernel has a large purple sector with no colorless spots within its borders, indicating that the loss mechanism has stopped. (3) An *A Sh/a sh/a sh, Ac/-/-* kernel where a single loss occurred at an early division and the other sector was stable, with the exception that activity occurred once to produce a small colorless shrunken sector. (4) An *A Sh/A Sh/a sh, Ac/-/-* kernel with erratic loss pattern with small sectors, resulting from sequential early loss covering most of the kernel and a subsequent loss of the second *A Sh* chromosome.



Ds-3L2 (Image 42). Enlargement of kernel 2 in Image 3 (above), showing individual aleurone cells with variations in color intensity and morphological distortion due to interruption of *Sh2* function, both of which are difficult to interpret at this stage. Also a single small colored non-shrunken recovery spot embedded in the large colorless shrunken sector.

Chromosome 4S (Marker *Bt2*). The *Bt2* marker is near the centromere on the short arm of

chromosome 4 and has a phenotype similar to *Bt1* and *Sh2*.

***Ds-4S1*. Stocks not available.**
Chromosome breaking; position not known. With *Bt2* and *Ac* (both in 1 dose) against *bt2* as a tester, expresses as kernels with small pits and larger shrunken sectors that have an unusual form. **Origination:** a purple non-brittle kernel with small pits and a larger shrunken patch on a normal purple kernel ear, from the cross of *bt2 x Ac/-, Bt2/Bt2, Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot. The plant grown from this kernel was normal and produced a selfed ear that segregated *Bt2, Bt2* with *bt2* pits and sectors and *bt2* kernels. A homozygote for the *Bt2 Ds-4S1* segment and *Ac* in a subsequent progeny backcrossed on a *bt2* tester produced kernels with small pits and larger shrunken sectors that had an unusual form. These appeared as branched and meandering crevices in the kernel, much like *et1*. Because of the lack of precise borders between the mutant and nonmutant tissue it was not possible to determine twin spots or recovery spots that would indicate location with reference to *bt2*. This raises the question of cell autonomy.

Ds-4S1 (Image 43). Ear from the cross of *bt2 bt2* x *Ds-4S1 Bt2/Ds-4S1 Bt2, Ac/-*, with all purple kernels showing medium to small sized colorless brittle sectors and patches; the larger sectors are long, irregular in pattern and often branched.

***Ds-4S2*. Stocks not available.** Position unknown.

Ds-4S2 (Image 44). Ear from the cross of *Bt2 bt2 c1 c1 r1 r1* x Ac/- *Ds-4S2 Bt2/bt2, C1 C1, R1-sc Ds-10L2/R1-sc Ds-10L2* producing all faintly colored kernels due to one dose of the colored genes *C1* and *R1* in the 3N endosperm. Some appear to be colorless because *Wc* (white cap) is also segregating. Also several *Bt2* kernels show a typical mosaic pattern of loss of *Bt2* due to *Ds* breakage; namely, narrow, branched indentations and scattered pits (see arrows at left end of ear).

Chromosome 4L (Marker *C2*). The *c2* cases are especially informative for analysis of chromosome 4L, due to the wide variation in gene dosage-related color intensity. Kernels with 1 dose of *C2* (*C c c*) in the 3N aleurone are dilute purple; those with 2 doses (*C C c*) are moderately dilute; and those with 3 doses (*C C C*) are fully colored. Thus, three levels of color can be observed in addition to colorless. In general, the mosaic kernels were dilute with small to medium-sized sharp-bordered colorless patches scattered randomly on the dilute background. The angular shape and sharp borders of sectors with dilute background, and the rounded corners and fuzzy outlines of extreme dots and sectors, are the result of anthocyanin diffusing away from the intense areas, but not into or away from the dilute areas. These patches were associated with even smaller sharp-bordered angular patches with irregularly intense color. They are observed on the dilute side of the border between the dilute and colorless sectors. Most of these intense sectors have more than one level of intensity and some tiny colorless spots within their borders.

These observations can be interpreted using McClintock's (1941) breakage-fusion-bridge cycle model. The single dose, unchanged condition, is uniform dilute aleurone. When a *Ds*-induced break occurs distal to *C2* and fusion of the ends follows, then a bridge is formed with two *C2*'s in tandem. When this bridge is broken, sometimes the two *C2* alleles go to one daughter cell and none to the other. Since 3N aleurone can develop quite well with only two intact chromosomes, the deficient daughter cell survives to produce descendant tissue lacking *C2* and will therefore be colorless. The clone of cells descending from this cell will remain colorless. The other daughter cell receives the duplicate segment and will therefore be moderately colored. Subsequent repetition of breakage and healing will repeat the process,

only this time the sectors will be much smaller and the duplicate clone will be of full intensity; hence, the characteristic twin spots.

If, however, the *Ds* breaker is proximal to the *C2* locus, the first break would cause the loss of the *C2* locus from the centric chromosomes in both daughter cells while retaining both *C2* genes in the acentric fragment. The distribution of *C2* function will therefore depend on the fate of the fragment in subsequent mitosis, and the expression of *C2* will depend on the behavior of the locus in a piece of chromosome without a centromere. If the locus functions in the fragment, then smaller clones of intense pigment should remain in cell lineages as long as the fragment replicates. The fate of the *C2* locus will depend on what happens to the fragment carrying the locus: does it replicate and/or did the daughter fragment get dislocated in the subsequent cell lineage. Replicated fragments would appear as recovery spots (single islands or chains of islands of intense pigment) within the colorless lineages. Thus, smaller sectors with twin spots indicate a distal locus location. Larger sectors with no twin spots but with recovery spots indicate a proximal site. In this case, the location of *Ds* must be distal to the *C2* locus.

The behavior of the acentric fragments may depend on a number of characteristics, such as whether the segment replicates and/or repairs, is attracted to other genetic material, or there may be sites on the fragment that have a propensity to do these things. Also there may be sites on chromosomes which have acentric properties. If this is true, large fragments are more likely to carry these sites than small ones.

***Ds-4L1*. Maize Genetics Coop Stock Number: T3312O**

Chromosome breaking; position distal. With *C2* and *Ac* (both in 1 dose) against *c2* as a tester, expresses as dilute purple kernels with small to medium sharp-bordered colorless patches scattered about on the dilute background. The patches are associated with smaller angular patches of irregularly intense color and are located on the dilute side of the border between the dilute background and the colorless sectors. Most of the intense sectors have variable levels of intensity. These kernels have a classical twin spot appearance and indicate a distal location for this *Ds*. **Origination**: a single dilute purple kernel with medium to tiny colorless sectors and associated small variably intense colored dots on a dilute purple ear, from the cross of *c2 c2* x *Ac/-, C2 C2, Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot. The plant grown from this kernel was normal and produced a selfed ear that segregated for: full to medium purple, dilute with colorless and associated intensely colored patches, and colorless. Outcrosses of the progeny of this mosaic class of kernels (presumably *Ac, C2 Ds-4L1/c2*) onto a *c2* tester produced: dilute purple, dilute purple mosaic, and colorless kernels indicating *Ds-4L1* is near *C2* and responds to *Ac*.

Ds-4L1 (Image 45). Ear showing the original *Ds-4L1* colorless dotted kernel (in the middle of the area indicated by black marker) showing dilute purple kernels from the cross of *c2 c2* x *Ac/-, C2 C2, Ds-10L2 R1-sc/Ds-10L2 R1-sc,* and a single mosaic kernel with many small and medium sized colorless dots and patches on a dilute background, the larger of which often have smaller dark-colored dots along and outside their borders (twin spots).

Ds-4L1 (Image 46). Enlargement of the original *Ds-4L1* kernel showing patches of colorless tissue and in most cases a smaller intense bit of tissue attached to, but on the outer side of, the border with normal pale aleurone (twin spots) resulting from a distal break, followed by the breakage-fusion-bridge cycle, which results in distribution of breakage products in subsequent daughter cells.

***Ds-4L3*. Maize Genetics Coop Stock Number: T3312P. No image.**

Chromosome breakage and suppression of resident locus phenotype; position at *C2* locus. With *C2* and *Ac* (both in 1 dose) against *c2* as a tester, expresses as dilute kernels with many tiny dark dots and fewer tiny colorless dots (not necessarily associated as twin spots) on the dilute background. Occasional large colorless sectors have a small intense sector along their borders. These characteristics suggest a location at or near the *c2* locus. **Origination**: a single dilute kernel with large colorless sectors on a dilute purple ear from the cross of *c2 c2* x *Ac/-, C2 C2, Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot. The plant grown from this kernel was normal. When outcrossed as a pollen parent to a *c2* tester, the resulting ear showed kernels with dilute purple, dilute purple with colored dots, and occasional large colorless sectors, and colorless kernels. When grown out, the mosaic kernels produced a selfed ear that had 3/4 colored and dilute and 1/4 colorless kernels. The dilute kernels had occasional large colorless patches and colored frequent dots. An outcross on a *c2* tester gave dilute with colored dots and colorless kernels. The dilute kernels with dots from the above crosses had many tiny dark dots and fewer tiny colorless dots. Occasionally there were twin spots of some colorless dots with adjoining smaller dark purple dots but this may have been coincidental. Also occasional kernels had large colorless sectors (half kernel), and these often had adjacent intensely colored twin spots. *Ds-4L3* appears to be at or near the *C2* locus causing suppression, mutability and some chromosome breakage resulting in losses at the *C2* locus.

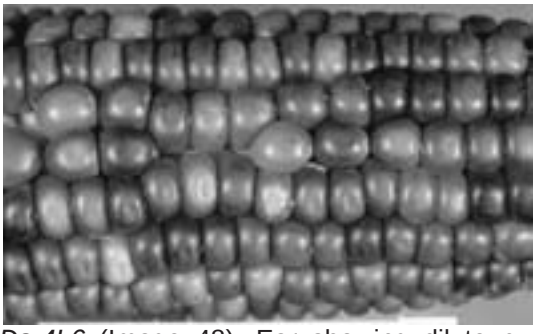
***Ds-4L4*. Maize Genetics Coop Stock Number: T3312Q. No image.**
Chromosome breaking; position distal. With *C2* and *Ac* (both in 1 dose) against *c2* as a tester, expresses like *Ds-4L1* but fewer small to medium losses and marked large sectors and fewer sectors. **Origination**: a single dilute purple kernel with various sizes of colorless spots on a dilute purple ear from the cross of *c2 c2* x *Ac/-, C2 C2, Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot. The plant grown from this kernel was normal and produced a selfed ear which segregated for purple, dilute colorless mosaic and colorless kernels. The mosaic kernels are like *Ds-4L1* but had many fewer medium and smaller events of color losses, yet about the same number of large (1/2 +/-) sectors. Also those colorless dots that did appear were less likely to have associated darker twin spots. No intensely colored satellite islands in the colorless sectors were seen, indicating a distal location for *Ds-4L4*.

***Ds-4L5*. Stocks not available. No image.**
Chromosome breaking; position distal. With *C2* and *Ac* (both in 1 dose) against *c2* as a tester, expresses like *Ds-4L1* but fewer and smaller colorless sectors (later events). **Origination**: a single dilute purple kernel from the cross of *c2 c2* x *Ac/-, C2 C2, Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot. Dilute purple kernels with small to medium sharp-bordered colorless patches are scattered about on the dilute background. The patches are associated with smaller angular patches of irregularly intense color and are located on the dilute side of the border between the dilute background and the colorless sectors. Most of the intense sectors have variable levels of intensity. These kernels have a classical twin spot appearance, and indicate a distal location for this Ds.

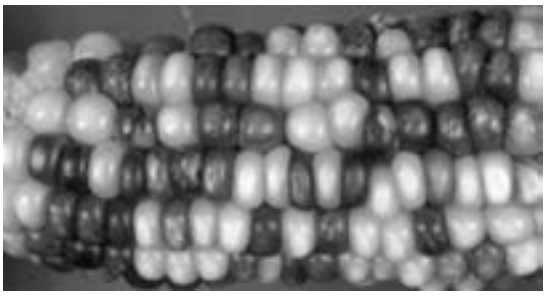
***Ds-4L6*. Maize Genetics Coop Stock Number: T3312S**
Chromosome breaking; position distal. With *C2* and *Ac* (both in 1 dose) against *c2* as a tester, expresses as dilute purple kernels with small to medium sharp-bordered colorless patches scattered about on the dilute background. The patches are associated with smaller angular patches of irregularly intense color and are located on the dilute side of the border between the dilute background and the colorless sectors. Most of the intense sectors have variable levels of intensity. **Origination**: a single dilute purple kernel with many colorless sectors and associated intense colored patches on a dilute purple ear from the cross of *c2 c2* x *Ac/-, C2 C2, Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot.

The origin kernel produced a normal plant which was selfed and crossed on a *c2* tester. The selfed ear (see image below) was *P1-vv* and segregated purple, dilute purple, dilute purple with colorless sectors and associated intense colored spots and colorless kernels. The outcross of this selfed plant on *c2* produced ears with dilute, dilute colorless mosaic, and colorless kernels. Mosaic kernels had many small to medium size colorless sectors associated with smaller intense purple spots on the dilute side of the border between the parental dilute tissue and the colorless loss tissue. Occasionally these

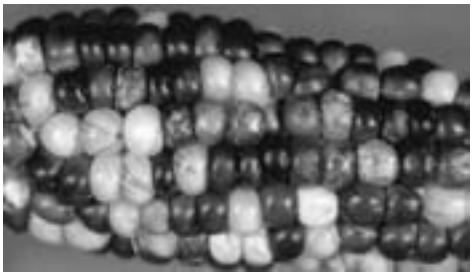
spots projected out into the colorless sector but this appeared to be a chance distribution of cells from the original two daughter cells. The frequent appearance of typical twin spots of colorless and smaller purple spots suggests a probable distal location for *Ds-4L6*.



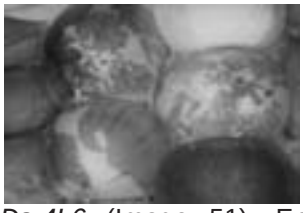
Ds-4L6 (Image 48). Ear showing dilute purple kernels from the cross of *c2 c2* x *Ac/-, C2 C2, Ds-10L2 R1-sc/Ds-10L2 R1-sc*, and a single mosaic kernel with many small and medium sized colorless dots and patches on a dilute background, the larger of which often have smaller dark-colored dots along and outside their borders (twin spots).



Ds-4L6 (Image 49). Ear from the cross of *c2 c2* by the original *Ac/-, C2 Ds-4L6/c2* plant, showing mosaic dark purple and pale (the unusually dark color of these is due to a color modifier segregating in the stock) and nonpurple kernels, with medium to tiny losses of color with tiny dark twin spots.

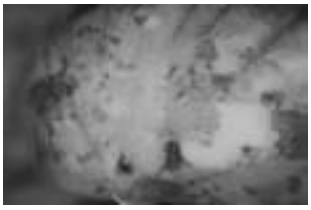


Ds-4L6 (Image 50). The selfed ear of an *Ac/-, C2 Ds-4L6/c2* plant showing several unique phenotypes expressed by different combinations and dosage of *Ac*, C2, and *Ds* in the 3N endosperm and 2N maternal pericarp. The dilute kernels are *C Ds/c/c*, most of which have *Ac,* and are mosaic for colorless (*C Ds/-/-, Ac*/-) and show distinct small dark dots (*C Ds C Ds/c/c* twin spots) and a few intense dots (*C Ds C Ds/C Ds C Ds/c/c*, second cycle twin spots). The intermediate kernels (*C Ds/C Ds/c*), which have *Ac*, are dilute, mosaic and have coincidental colorless spots and intense (*C Ds C Ds/C Ds C Ds/c*) twin spots. The dark colored kernels which have *Ac* are dark with lighter small dots and occasional colorless dots from coincidental loss of all three *C* alleles. The other kernels, lacking *Ac* or with 3 doses of *Ac*, are simply dilute, intermediate, and full-colored. The colorless kernels are *c/c/c* with all combinations of *Ac*. The whole ear is covered by the same 2N maternal pericarp, as shown by the long red streaks from the *P-v* allele.



Ds-4L6 (Image 51). Enlargement of kernels taken from the ear in Image 50 showing several kinds of events that occur. For some, an interpretation of the mechanism is easily derived, for others it remains obscure. The lower left kernel has a large dilute area adjacent to a large colorless area, suggesting that *C Ds* was lost at the first breakage event and that most *Ac C Ds* activity was stopped in the other daughter cell, indicating loss of *Ds* but not *C.* The kernel directly above is probably *C Ds/C Ds/c* and has multiple events (losses and chains of dots.

Ds-4L6 (Image 52). Another enlargement of kernels taken from the ear in Image 50 showing varying expressions of *Ds* events. The whole kernel on the left has typical distal location in a *C Ds/c/c* dilute loss; i.e., small colorless loss of *C* with frequently associated intense colored spots. The kernel on the right is very similar but has more intense pigmentation.



Ds-4L6 (Image 53). Enlargement of one of the kernels from the ear in Image 50, showing many losses and twin spots expressed at the cellular level.

***Ds-4L7*. Maize Genetics Coop Stock Number: T3312T. No image.**
Chromosome breaking; position distal. Like *Ds-4L4*. **Origination**: a single dilute purple kernel from the cross of *c2 c2* x *Ac/-, C2 C2, Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot.

Chromosome 5S (Marker *A2*): The recessive marker stock *a2 bt1 pr1* was used. This allowed us to cover both arms, *a2* for 5S and collapsed brittle kernel for 5L. Normal purple flint kernels were observed for colorless aleurone changes.

***Ds-5S1*. Maize Genetics Coop Stock Number: T3312V**
Chromosome breaking; position proximal. With *A2* and *Ac* (both in 1 dose) against *a2 bt1* as a tester, expresses as purple nonshrunken kernels with many large and medium sized colorless sectors but very few tiny sectors. The colored areas adjoining the large colorless sectors are uniform in color and have very few included small colorless spots. The large colorless sectors have striking small intensely colored islands of color within their borders. These appear as classic recovery spots. **Origination**: a single purple and colorless mosaic kernel on a full purple ear from the cross of *a2 bt1 pr1/a2 bt1 pr1* ears by *Ac/-, A2 Bt1 Pr1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot. The plant grown from this kernel was normal and produced a selfed ear segregating for purple nonbrittle, purple nonbrittle with mosaic patches of colorless aleurone, and colorless brittle kernels with occasional appropriate crossovers between *A2* and *Bt1*. Pollen from this plant crossed on an *a2 bt1* plant produced an ear segregating for: 1/4 purple nonbrittle, 1/4 purple nonbrittle with colorless nonbrittle sectors, and 1/2 colorless brittle kernels with the appropriate number of crossover types. Mosaic kernels from the backcross ear had many large (average 1/2 kernel) or intermediate (1/16 to 1/4 kernel) colorless sectors, and very few tiny colorless sectors. The purple tissue adjoining the large colorless areas was mostly uniform in color intensity and had very few included smaller losses of color. In the large colorless sectors there were often intense single purple spots as well as occasional recovery spots (chains of spots). Kernels with medium or small patches had some smaller subsequent losses of color in the adjoining purple aleurone and also had small intensely-pigmented patches on the border between the colored and colorless patches. There were very few observed changes of *Bt1* and *Pr1*. The frequent large sectors, the absence of twin spots and the clear expression of recovery spots suggest a proximal location for this *Ds* site.

Ds-5S1 (Image 54). Selfed ear of the original *Ac/-, A2 Ds-5S1 Bt1* Pr1/*a2 bt1 pr1 Pr1/a2 bt1 pr1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* plant with a 3:1 segregation for *a2 bt1 pr1*, showing 3 colored and colorless mosaic kernels (*A2 Ds Bt1/a2 bt1/a2 bt1*) with large colorless sectors, resulting from early *Ds*-induced loss of *A2* but not *Bt1*. *Ac* was present but not at the *P1* locus.

Ds-5S1 (Image 55). Selfed ear of an *Ac/-, A2 Ds-5S1 Bt1* Pr1/*a2 bt1 pr1 Pr1/a2 bt1 pr1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* plant, with 3:1 segregation for *a2/bt1/pr1* showing appropriate linkage and crossover classes and segregating for *Ac*. The several colored and colorless mosaic nonbrittle kernels with large colorless sectors are the *Ac/-/- A2 Ds-5S1 Bt/a2 bt/a2 bt* endosperm class. The nearly complete absence of concurrent colorless brittle (*A2 Bt1* losses)

proves that these two genes are on opposite sides of the centromere.

Ds-5S1 (Image 56). Ear from the cross of *a2 bt1 pr1/a2 bt1 pr1* x *Ac/-, A2 Ds-5S1 Bt1 pr1/a2 bt1 pr1* with a 1:1 segregation for *Ac* and the chromosome-breaking with linked markers *A2* and *Bt1*. The colored and colorless non-brittle mosaic kernels (*A2 Ds-5S1/a2 bt/a2 bt*) have large colorless sectors on more than one-half of the kernel surfaces. These have small islands of intensely colored tissue (recovery spots).

***Ds-5S2*. Maize Genetics Coop Stock Number: T3312W**

Chromosome breaking; position proximal. With *A2* and *Ac* (both in one dose) against *a2 bt1* as a tester, expresses like *Ds-5S1* but with more satellite islands of color, including more persistent acentric fragments. Large colorless sectors had many more colored patches and chains of intensely pigmented recovery spots within their borders. Also, seedlings from mosaic kernels had rough leaves throughout life, indicating more complex behavior. **Origination**: a single purple and colorless mosaic kernel on a full purple ear from the cross of *a2 bt1 pr1* ears x *Ac/-, A2 Bt1 Pr1/A2 Bt1 Pr1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* in an open-pollinated detasseled plot.

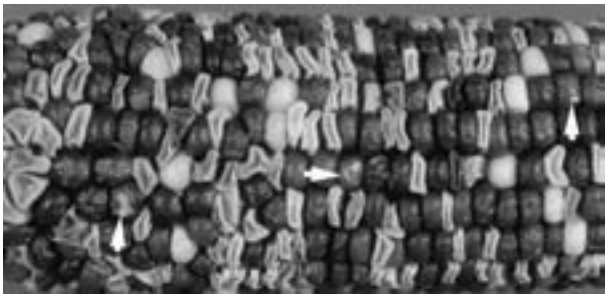
Ds-5S2 (Image 57). Selfed ear of the original *Ac/-, A2 Ds-5S2 Bt1 Pr1/A2 Bt1 Pr1, Ds-10L2 R1-sc/Ds-10L2 R1-sc* plant, with a 3:1 segregation for *a2 bt1 pr1*, showing 3 colored and colorless mosaic kernels (*A2 Ds Bt1/a2 bt1/a2 bt1*) with large colorless sectors, resulting from early *Ds*-induced loss of *A2* but not *Bt1*.

Chromosome 5L (Marker *Bt1*): The recessive marker stock *a2 bt1 pr1* was used. This allowed us to cover both arms, *a2* for 5S and collapsed brittle kernel for 5L. Normal purple flint kernels were observed for colorless aleurone changes.

***Ds-5L1*. Maize Genetics Coop Stock Number: T3312U**

Chromosome breaking; position distal to *Bt1* and proximal to *Pr1*. With *Bt1 Pr1* and *Ac* (both in one dose) against *a2 bt1 pr1* as a tester, expresses as purple kernels with large medium and small red (*pr1*) shrunken (*bt1*) sectors. **Origination**: a purple colored kernel with many variably-sized shrunken (*bt*), usually red-colored sectors on a full purple ear, from the cross of *a2 bt1 pr1* ears x *Ac/-, A2 Bt1 Pr1/A2 Bt1 Pr1, Ds-10L2 R1-sc/Ds-10L2 R1-sc.* The plant grown from this kernel was normal and produced a selfed ear with kernels showing: purple non-bt; purple non-bt with many sectors of shrunken red tissue; colorless shrunken; and the appropriate number of crossovers between *a2 bt1* and *pr1*.

Plants from the sectored kernels were crossed onto an *a2 bt1 pr1* tester. The resulting ear (see below) had colored, normal brittle mosaic and colorless bt kernels in a 1:1 ratio with appropriate crossovers for the markers present. However, *pr* separation was nearly impossible. *Ds* events occurred for 41 of 42 *a2 Bt* crossovers and 351/360 *A2 Bt* parentals, indicating that *Ds* is 2 crossover units distal to *Bt1* and proximal to *Pr1*. Non-brittle mosaic kernels had mostly red colored shrunken sectors due to loss of *Bt1* and *Pr1*. Rare (< 0.5%) colorless nonbrittle sectors on colored *Bt1 bt1* mosaic kernels indicate simultaneous losses of genetic material on both sides of the centromere, perhaps whole chromosomes. Occasional (over 2%) colorless shrunken sectors indicated the loss of the whole chromosome 5. Crosses where *P1-vv* (*Ac*) segregates proved that sectoring is closely associated with the *P1-vv* allele. The high frequency of large sectors (early events) indicates proximal location of *Ds*, which is confirmed by 3-point linkage data.

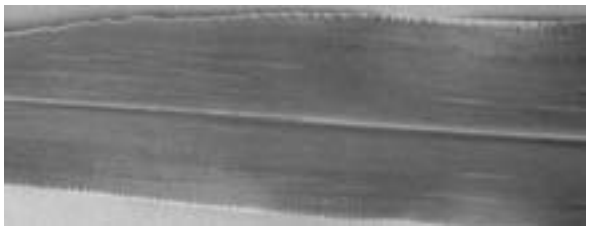


Ds-5L1 (Image 58). (Note, the symbol "***–o–***" indicates the position of the centromere*.)* Ear from the cross of *a2 bt1 pr1/a2 bt1 pr1* x *Ac Ac, A2* ***–o–*** *Bt1 Ds-5L1 Pr1/a2 bt1 pr1* showing colored *A2 Bt1* kernels with *bt1* sectors, and colorless *bt1* kernels in a 1:1 ratio with the appropriate number of crossovers between *a2 Ds* and *bt1*. Note that 351/360 colored *A2 Bt* parentals are sectored, and 9 are not; also that 41/42 colorless *a2 Bt1* crossovers are sectored. There are no colorless non-*Bt* sectors on the ear. There are, however (note arrows) several kernels with colorless *bt1* (loss of *A2* and *Bt1*) sectors, indicating involvement across the centromere. This is probably resulting from the loss of the entire chromosome. One kernel sector (arrow on left) with double loss of both *A2* and *Bt1* is comparatively quite large, hence may be considered a loss of the whole chromosome. Most of the kernels have greater than half red areas with many more smaller *bt1* losses within their borders. All of the *bt* sectors are small (less than one quarter of the kernel's surface), indicating a delay in expression as expected from breakage-fusion-bridge losses. The larger non-loss purple sectors have no further losses within their borders. If this logic is correct, then *Ds* is located between *Bt* and *Pr* and about 2 map units distal to *Bt* from the centromere. The kernels are mostly red due to the fact that proximal losses of *Pr1* are frequent and early.

Ds-5L1 (Image 59). A selfed ear of the original *Ac/-, A2* ***–o–*** *Bt1 Ds-5L1 Pr1/a2 bt1 pr1* plant segregating for linkage markers with *Ds* and independently of *Ac*, showing generally late losses for *Bt* and early losses of *P-r* and no losses of *A2*.

Chromosome 7L (Marker *O5*):
The EMS-induced defective kernel mutant *collapsed*-76B* is allelic to *o5* (J. Beckett, personal communication), but differs significantly in that *cp*-76B* has an extreme collapsed (*cp*) endosperm and *o5* has an opaque plump kernel. The mutant also has a yellowish white lethal seedling phenotype. Kernels have many pits and various sizes of collapsed endosperm sectors when in heterozygote with the mutant allele *o5-76B* (hereafter referred to as *cp* for brevity), the *O5-Ds2* segment in a *cp/cp/Cp/Ds* endosperm configuration (*cp* from the female), and with *Ac* present. The sectors did not have precise borders, so that when a majority of the seed kernel surface was in mutant sectors the remaining non-mutant areas tended to bleach out, suggesting a lack of cell autonomy.

***Ds-7L1*. Stocks not available.**
Chromosome breaking; position distal. With *O5* and *Ac* (both in 1 dose) against *o5-76B* as a tester, expresses as normal kernels with large sectors of collapsed tissue and with pits and irregular indentations of the kernel surface. Kernels with larger sectors of mutant tissue appear as completely mutant though less extreme. Seedlings grown from these kernels are green with small elongated yellowish white sectors on the leaf blade. **Origination**: a single collapsed endosperm mosaic kernel on a normal ear, from the cross of *+/cp*-76B* plants (from a segregating F2) by *Ac, Cp Cp, Ds-10L2 R1-sc/Ds-10L2 R1-sc* pollen in an open-pollinated detasseled plot.



Ds-7L1 (Image 60). The underside of a leaf of the original *Ac/- O5 Ds-7L1/o5* plant showing several long pencil-lead sized pale green sectors, ranging down to a few cells in length, indicating loss of *O5* from *Ds* breakage action. These sectors are the result of having a single

layer of white cells overlaid by one or two layers of normal green cells.

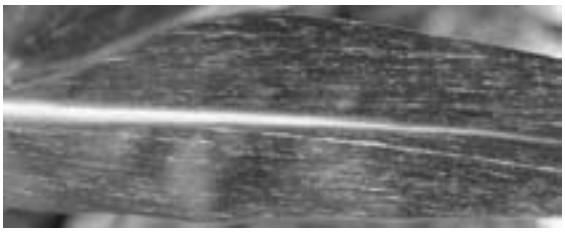
Ds-7L1 (Image 61). A selfed ear with all colored kernels from the original *Ac/- O5 Ds-7L1/o5* plant showing semisterile seed set (due to failure of homozygous *o5* kernels, which can be extremely collapsed, to become established on the ear) and segregation for purple kernels versus pitted etched mosaic kernels (probably *Cp Ds/cp/cp*) indicating frequent late loss of *Cp* in the endosperm due to *Ds* breakage on the distal side of the *O5* locus. Most of the combinations of the 3N endosperm that have more than one dominant *Cp* allele or more than one *Ac* or no *Ac* at all will have no visible *cp* sectors because of the characteristic late occurrence of the breaking event in this case. The presence of *Ac* is confirmed by the red P-v streaks on the pericarp of many of the kernels.

Ds-7L1 (Image 62). Top leaf surface of an *Ac/-, Cp Ds-7L1/cp* plant showing many small elongated lighter green and whitish sectors ranging from pencil-lead sized down to a very few cells. Some of the larger ones are almost white, like the *cp cp* seedling phenotype.

Ds-7L1 (Image 63). Underside surface of the same leaf as Image 3 above, showing the same phenotype except that the nearly white expression of the longer sectors is more pronounced. Also there is some indication of latent necrosis of the larger sector which is characteristic of the allele *cp*-76* used in these experiments. Note that none of these sectors have an effect on the structure of the leaf as seen in *Ds-1S4* (Images 23-26 above).

Ds-7L2. **Stocks not available.**
Chromosome breaking; position proximal? **Origination**: a single colored pitted, kernel on a normal colored ear, from the cross of *+/cp*-76B* plants (from a segregating F2) by *Ac/-, Cp Cp, Ds-10L2 R1-sc/ Ds-10L2 R1-sc* pollen in an open-pollinated detasseled plot. Kernel sectors produced with this case appear as though the collapsed phenotype is not confined to areas with a mutant genotype (*cp/cp/-)* but spreads into the area of nonmutant (*cp/cp/Cp*) genotype. Because of a lack of precise chimeral borders on the kernel, etc. it was not possible to confidently determine the position of *Ds* on chromosome 7L. However, the high frequency of relatively large leaf sectors suggests a proximal location. Seedlings produced from planting the mosaic kernels grew very well and were green with many tiny elongated yellowish white sectors scattered at random on the leaf surface. The mutant sectors were composed of apparently healthy and morphologically normal cells that lacked chlorophyll and had reduced carotenoid pigments. In the larger sectors the cells bleached into white and senesced earlier than those in the adjoining non-mutant tissue. Plants were green with many small narrow yellowish white sectors of otherwise normal healthy tissue (Image 64, below). The larger sectors bleached to white and senesced earlier than the normal adjoining tissue, showing that *o5* has a yellowish

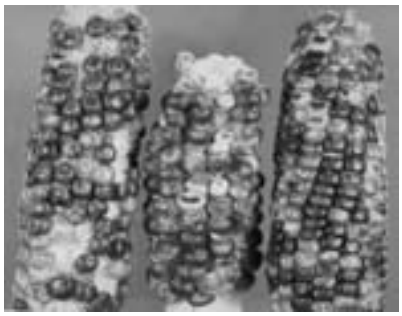
white morphologically normal but lethal plant phenotype.



Ds-7L2 (Image 64). Top leaf surface of an *Ac/-, Ds-7L2 Cp/cp* plant showing many tiny to medium size elongated sectors of pale green to almost white tissue, resulting from the loss of the *Cp*-carrying 7L segment revealing the collapsed phenotype of the hemizygous *o5* allele. Note the largest single sector is almost white and shows signs of early necrosis.

Ds-7L2 (Image 65). Undersurface of the same leaf from Image 64 above.

Ds-7L2 (Image 66). Like image 65, a mature plant, showing typical plant expression of *Ds-7L2*.



Ds-7L2 (Image 67). Three ears; on the left the selfed ear of *Ac/- Ds-7L2 Cp/cp R1-sc Ds-10L2/R1-nj* with poor seed set and segregating for extreme mosaic kernels (*Ds Cp/cp/cp*). These did not develop properly under field crop conditions but show enough to prove the mutant. The middle ear is from the cross of *Cp/cp, R-nj R-nj* x *Ac/- Ds-7L2 Cp/cp R1-sc Ds-10L2/R1-nj* with segregation for normal, normal and collapsed mosaic (*Ds Cp/cp/cp*) and extreme collapsed (*cp/cp/cp*). The one-dose *Ds Cp* kernels show evidence that the *Cp* endosperm sectors sustained the *cp* sectors, allowing kernels to be more nearly normal than expected (non-cell autonomy). Ear on the right is from the cross of *r1 r1* x *Ac/- Ds-7L2 Cp/cp R1-sc Ds-10L2/R1-nj* showing various segregants of mosaicism due to *Ds-10L2* vs. *R-nj*, and also full colored non-mosaic kernels (non-*Ac*).

Chromosome 8L (Marker *pro1*):

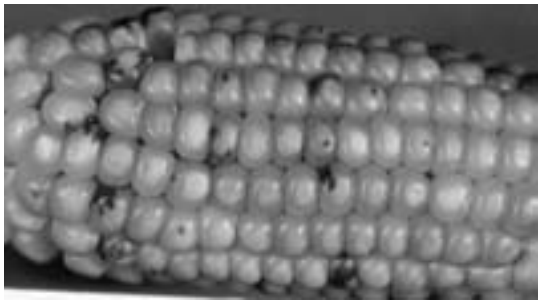
***Ds-8L1*. Stocks not available.**

Position unknown.

Ds-8L1 (Image 68). Self of original *Ac/-, Ds-8L1 Pro1/pro1*, *Ds-10L2 R1-sc/r* segregating for *Ds-8L1 Pro1/pro1* and *Ds-10L2 R1-sc* showing good cases of large *Cp* vs *cp* mosaicism, indicating early loss of *Ds Pro* (3N *Ds-8L1/pro/pro* kernels). Many kernels of the homozygote *pro1* class failed to set seed, causing a semi-sterile appearance of the ear. The *Ds Pro/pro/pro* class included the mosaic kernels which have reduced clarity of expression because of cross-feeding of mutant tissue by non-mutant sectors (non-cell autonomy).

Chromosome 9S (Marker *C-I Ds by McClintock*): Marker described in detail by McClintock (1951).

***Ds-9S1*. Maize Genetics Coop Stock Number: T3312Y**
Chromosome breaking; position may be proximal. *Ds* in the standard position, described in detail as *C-I Ds* by McClintock (1951).

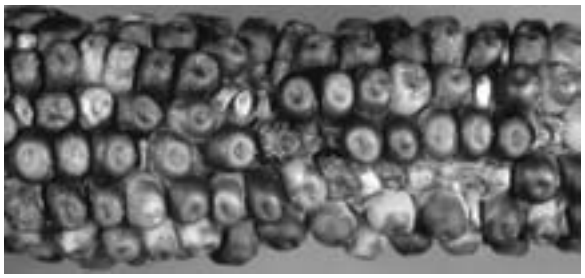


Ds-9S1 (Image 69). Ear from the cross of a purple kernel *C1 C1, Ac Ac* plant by homozygous *C1-I Ds-9S1 wx* (*C-I* is a dominant allele of the *c* locus that inhibits formation of color by the *C1* allele), showing mostly colorless kernels with many tiny colored dots, typical of two dose *Ac* action (delayed function from homozygous *Ac* maternal plant) and a few kernels with various sized colored sectors expressing earlier function of *Ds Ac*. Compare this behavior with that of *Ds-2S4* (and see Image 70 below).

Ds-9S1 (Image 70; see also *Ds-2S4*). A double-pollinated ear of an *Ac/-, b-dm Ds-2S4/b* plant. The top half silks were pollinated by *bb rr* pollen, and show a 1:1 segregation for purple vs colorless, indicating that the 2-dose (*b-dm/b-dm/b*) kernels are full-colored and not pale. The bottom half, pollinated by homozygous *C1-I Ds-9S1* no *Ac,* has two classes of kernels: 1) those that are colorless have no dots because they lack *Ac*; 2) those with a pale background composed of many tiny pale two- to four-celled dots and fewer darker and larger dots and sectors and a scattering of intense full-colored dots. These probably are the *b-dm/b-dm/b, Ac/-/-, C/C/C-I Ds* kernels.

Chromosome 9L (Marker *Dek13*): The mutant *dek*-N744* is a thin collapsed kernel with nonviable embryo; it has been designated *Dek13*.

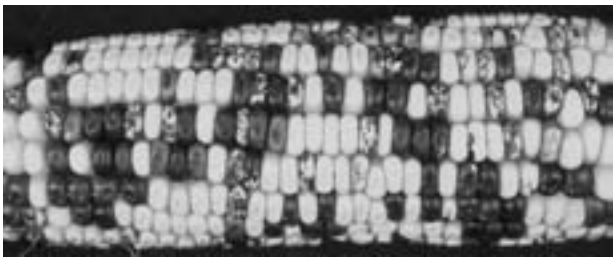
***Ds-9L2*. Stocks not available.**
Position unknown.



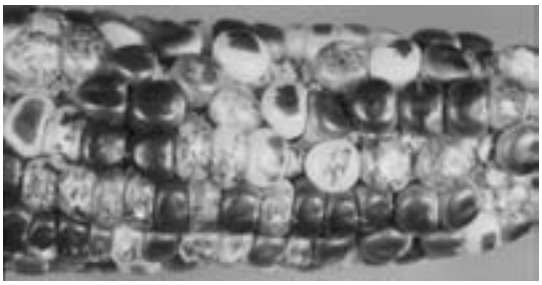
Ds-9L2 (Image 71). A selfed ear of *Ac/- Ds-9L2 Dek13/dek13, Ds-10L2 R1-sc R-nj*, showing several expected classes including *Ds-9L2, Dek13/dek13/dek13, Ac/-/-* (medium sized collapsed sectors and dots) and *Ds-10L2 R1-sc/R-nj R-nj* (mosaic *R-nj*) or *R-sc/R-sc* (all colored). It is impossible to tell whether the position of *Ds* is proximal or distal to the centromere. This is a good case but has no progeny.

Chromosome 10L (Marker *R1-sc*):

***Ds-10L2*. Maize Genetics Coop Stock Number: T3312Z**
Chromosome breaking; position proximal (also known by *Ds-2 R-sc, Kermicle)*. With *R1-sc* and *Ac* (both in 1 dose) and also *b1* against *b1 r1* as a tester, expresses as mosaic kernels with many large colorless sectors and irregular patches of colored tissue, the latter with chains and clusters of recovery spots of varying color intensity. Proved by linkage to be approximately 10 crossover units proximal to *g1* (J. Kermicle personal communication.) Note: see also Image 1 at *Ds-2S3* for another image and description relating to *Ds-10L2*.

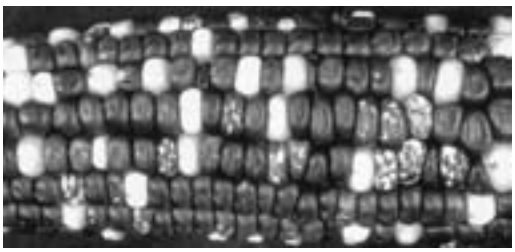


Ds-10L2 (Image 72). Ear from the cross of *r r x Ac/-, Ds-10L2 R1-sc/r* segregating 2 colorless:1 full purple and 1 colored-colorless mosaic kernels. These mosaic kernels have many medium to large colorless sectors, separated by chains of colored dots and including some intense islands of color within their borders, indicating a proximal location of *Ds* between the centromere and the *R* locus.



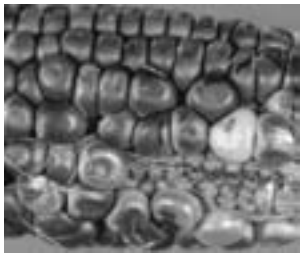
Ds-10L2 (Image 73). Ear from the cross of *r r x Ac/-, Ds-7L2 Cp/cp, Ds-10L2 R1-sc/r-nj* segregating for *Ds R-sc Ac* (mosaic kernels) and for no *Ac* (full colored kernels and for *R-nj* colorless kernels with colored crown). Note colorless kernels with mosaic crown are the result of a crossover putting *Ds* and *R-nj* on the same chromosome. Other single kernels with variant expression of *Ds R-sc* are also evident.

***Ds-10L4*. Stocks not available.**
Chromosome breaking; position proximal. Also known as *Ds-4 R-sc (Kermicle*). Expresses like *Ds-10L2*. Proved by linkage to be approximately 10 crossover units proximal to *g1* (J. Kermicle personal communication.)



Ds-10L4 (Image 74. . Selfed ear of an *Ac/-, Ds-10L4 R-sc/r* plant segregating for purple normal, colored-colorless mosaic, and colorless kernels. The mosaic kernels are of two classes: 1) those with frequent colorless sectors (*Ds R-sc/r/r* 3N aleurone) with chains of dots and recovery spots and 2) those with rare small colorless sectors (*Ds R-sc/Ds R-sc/r* 3N aleurone).

***Ds*-10L5. Stocks not available.**
Chromosome breaking, suppressing, both, or creates new *Ds* cases; *Ac* at the *R1* locus. Events are early or late depending on *Ac* dosage and position.



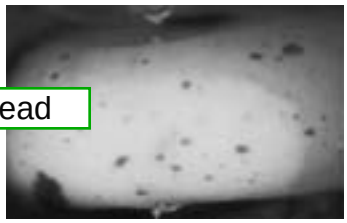
Ds-10L5 (Image 75). An ear with purple kernels from the cross of *a2 Bt1 a2 bt1, R-scm x A2 Bt1/A2 Bt1 Ds-10L2 R1-sc/Ds-10L2 R1-sc* pollen stock in an open-pollinated detasseled plot showing the original colorless kernel with a dilute purple sector and several dilute purple dots. This kernel, thought to be an *a2-m Bt1* case, produced a normal plant which when selfed produced an ear segregating for *a2 bt1* (see image 2 below). In subsequent tests, progeny from this plant were crossed onto an *r* tester and an *a2 bt* tester which showed that the transposon activity was at the *R1* locus and not the *A2* locus; therefore the female parent plant was *R1-scm/r1*.

Ds-10L5 (Image 76). Selfed ear of the original *Ac/- A2 Bt1/a2 bt1, Ac-10L5 r-m/R-scm* plant segregating for: 1) purple *Bt* kernels which included normal purple, colored and colorless mosaics of various types, and colorless kernels with several levels of dotting frequency; 2) *Bt* with colorless sectors; 3) non-*Bt* colorless with different levels of dotting; 4) and colorless *bt* without dots. No colorless *Bt* kernels without

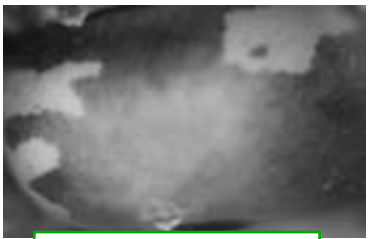
dots were visible. Also present were the usual *A2 Bt1* crossover classes, including an *a2 bt1* dotted case. The occurrence of colored kernels with colorless sectors indicates chromosome breaking. The colorless kernels that have dots are characteristic of suppression at the *R* locus, with revertant events due to *Ds* loss. This behavior resembles that seen when *Ac* moves into the locus. Note that all except the expected (*R R R*) class have either breaking or suppression activity. This is a strong case for *Ac* at the *R* locus.

Ds-10L5 (Image 77). Enlargement of several kernels from the original *Ac/- A2 Bt1/a2 bt1, Ds-10L5 r-m/r* ear showing (left to right), 1) a purple brittle crossover kernel (*A2 bt1*); 2) a colorless kernel with colored dots (*r-m/r-m/r-m*); 3) a pale colored kernel with darker dots showing through and with two large colorless sectors that have colored dots (*R/r-m/r-m*); 4) a mosaic kernel with a large colored sector with colorless loss areas and a large colorless sector that includes chains of dots and single intense dots within its borders (possibly *R/R/r-m*); 5) a mosaic kernel made up of chains of repeated *R* loss.

image 77 instead



Ds-10L5 (Image 78). Enlargement of kernel 2 from image 3 above.



Ds Image 77 instead rgement of kernel 3 from image 3 above.

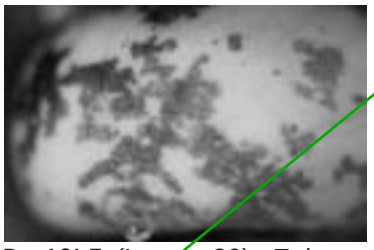


image 77 insteda

Ds-10L5 (Image 80). Enlargement of kernel 5 from image 3 above.

Ds-10L5 (Image 81). Top: Ear from the cross of *r1 r1 x* Ac/-, Ac-*10L5 r1-m/Ac-10L5 r1-m* with more than 3/4 of the kernels showing fairly uniform characteristic colorless kernels with tiny small dilute purple dots. Five of the colorless kernels have no dots. Seventeen kernels are solid purple and may have originated from one or more stable transposon revertants to *R* in the tassel. Nine kernels have a large sector of colored aleurone resulting from early transposition in the developing kernel. The whole diluted kernels and one mosaic kernel suggest that the transposon revertants are stable (not prone to frequent change) and are at various levels of gene function. Bottom: Selfed ear of an *r1 r1 x Ac/-, Ac-10L5 r1-m/Ac-10L5 r1-m* plant showing mostly colorless kernels with roughly three times as many dots as the outcross onto *r r* in the top ear. Also note reduction in occurrence of large sector revertants.

***Ds-10L6*. Stocks not available.**
Chromosome breaking, suppressing, both, or creates new *Ds* cases; *Ac* at the locus. Events are early or late depending on *Ac* dosage and position.

Ds-10L6 (Image 82). Selfed ear of the original *Ac/- A2 Bt1/a2 bt1, r1-m2 Ac-10L2/R-scm* plant segregating 3:1 for *A2 Bt1/a2 bt1*. The *Bt* kernels (except for occasional crossovers) all have full color, colored and colorless mosaics, and/or colorless kernels with colored dots. A few tiny red streaks on the pericarp indicate the presence of two *Ac*: one at the *P1* locus (*P1-vv*) and the other apparently at the *R1* locus (*r-m*). The *Bt* kernels include all purple (*R/R/r-m*), purple and colorless mosaics of various types (*R/r-m/r-m* with the *Ac* at *r-m* somehow affecting the *R* allele, or a large sector unstable phase of *Ac* activity at *r-m* in the *r-m/r-m/r-m* kernels) and colorless kernels with frequent small dilute dots and occasional small dilute sectors (*r-m/r-m/r-m*). All the characteristics seen in this case agree with the known behavior of *Ac* at a gene site.

Acknowledgements. The author gratefully acknowledges support from the National Science Foundation via supplemental funding to MG Neuffer via NSF grant 0743804. Dr. Jerry Kermicle provided the original two stocks of chromosome breaking *Ds*. The author is grateful to Carolyn Lawrence, Sarah Hake, and Mary Schaeffer for their support, advice, and dedication to the maize community. Thanks also to Kelly Dawe and Hugo Dooner for reading the material and providing many helpful suggestions. Lou Butler assisted in gathering the data, editing the text, preparing the artwork, and formatting this material for posting online.

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Maize Genetics Cooperation • Stock Center

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8,305 seed samples have been supplied in response to 458 requests for 2013. These include 178 requests received from 28 foreign countries. This was the third record-breaking year in a row for requests, but only a bit higher than last year. Interest in reverse genetics tools, such as the UniformMu sequence indexed stocks, continues to grow. Presently, requests for UniformMu stocks represent more than 50% 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, kernel starch quality traits, plant architecture traits, 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 later than we prefer, but excellent weather during the growing season allowed for a normal pollination season. There were sufficient stands for a good increase in most instances. Supplemental irrigation was required after the planting of our second crossing nursery in order to minimize bird damage, and again late in the season during a post-pollination dry spell. Moderate temperatures and low plant stress resulted in excellent yields from most pollinations.

Special plantings were made of several categories of stocks:

1. Plantings were made of donated stocks from the collections of Bong-Ho Choe (*rlc1*), Vicki Chandler (*mop1*, *mop2*, *mop3*, *rmr1*, and *rmr2* alleles), Andrea Gallavotti (various inflorescence mutants), Inna Golubovskaya (*mac1-Y211*), Sarah Hake (*kn1*, *Lgn1*, *Ts6*, *ifa1*, *mwp1*, and *ra2* alleles), Thomas Hartwig (*na2*, and *url1* alleles), Jay Hollick (various *B1* and *Pl1* stocks), Don McCarty (*vp8* alleles), Peter Rogowsky (*ppr2263*), Bao-Cai Tan (*emb17*), Clint Whipple (*tasselsheath* mutants), Mark Williams (*clpp1-ys*) 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 *vp5*, *te1*, and *oro1* loci. The isoprenoid biosynthesis mutant *zb7* and the luteus seedling mutant *l17* were found to be allelic to *lw1*.

3. Due to lack of personnel, we continue to provide only bare-bones curation of the A-A translocation stocks that were previously maintained by Janet Day Jackson. We are continuing to grow up recent outcrosses of translocation stocks in our observation fields to score for male and female semisterility.

4. Stocks produced from the NSF project “Regulation of maize inflorescence architecture” (see: http://www.maizegdb.org/MIP/) were grown again this summer. Approximately 450 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, 1,546 families of 2011 and 2012 EMS seed increase materials were grown for adult plant observation and 215 fami-

lies 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.

5. Critical plantings of a limited number of stocks were made in our greenhouse facilities. Current funding levels did not allow for a winter crop in Puerto Rico.

We currently have 8,849 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 an additional 228 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). Changing web standards and browser updates have caused considerable disruption with certain parts of the curation tools, but work on the tools continues. Moving to a more modern way of handling communication between various parts of the curation tools is the current major task. The harvest notes tool has been completed and this year's planting data was the first year to fully benefit from the harvest notes tool. Getting data from the pedigree records to harvest notes and finally into inventory is now streamlined, manual data entry has been reduced dramatically and data accuracy has improved. The family tree tool has been mostly integrated with the curation tool's pedigree pages and new features, like the ability to compare family trees for more than one pedigree at a time, are in the works. 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.illinois.edu/ph/www/maize).

Marty Sachs	Philip Stinard	Shane Zimmerman	Josh Tolbert
Director	Curator	Agric Sci Res Tech (Plants)	Information Tech Specialist

ADDITIONS TO OUR CATALOG OF STOCKS SINCE MNL86
(For a complete list of our stocks, see: http://maizegdb.org/cgi-bin/stockcatalog.cgi)

Chromosome 1 Markers
121AB ms14-BS21
133D Ts6-GN2230
133E Kn1-174::Mu
133F Kn1-Z
133G Kn1-169::Mu
133H Kn1-168
133I vp8-umu1
133J vp8-umu3

Chromosome 2 Markers
223B B1-615 Pl1 r1
227M ms33-Stan1
5303E B1-I||B1-Peru-D2
5303G B1-Peru||B1-I-D8
5303N B1-Peru||B1'-pg2
5303P B1-Peru||B1'-pg9
5303R B1-Peru||B1'-pg12
5303S B1-Peru||B1'-pg16
5303X B1-I||B1-Peru-L1
5303Y B1-Peru||B1-I-D5
5303Z B1-Peru||B1'-pg8

Chromosome 3 Markers
302AC d1-SGL
308C ra2-DM
310I na1-3
333BA te1-87-2490-22

Chromosome 5 Markers
506M sxd1-1
510H am1-485
510I emb17-1

Chromosome 6 Markers
601A tdy1-D6
621H tsh1-2

Chromosome 7 Markers
715F mwp1-2

Chromosome 8 Markers
810D vt2-04Mo-A619xB73GN-210

Chromosome 9 Markers
906Q ms25
906R ms25-BS5
910J Sh1 sus1
910K sh1 sus1
939D clpp1-ys

Chromosome 10 Markers
X04H mac1-Y211
X11J rlc1
X19EA R1-r(Ecuador1172) Lc1 Pl1
X336D r1-r(S1::dSpm; S2::Ds) isolate 1
X35Z Rp1-I

Multiple Genes
M342A B1-I Pl1-Rhoades R1-r
M342B B1' Pl1-Rhoades R1-r
M342C B1-I Pl1' R1-r
M342D b1 Pl1-Rhoades R1-r
M342E b1 Pl1' R1-r
M342F b1 Pl1' r1-r
M342G B1-I Pl1-Rhoades c1 R1-r
M342H B1-I Pl1' c1 R1-r
M342I b1 Pl1-Rhoades r1-r
M342J b1 Pl1-Rhoades c1-Hbr r1-r wx1 T6-9(043-1)
M342K b1 Pl1-Rhoades c1-Hbr r1-g wx1 T6-9(043-1)

Recombinant Inbred
Z039 B73xCML254 RILs
Z040 CML254xB97 RILs
Z041 B97xKi14 RILs

Toolkit
T3306O Rhoades K10 high knob derivative

Stocks Characterized Only by Phenotype

floury endosperm
6405D fl*-04HI-A632TR-11

grainy leaf
6606E GrNl*-N2536
6606F LesGr*-N2544

lesion
6606FA Les*-N2554
6606K Les*-N2641

male sterile
5405A dv*-IG5505
5406H dsy*-9303
5406I dsy*-9305

miniature kernel
138-42 mn*-MTM4910
nana plant

6607K na*-EMS939

oil yellow plant
6606G Oy*-N2553

pigmy plant
6607L py*-EMS796

rosette
6607J d*-EMS692

semidwarf
6606D VSdw*-N2525
6606H WiSdw*-N2560

short plant
6603I PgD*-N2542

torn leaf
6606J Ylfb*-N2612

yellow stripe leaf
6606I Ys*-N2603

Annual Report 2013

New Project Plan. The 2013-18 USDA ARS project plan for MaizeGDB was approved following review by OSQR. The main objectives are as follows:

(1) Support stewardship of maize genome sequence and forthcoming diverse maize sequences;
(2) Create tools to enhance access to expanded datasets that reveal gene function and datasets of genetic and breeding analysis;
(3) Allow researchers access to larger sets of data;
(4) Provide community support services, training and documentation, meeting coordination, and support for community elections and surveys;
(5) Identify and curate key datasets that will serve to benchmark genomic discovery tools for key agronomic traits, especially response to biotic and abiotic environmental stressors (Columbia, MO).

Genome assembly stewardship will involve a new collaboration with the "Genome Reference Consortium (GRC, Church DM et al (2011) PLoS Biol 9:e1001091). The GRC includes NCBI, EMBL-EBI, The Genome Institute at Washington University, The Wellcome Trust Sanger Institute, and was formed to deal with complex allelic diversity in mammalian model organisms. In this work we are collaborating with Gramene and the Arizona Genomics Institute. GRC tools will be used to represent diversity in maize genome and to enable the community of maize researchers to improve the reference genome sequence in real-time. Toward this end, MaizeGDB has aligned the BAC fragments in each GenBank BAC record with the current assembly, with submission of these updates by The Genome Institute at Washington University. Annotation tools have been released on the newly designed MaizeGDB site that track community supplied documentation towards improving the assembly. More information about the GRC process may be found here: http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/

New Data. New *diversity data* include: new Mo17 SNP data from JGI (6.5 million SNPs); Illumina MaizeSNP50 data (55,000 SNP, with ~40,000 genetically mapped on inter-mated panels (IBM, LHRF) and/or double haploid panels (Ganal et al 2011; Bauer et al 2013); HapMap2 (55 million SNPs over 103 lines, including landrace inbreds and teosinte.; Chia et al 2012 Nature Genetics 44: 803-807; Hufford et al. Nature Genetics 44:808-811). MaizeGDB stores genotypes for genetically mapped SNP, and is working toward a comprehensive representation in collaboration with the maize Diversity Panzea project (Ed Buckler), and iPlant. These will include current data on the HAPMAP studies and a recently published study of Midwest Germplasm (Romay MC et al 2013 Genome Biology 14:R55)

Gene function annotation data include some 50 current publications selected by the database Editorial Board; the fluorescent fusion protein lines and confocal microscope expression images from the Maize Cell Genomics Project (http://maize.jcvi.org/cellgenomics/index.php); Grassius (http://grassius.org/) transcription factor family assignments to gene models (Yilmaz A. et al 2009. Plant Physiol 149:171-180.), manual and computed updates of BioCyc corn metabolism databases that are hosted by MaizeGDB; Gene Ontology (GO) annotations performed to support a text mining experiment in collaboration with PIR, NCBI and curators for several other genome databases (TAIR, WormBase, FlyBase, RatDB).

Syntenic orthologs are now represented for *Sorghum biocolor*, *Setaria italics*, *Oryza sativa*, and *Brachypodium*, courtesy of J Schnable (Schnable J and Freeling M 2011 PlOs One 6:e17855). Numerous links are maintained to outside resources for data about orthologs, notably Phytozome, Gramene, and Arabidopsis.

Atlas RNA-Seq and proteomics data are anticipated soon. RNA-seq data will update the Kaeppler Gene Atlas based on Nimblegen microarrays, and includes several new tissues. The proteomics atlas is being provided by Steve Briggs (Walley AW et al PNAS 110:e4808-17, http://maizeproteome.ucsd.edu/), and will include many tissues, organelles, and protein modification data, much not yet accessible online.

Outreach. Training workshops were provided at the Genetics of Maize Microbe Interactions Workshop, Danforth Center, Feb 26-28, 2013; the University of Missouri-Columbia, Feb 28, 2013; the Maize Genetics Conference, Pheasant Run, IL, March 14, 2013. An outreach booth for several Plant Genome database resources was sponsored by MaizeGDB at the 2013 PAG meetings, San Diego, CA. Oral presentations were presented at the PAG meetings, and at the Maize Genetics Conference, along with numerous (over 12) posters. Three podcasts were prepared in collaboration with the National Corn Growers Association where the target audience is the general public. The topics this year cover phenotypes, SNPs, and proteomics data at MaizeGDB.

2013 Publications

Arighi CN et al 2013 Database (Oxford) An overview of the BioCreative2012 Workshop Track III: Interactive text mining task.
Cooper L et al 2013 Plant Cell Physiol 54(2):e1 The plant ontology as a tool for comparative plant anatomy and genomic analysis
Ghaffari et al 2013 Chromosoma 122:67-75. Maize chromosomal knobs are located in gene-dense areas and suppress local recombination.
Monaco M et al 2013 The Plant Genome 6(1):12 Maize Metabolic Network Construction and Transcriptom Analysis.

Guidance is generously provided by many members of the community, and especially by the following groups of persons:

(1) *MaizeGDB Working Group*: Alice Barkan, Qunfeng Dong, David Jackson, Thomas Lubberstedt, Eric Lyons, Adam Phillippy (*Chair*), Marty Sachs (ex officio), Mark Settles, and Nathan Springer);
(2) *Maize Genetics Executive Committee*, with elected membership: Jeff Bennetzen (*Chair*), Rebecca Boston, Ed Buckler, Carolyn Lawrence, Marja Timmermans, Nathan Springer, James Birchler, Sarah Hake, Paul Chomet, Sherry Flint-Garcia; and appointed membership, Brent Buckner, Ruairidh Sawre, Frank Hochholdinger, and Jinsheng Lai.
(3) *Maize Genetics Nomenclature Subcommittee*: Tom Brutnell, Hugo Dooner, Charles Du, Toby Kellogg, Carolyn Lawrence, Marty Sachs (*Chair*), Mary Schaeffer, Phillip Stinard
(4) *Editorial Board*: Lewis Lukens, Ruairidh Sawers, James Schnable, Thomas Slewinski and Amanda Wright.

Core Funding for MaizeGDB is from the USDA ARS, with additional support from the NSF and the NCGA (National Corn Growers Association).

Submitted Dec 6, 2013, by the MaizeGDB team: Mary L Schaeffer, Carson Andorf, Darwin Campbell, Ethalinda Cannon, Jack Gardiner, Lisa Harper, John Portword, Taner Sen, Carolyn Lawrence (Director).

Maize Genetics Executive Committee (MGEC) 2013/14

(prepared by editor of MNL from material posted at MaizeGDB http://www.maizegdb.org/mgec-activities2013.php)

1. Prepared a statement to the Interagency Working Group on Plant Genomes. It follows this report.

2. Founded the McClintock Prize for Plant Genetics and Genome Studies. This prize will be awarded each year to one or more of the most creative minds and productive scientists in the study of plant genome structure, function and evolution, including the analysis of gene regulation and epigenetics. To be eligible, a scientist must be currently active and not a current member of the Maize Genetics Executive Committee. This award is for career scientific accomplishments, not for community service or as a way to assist the development of a junior scientist. Awards will be made only to a single scientist each year, except in exceptional cases where two nominees contributed approximately equally in the same subject area. Each recipient will be invited to receive the award and make a research presentation at the annual Maize Genetics Conference. For more information about nominations, see the website http://mcclintock-prize.maizegdb.org/.

3. Surveyed the community about future needs. Survey results are posted online (http://maizemeeting.maizegdb.org/mgec-survey14/report2014.php). In brief, the top four research directions selected indicate a continued primary interest by this community in *gene function* and genetic engineering: (a) advance functional studies of maize genes, gene families, and networks; (b) increase high-throughput phenotyping capabilities for maize; (c,d) generate additional sequence to improve quality of the B73 reference genome sequence, and to include diverse maize genotypes and/or species closely related to maize. Regarding community resources, the Stock Center is indispensible and a $50 fee is unlikely to reduce use. Concerning education, the top needs selected included increased support for training in maize genetics, genomics, and bioinformatics and support for diverse bioinformatics "workshops" at Maize Genetics Conferences and other locations.

Response to IWGPG request for input re NPGI Five Year Plan
Maize Genetics Executive Committee
March 26, 2013

Executive Summary

The grand challenge to produce as much food in the next 40 years as in the previous 10,000 years requires rapid deployment of research discoveries from the laboratory to the field. As the leading U.S. commodity with a rich history of foundational discoveries from genetics research, maize must be a top target. Investments made under the NPGI in the last two decades have yielded the core genomic resources to make maize the preeminent monocot model for translational research. When genome sequencing was expensive, C3 rice had a role in basic plant research; however, it is a poor model for conducting translational research under U.S. environments and field growth conditions. Therefore, C4 maize dominates and offers direct translation of scientific discoveries to increased field productivity critical to sustain our production of food, fiber and fuel. This unique system that spans basic to applied research is underpinned by investments by federal funding agencies that have resulted in a completed genome sequence, a detailed understanding of natural variation, and a growing set of resources for reverse-genetic analysis of gene function. The maize community through surveys and community forums has identified key areas in which resources are needed to capitalize on these past investments and continue to increase the productivity of this important crop plant. Anchoring these needs are a strong, global infrastructure for data management that includes quality metrics and public access; foundational tools/resources (facile maize transformation, gene targeting, functional annotation, rapid phenotyping); and grants/fellowships to support the cross-training of students in interdisciplinary research. This overarching infrastructure is critical as we now move toward translating genomic data to an improved understanding of plant processes and predictive models of how genetic variation will influence field performance. The NPGI has proven to be an outstanding mechanism for guiding plant genomics from basic discoveries to deployment of science-based production agriculture. The Maize Genetics Executive Committee appreciates the opportunity to offer suggestions for the 2014-2018 plan.

Foundational Tools

Past investments by federal funding agencies in the U.S. have resulted in the development of foundational tools for research in maize. However, surveys of the maize research community make clear the community's broad agreement that additional such tools are necessary to enable basic, translational, and applied research. In a 2012 survey of the maize community there was very strong support for (1) increased functional annotation of maize genes, (2) a more robust system for transformation and production of transgenic maize, and (3) enhanced reverse-genetics resources. Support for a multi-national, well-coordinated ZeaENCODE-like project would provide valuable functional annotation data for the maize genome that would be widely used in the maize community. An improved ability to transform maize would increase our ability to probe the phenotypic consequences of altering genes, enabling the targeted creation of precise changes (including both transgenic and non-transgenic alterations to the genome — e.g., TALENs) to identify and provide useful agronomic characteristics. A more complete reverse-genetics resource that provides sequence-indexed mutations in nearly all maize genes would enable the understanding of gene function in maize in a manner that is currently unavailable. There is also a rapidly growing demand for high throughput phenotyping tools to enable large-scale field trials for agronomically meaningful traits. However, traits screened by biochemical or physiological assays typically require destructive sampling of a specific tissue that is transported to and assayed in the lab. These more traditional methods are simply not feasible for large sample numbers. Some measurement systems have recently become available to improve field measurements for complex physiological traits, but additional new tools are required for rapid, non-destructive sampling in the field. Together, these tools would revolutionize the pace by which the products of maize research can be deployed into farmers' fields.

Integrating Plant Processes

A major goal for the post-genome sequencing era is to gain a full and holistic understanding of how plants grow, develop, and reproduce. This information will integrate genome datasets of the last decade to help translate the knowledge of sequenced-based plant biology to crop improvements in the field. Such a goal can be accomplished only if we have a comprehensive understanding of plant processes that span from the cellular to the organismal level throughout the plant life cycle. This effort will require a detailed developmental and physiological understanding of how cells function to make tissues, how the tissues are organized into structures and organs, how changes in metabolic flux influence cell functions, and how these processes coordinate to produce an integrated whole. Starting with the farmer planting a seed, we need to determine the regulatory mechanisms of seed germination and seedling emergence at the cellular and physiological level. What are the different tissues in the seed? How are they coordinated? What prevents or delays germination? How are activities of the root, shoot, and endosperm coordinated? As the plant grows and produces seeds, we need a mechanistic understanding of how leaves and branches initiate, how cells remember their developmental fate, how physiology is coordinated at the tissue and whole plant level, how metabolic resources are allocated, and how biotic and abiotic factors combine to trigger growth, development, and reproduction. The information needed to completely describe these plant processes will couple

careful observations and experiments starting at the microscopic level with new information about the genome, regulome, transcriptome, proteome, and metabolome. Cross-disciplinary approaches will be needed to generate, analyze, and synthesize the large datasets that result from deep sequencing at the genetic and epigenetic levels. When integrated over developmental time, in different tissues, and under different environments, this information will provide a basis for selecting traits of interest by targeted phenotyping and for validating predictive models. The resulting knowledge will lead to new decision tools for improving maize and other economically important plants.

Genomes in the field

Genomic sequencing technology is providing tremendous insight into how the natural variation of maize performs on average across environments. However, as the great drought of 2012 illustrated: *there are no average environments*. While a couple of advanced breeding efforts showed promise in this arena, we do not have varieties of maize that withstand the full range of climatic variation that we will likely encounter over the next decades. This lack of varieties stems from a lack knowledge in four areas: (1) how genetic variation performs in specific environments, (2) how physiology varies across genetic and environmental variation, (3) how to develop crop models that integrate genetic variation, physiology, and environments together, and (4) how to develop genome wide predictions that include biological and physiological mechanisms. A better understanding of the basic science of how genomes perform in their environment will facilitate tremendous applications to agriculture, and with side benefits in medicine and conservation.

We advocate developing a research ecosystem that promotes extensive research in how genetic variation performs across environmental variation. Key aspects of the proposed research ecosystem would be (1) extensive field trialing with ~100 environments per year for 10 years (substantial portion of the locations would be through private sector collaboration), (2) integrated genomic and physiological analysis, (3) development of high throughput and innovative phenomics tools to measure physiology and the environment, (4) core national effort to make germplasm available, run central environments, and provide bioinformatics to enable numerous group projects focused on physiology, agronomy, genetics, and modeling to coordinate their efforts.

This is the time to make the shift to understanding how genomes perform in the field. Maize is already a superior model species for population and quantitative genetics and serves as the preeminent model for outcrossing plant species. A national effort initiated at this time would leverage new public personnel in breeding and newly emerging, amazing technologies to achieve both basic and translational research in how genomes interact with the environment.

Coordination and Informatics

In order to select target genes for functional characterization and to effect crop improvement, significant coordination is required, not only by researchers involved in generating data through diverse, independent projects, but among informatics groups that manage and analyze genome-scale information. This translates into coordination and broad data management needs that involve documentation of ongoing project goals and timelines as well as data including, e.g., genomics, functional genomics, and diverse annotations of phenotype. These needs can be met by developing, documenting, and requiring the use of gold standard datasets and best practices. The availability and pervasive use of such guidelines enables broad access to materials that serve as benchmarks to check computational pipelines and supports effective data collection that enables the simultaneous use of diverse datasets generated by multiple project teams. To benefit from the work of others in this area, engagement with outside groups, notably the NSF-funded Genomic Standards Consortium (GSC; http://gensc.org/gc_wiki/index.php/Main_Page), already deeply involved in these sorts of endeavors is key. It is noteworthy that the GSC has created a peer-reviewed venue called Standards in Genomic Sciences (SiGS; http://standardsingenomics.org/) to report work in this area, thus ensuring that such foundational endeavors are encouraged and valued by academic standards (e.g., publications to develop and deploy standards count toward tenure review). The GSC has made requests for plant researchers' involvement in their work already. By participating to enable the expansion of existing gold standards and best practices to accommodate plant-specific requirements, engagement by maize researchers will benefit both plant biology and the GSC. The maize community database MaizeGDB (http://www.maizegdb.org) is committed to serving as a centralized clearinghouse for access to maize-specific standard practices by deploying a "Standards" data center with linkages to outside groups and datasets that are not specific to maize, where appropriate and available.

Cross-training

Technological advances in genomic tools have fundamentally and rapidly changed how students must be educated and trained for careers in agriculture and life sciences. Team-building, communication and analytical skills essential for success in today's and tomorrow's complex research endeavors must overlay foundational disciplinary knowledge in core areas of biology, genetics, genomics, statistics, physiology, etc. The maize community has long embraced and established a strong track record of success stories for cooperative research, sharing of resources and mentoring of students, postdocs and junior faculty. Meeting today's global challenges requires a workforce able to move seamlessly across traditional disciplinary boundaries to use large datasets, develop and improve analytical tools

and expand our understanding of fundamental biological, physiological, biochemical and developmental processes. Support through research assistantships and training grants for students, postdoctoral grant programs and re-tooling opportunities for established faculty are critical for building interdisciplinary bridges and preparing a new generation of researchers who can leverage existing resources and build new ones to solve complex crop production problems.

International Challenges and Opportunities

The maize community is fortunate to have such a tremendous community of scientists who support the long-standing tradition of cooperation that is made possible by intentional collaboration and resource-sharing. While in the past this has been mostly focused among U.S. scientists, research in China, Europe, and the developing world plays an increasingly important role in the advancement of maize genetics. Although this global community of scientists has expanded the horizon for opportunities that promise to accelerate scientific advancement, lack of international agreement on germplasm access and data sharing impede effective collaboration and prevent worldwide resources from being leveraged most effectively. Specifically, agreements need to be in place to share genetics stocks that currently are encumbered by MTAs. Although advanced breeding stocks are anticipated to maintain IP limitations, uniform methods to enable sharing older (>20 years) breeding germplasm are needed. Additionally, genomic-phenomic resources (whole genome sequence, RNA profiles, and field phenotypes) are not currently shared through publically recognized venues (e.g., http://www.insdc.org/; the International Nucleotide Sequence Database Collaboration, INSDC, that coordinates the activities of DDBJ, ENA, and GenBank) by many groups outside the U.S. Alignment of U.S. open data approaches with European and Chinese research would help even the playing field in this arena to accelerate science (as recognized by the G-8 through their upcoming International Conference on Open Data for Agriculture; see https://sites.google.com/site/g8opendataconference/home). Many of the issues with data and germplasm sharing are not caused by individual scientists, but rather by high level policy decisions with consequences that inhibit science. Policies that support and even require international data and germplasm sharing are needed to support the success of future international agreements.

Diversity of Grant Portfolios

Global access to public genomic resources and analysis tools continues to generate tremendous returns on investment as scientists mine datasets and develop hypotheses about important biological processes. Creative researchers working at small colleges or as individual principal investigators provide the spokes extending from this hub of genomic resources to test hypotheses, provide excellent training for students and postdocs, and make seminal advances in plant sciences. Such leveraging of public data, however, requires that granting agencies support single investigator awards for smaller scale projects that draw upon rather than generate genomic resources. We strongly support individual research grant programs that fund established as well as new investigators.

The MaizeGDB 2013 Editorial Board included Lewis Lukens, Ruairdh Sawers, James Schnable, Thomas Slewinski and Amanda Wright. Each month, board members select papers for curation at MaizeGDB, and additionally provide a commentary, attached to the reference record at MaizeGDB. The papers and commentaries are provided below.

Boerner S et al 2012. PLoS One 7:e43047. Computational identification and functional predictions of long noncoding RNA in Zea mays.

Editorial Comment. Long noncoding RNAs behave in many ways like traditional genes often showing similar patterns of chromatin state and sequence conservation as observed for protein-coding genes. Many of these long noncoding RNAs appear to function in the regulation of either chromatin states or gene expression, however most of the genome wide studies conducted to date have focused on mammalian systems. This study used two different computational approaches to identify maize transcripts unlikely to code for protiens. Unlikely mammalian systems where nearly half of unique transcripts appear to be non-protein-coding, more than six out of seven maize transcripts apparently codes for protein. Any analysis where plant genomes appear to be globally different from mammalian genomes is interesting, however this paper also brings up an important issue for maize researchers to be aware of in the current set of maize gene model annotations. Four-hundred and seventy of the long noncoding transcripts identified in this study corresponded to sequences in the maize genome currently annotated as protein-coding genes (with short ORFs and no homology to any known sequence). This suggests maize researchers who identify candidate genes with small ORFs and no homology should be cautious about assuming these genes code for protein at all. *James Schnable 2013*

Bommert P et al 2013. Nature Genetics. 45:334-7. Quantitative variation in maize kernel row number is controlled by the FASCIATED EAR2 locus.

Editorial Comment. The ear of modern maize is one of the most striking examples of domestication at work, and, perhaps not surprisingly, the loci controlling variation in ear architecture have been a target of genetic study from the time of the first experiments to understand the differences between maize and teosinte. Despite this long history of investigation, the underlying genes controlling ear architecture have remained broadly unknown. Now, in their article, Bommert et al demonstrate that single-nucleotide polymorphisms in the gene *fascinated ear2* are sufficient to result in an increase in row number in the W22 background. Significantly, this increase in rows is accomplished without the loss of row-ordering typical of the previously characterized null mutants. The authors go on to present a model for their observations based on changes in inflorescence meristem size. Where the teosinte ear has 2 rows of kernels, early domesticates quickly developed multiple kernel rows, from the basic 8-row types (actual row number varies), such as Nal-Tel, for which the ear architecture of early specimens is wonderfully preserved in pre-Colombian ceramics, through to the high-row numbers of the small-grained ancient popcorns, such as Palomero Toluqueno. While Bommert et al do not present evidence for the involvement of *fasciated ear2* in this early diversification, they do suggest that variation in the gene played a role in later line improvement. *Ruairidh Sawers, 2013*

Burton, AL et al 2013. Crop Sci 53:1042-1055. Phenotypic diversity of root anatomical and architectural traits in Zea Species.

Editorial Comment. The domestication of cultivated maize from wild teosinte has become a text-book example of how far, and how fast, morphology can change in response to intensive selective pressure. Indeed, the extent of the difference between maize and teosinte is such that it was considered for a long time inconceivable that the two were any more than distant relatives. The story of maize domestication, however, has always been that of change to the aerial portions of the plant. What of the root system? Can so much be happening above ground without impacting the plant's "hidden half"? Here, Burton and colleagues provide perhaps the first detailed survey of the diversity to be found in maize and teosinte root systems. The authors characterize a panel of 256 accessions, including landrace and teosinte diversity, measuring a range of anatomical (e.g. area of cortex, steele, aerenchyma) and architectural (e.g. root number, root length) traits after 28 days of greenhouse growth. Intriguingly, the authors find significant differences between teosinte and landrace groups for a number of traits. It interesting to speculate on the impact of domestication on the root system, and the role root system adaptation may have had to play on the dispersal of maize. With respect to roots, there is clearly no "big bang" moment analogous to the formation of the cob: perhaps, a better analogy would be with the tassel (male

inflorescence). Regardless, Burton and colleagues provide evidence that both landraces and teosintes represent an important source of root phenotypic variation, a source that may have much practical utility in future maize improvement. *Ruairidh Sawers 2013*

Derrer C et al 2013. Plant Cell 25:3010-3020. Conformational changes represent the rate-limiting step in the transport cycle of maize SUCROSE TRANSPORTER1.

Editorial Comment. SUT1 is a symporter that allows the accumulation of high concentrations of sucrose in phloem cells by coupling the unfavorable transport of sucrose with the favorable transport of protons across cell membranes. Building on previous work investigating the molecular mechanisms of ion-coupled transporters in E. coli, animals, and plants, Derrer et al propose a model for the early stages of SUT1 mediated transport. The authors identified sucralose, the artificial sweetener commercially sold as Splenda, as a competitive inhibitor of SUT1. When sucralose is bound to SUT1, SUT1 can still bind protons, but the transport of the protons across the membrane is inhibited. They used voltage clamp fluorometry (VCF), a technique that detects conformational changes in a protein, to determine that sucrose transport is coupled to the movement of an extracellular loop and sucralose inhibits this conformational change. VCF works by fusing a flourophore to an accessible amino acid with subsequent changes in fluorescence intensity indicative of movement of the fluorophore and thus a conformational change of the protein. Their final model is that SUT1 continuously changes conformation so that the binding site is alternative exposed the interior and exterior of the cell. Upon proton binding, the current conformation is stabilized allowing for binding of the sugar. Overall, this study provides a fascinating view into the intricate workings of a transporter critical for the movement of sugars from source to sink tissues in plants. *Amanda Wright 2013*

Dong Z et al 2013. Plant Physiol 163:1306-1322. Maize LAZY1 mediates shoot gravitropism and inflorescence development through regulating auxin transport, auxin signaling and light response

Editorial Comment. In this paper, Dong et al report the phenotypic characterization and cloning of the maize ZmLA1 gene. This thorough analysis places ZmLA1 at the intersection of gravitropism, inflorescence development, light and auxin signaling. A classic maize mutant, *la1* plants have a prostrate stem phenotype and the shoot fails to exhibit a robust gravitropic response. In addition, tassel and ear development are abnormal. In other plants, disruption of *ZmLa1* homologues results in less severe gravitropic defects while the inflorescences develop normally. In maize, *ZmLa1* is expressed in ears and tassels as well as the nodes of the stem and expression increases in the dark. In onion cells, ZmLA1-GFP localizes to the nucleus and plasma membrane. Basipetal polar auxin transport is enhanced in the ears, tassels, and coleoptiles of the mutants while lateral polar auxin transport is decreased in the *la1* coleoptiles suggesting that ZmLA1 normally acts to inhibit basipetal polar auxin transport and enhance lateral auxin transport. A yeast two-hybrid screen identified 8 interacting proteins including two known to be involved in auxin signaling. RNA-SEQ experiments identified 646 up-regulated genes versus 285 down-regulated genes in *la1* plants compared to wild type suggesting that ZmLA1 also functions as a transcriptional repressor. Some of the differentially expressed genes are involved in auxin response or transport. *Amanda Wright 2013*

Eichten S et al 2013. Plant Cell 25:2783-2797. Epigenetic and genetic influences on DNA methylation variation in maize populations.

Editorial Comment. In this study, Eichten et al profile DNA methylation levels across a set of maize genotypes by enriching for methylated DNA fragments and hybridizing the fragments to a microarray containing 2.1 million probes surveying the low-copy portion of the maize genome. They make a number of interesting discoveries, including the following. First, the DNA methylation levels of many chromosomal regions differ. They identify 1966 regions where at least three of the 19 lines have DNA methylation patterns that differ from other lines. 1754 regions have discrete methylation patterns in one or two lines only. The rare variants tend to be hypomethylated relative to the common variants. Second, Eichten et al find that most of the differentially methylated regions are heritable, and for many regions, the methylation state is cis regulated. Many differentially methylated regions are associated with nearby transposons. Finally, Eichten et al suggest a functional importance for differentially methylated regions because DNA methylation levels at a number of regions correlate with transcript abundance at nearby genes. High levels of methylation within regions that overlap genes' transcription start sites are negatively correlated with transcript abundances. Naturally occurring chromatin differences likely explain a proportion of the genetic variation within plant populations. This is especially true in maize because its genome has a high number of transposable elements, and many genes are located close to transposons. This work offers a fascinating snapshot of DNA methylation variation and offers a number of areas for further inquiry. *Lewis Lukens 2013*

Erhard KF et al 2013. Plant Cell 25:808-819. Maize RNA polymerase IV defines trans-generational epigenetic variation.

Editorial Comment. The *c1* (*colored alurone1*) and *pl1* (*Purple plant1*) genes are two long studied genes involved in the regulation of the pigment anthocyanin in maize. These genes were some of the early "duplicate genetic factors" that first suggested a polyploidy somewhere in the history of maize. As the name suggests, *c1* alleles tend to be identifiable by changes in kernel pigmentation, while *pl1* alleles result in changes in pigmentation in other tissues like

leaves and anthers. While working with a mutant involved in the epigenetic regulation (*nrpd1*), the authors started to notice purple pigmentation in kernels which became stronger the longer over multiple generations in an *nrpd1* mutant background. Afterwards even restoring a wild-type *nrpd1* allele did not correct the purple kernels phenotype. The authors verified that the *c1* alleles they were working with were truly null alleles and showed expression of the *pl1* gene in kernels. After observing this effect in multiple *pl1* alleles (but only those which contain transposon insertions upstream of the transcription start site) and similar pigmentation effects in other mutant backgrounds involved in siRNA biogenesis, the authors are able to conclude they have identified an example of transgenerational epigenetic regulation of tissue specific gene expression. While this particular example is dependent on knocking out key elements of maize's epigenetic tool kit, other cases may act as an additional source of heritable variation in maize. *James Schnable 2013*

Facette MR et al 2013. Plant Cell 25:2798-812. Parallel proteomic and phosphoproteomic analyses of successive stages of maize leaf development.

Editorial Comment. Facette at el. presents a comprehensive proteomics survey of the developing maize leaf. Using mass spectrophotometry, they identified ~12,000 proteins and ~3,500 phosphoprotiens within 4 developmentally distinct leaf zones including the cell division zone, cell differentiation zone, cell expansion zone, and mature blade tissue. Careful examination of the proteins revealed that while many proteins are found in all four zones, there is also a large number of proteins expressed in the division, differentiation, and expansion zones and excluded from the mature leaf blade. While the differentiation, division, and expansion zone have a similar complement of proteins, the identified phosphoproteins were more likely to have a zone specific distribution. Highlighting the usefulness of this data set for hypothesis generation, the authors took a close look at cell wall synthesis proteins as well as the proteins involved plant hormone production and response. They were able to identify expected as well as unexpected trends in protein and phosphoprotein distribution. Overall, the proteomic data discussed in this paper is a valuable community resource that complements existing mRNA expression data. *Amanda Wright 2013*

Goettel W and Messing J 2013. Theor Appl Genet 126:159-177. Paramutagenicity of a p1 epiallele in maize.

Editorial Comment. Paramutation is a fascinating phenomenon in maize genetics. Paramutation may occur between two alleles with identical nucleotides but differing in epigenetic state. In the heterozygote, one allele, the paramutagenic allele, can heritably silence the second, paramutable allele. The paramutable allele after exposure to the paramutagenic allele is termed the paramutant allele. Repetitive sequences are often associated with paramutation, and mutations in genes that function within the RNA directed DNA methylation pathway can disrupt paramutation. In this paper, the authors investigate the interaction and inheritance of *P1-pr* alleles with *P1-rr* alleles. The *P1-pr* allele produces a patterned pericarp, and the *P1-rr* allele produces a dark red pericarp. They find that in a heterozygote, the *P1-pr* allele represses the *P1-rr* allele. Interestingly, the repression is highly variable- hybrid seed ranges from nearly colourless to dark red. The pigmentation of ears from testcross plants derived from F1 plants correlated with the pigmentation of the hybrid ears. Thus, different epigenetic states of the paramutant allele within the hybrid population were transmitted through meiosis. The authors also analyze cytosine methylation within upstream regulatory sequences containing *p1* specific repeat and transposon sequences. Among F1 plants with variable pigmentation, cytosine methylation levels within the *p1* repeats was similar, although nominally higher among those plants with little colour. Among testcross populations of F1 plants, the average methylation of *p1* cytosines correlated with the absence of pigment. This work underscores how repeat-associated epigenetic changes can generate a range of novel traits and suggests that paramutation may be triggered by transposons adjacent to regulatory sequences. *Lewis Lukens 2013*

Guan, JC, et al 2012. Plant Physiol 160:1303-1317 Diverse roles of strigolactone signaling in maize architecture and the uncoupling of a branching-specific sub-network.

Editorial Comment. The plant hormone, strigolactone (SL), induces the germination of Striga sp. parasite plants and is required for establishing symbiotic mycorrhizae relationships. In addition to these roles, mutant analysis in diverse plant species has shown that SL signaling inhibits lateral branching. To investigate the role SL plays in establishing maize plant architecture, Guan et al identified a mutant that disrupts *carotenoid cleavage dioxygenase8* (*ccd8*), a SL biosynthetic gene. While *ccd8* mutants have twice as many axillary branches as wild-type plants, the branching phenotype is minor compared to that seen in SL pathway mutants in pea, Arabidopsis, and rice. In pea and Arabidopsis, SL signaling activates *teosinte-branched1* (*tb1*) homologues, which inhibits branching. In contrast, expression and *tb1*; *ccd8* double mutant analysis showed that SL does not regulate *tb1* in maize. Careful study of the *ccd8* mutant phenotype revealed that in addition to branching, SL signaling regulates plant height, stem diameter, nodal (adventitious) root system formation and inflorescent development in maize. The authors suggest that while *ccd8* was not a good domestication target in maize because it is single copy and has the associated pleiotropic phenotypes, SL signaling components maybe domestication targets in other grasses. *Amanda Wright 2013*

Guo X et al 2013. Plant Physiol 162:1359-1369. Non-redundant function of zeins and their correct stoichiometric ratio drive protein body formation in maize endosperm.

Editorial Comment. Zein proteins, which are storage proteins organized into protein bodies, make up a majority of the protein expressed in the maize endosperm. Zein proteins have a poor amino acid profile, which limits the ability of maize endosperm to serve as a quality protein source. In order to manipulate zein protein content to improve grain quality, it is important to have a complete understanding of zein protein function and how the zeins are organized into protein bodies. Many of the zein protein families contain multiple members so Guo et al (2013) used RNAi to knock down complete families in order to ascertain their function. In combination with previously published reports including a complementary zein RNAi study reported by Wu and Messing (2010), Guo et al proposes specific roles for zein proteins in protein body formation. They suggest that although the gamma zeins co-localize to the outer layer of the protein body, the 27kD gamma zein is needed for protein body initiation while the other gamma zeins are needed for protein body expansion. Additionally, simultaneous depletion of the two alpha zein classes results in smaller protein bodies pointing to a role in expansion for the alpha zein classes. Interestingly, when the RNAi constructs targeting all the zeins are combined, the resulting endosperm contains small numbers of normally shaped and sized protein bodies suggesting that fixed zein protein ratios are also critical for protein body morphology and expansion. *Amanda Wright 2013*

Hufford MB et al 2013. PLoS Genetics 9:e1003477. The genomic signature of crop-wild introgression in maize.

Editorial Comment. Maize is now known to have been originally domesticated from a subspecies of teosinte called *parviglumis* which grows natively in the lowlands of central mexico. However much of the modern range of maize cultivation is in environments very different from the native habitat of *parviglumis*. In the article the authors report on extensive gene flow from another teosinte subspecies (*mexicana*) which grows alongside maize in the mexican highlands, measured using microarray based genotyping of SNP markers. Despite limited viability of crosses between *mexicana* and domesticated maize, the authors found that gene flow occurs in both directions. However, adaptive introgressions have occurred almost exclusively from the wild teosinte subspecies into domesticated maize. This result is consistent with a model where these introgressed regions are involved in adaption to colder and drier climate of the mexican highlands. Two putatively adapted introgressed loci are potentially involved in the differences in macrohair development between lowland and highland teosintes. *James Schnable 2013*

Husakova E et al 2013. Ann Bot 112:417-428. Lateral root development in the maize (Zea mays) lateral rootless1 mutant.

Editorial Comment. The mutant *lateral rootless1* (*lrt1*) is one of only a handful of known maize root-system-architecture mutants. The mutant was first isolated for the absence of lateral roots during early post-embryonic development. Interestingly, this phenotype can be partially rescued by changing environmental conditions, perhaps most intriguingly by inoculation with mycorrhiza-forming fungi. Here, as part of an Annals of Botany focus issue on Matching Roots to their Environment, the authors provide new detailed histological data on the *lrt1* phenotype. Consistent with the earlier observations of an environmentally sensitive phenotype, the authors propose that the *lrt1* mutant does not affect the frequency of lateral root primordia formation per se; rather that, under standard conditions, mutant primordia arrest early during development, most commonly during root emergence. The authors hypothesize that this arrest is a result of a failure to penetrate unprepared surface cell-layers in the primary root. In support of this model, the authors report impaired root meristem function coupled with a disruption of both epidermal and sub-epidermal cell layers. In addition, they observe ectopic lignification and deposition of poly-phenolics in just those surface root layers through which lateral roots would emerge in a wild-type plant. While the *lrt1* mutant is still to be cloned, evidence continues to accumulate to suggest that the gene plays a role beyond blind development, and that it has a function in the integration of environmental and developmental signals. *Ruairidh Sawers 2013*

Jiang WK et al 2013. Plant Physiol 161:1844-1861. Prevalent role of gene features in determining evolutionary fates of whole-genome duplication duplicated genes in flowering plants.

Editorial Comment. As maize geneticists, we are often confronted with the maize genome's evolutionary past when we discover our favorite gene has a paralogue. These paralogous gene pairs often arise due to the whole genome duplication (WGD) events that are consistent features of the evolutionary past of many organisms, including the flowering plants. After a WGD genomes undergo fractionation, which includes genome rearrangements and gene loss. Previous studies have determined that duplicated genes can be retained and undergo neofunctionalization where one gene acquires a new fate or subfunctionalization where the duplicated genes split the function previously covered by the original gene. The study by Jiang et al examined duplicated gene pairs found within areas of synteny in 6 plant genomes (maize, sorghum, rice, soybean, poplar, and Arabidopsis) and looked to see if they could identify features that made both members of a duplicated gene pair more likely to be retained. They evaluated 18 features and were able to divide the retained genes into three groups based on shared features. Type I genes evolve slowly, are highly expressed, and are involved in processes essential to cell survival suggesting their retention could be due to dosage compensation where having equal amounts of the proteins within a functional network is beneficial.

Type II genes are structurally complex with many protein domains suggesting they are candidates for subfunctionalization. Type III genes are united by their high GC and GC3 (G/C present at the third codon position) content, fast evolution rates, and low expression levels suggesting they are candidates for neofunctionalization. In the end, this thorough study was able to identify evidence supporting many previously proposed evolutionary hypotheses as to the fate of duplicated genes following a WGD. *Amanda Wright 2013*

Kuntz M et al 2013. Nature Biotechnology 31:498-500. What the French ban of Bt MON810 maize means for science-based risk assessment.

Editorial Comment. Publications can have significant impacts political decisions regarding regulations and implementation of technology as well as choices of the general public in their selection of products and services. The recent controversy raised by a group claiming that transgenic maize and the associated herbicide both cause identical pathogenesis in mice (see Seralini et al 2012). Food and Chemical Toxicology) has shaken the public's acceptance of scientific method and objective research. Kuntz et al 2013 discuss the broad-sweeping impacts that this one report has had on both the public understanding of science and the national policies that conceived and enforced based on subjective interpretation of science. Surprising and truly unsettling trends are emerging in the public realm of science perception, not only in the fields of plant science and GM technology, but also in climate and health science (i.e. vaccination). However, such controversies also open up many opportunities for scientists to reach out and educate the the general public to increase the understanding and acceptance of science facts while reducing science fears which are gaining more and more prominence in many societies. *Thomas Slewinski 2013*

Labonne JDJ et al 2013. Epigenetics 8:398-408. Changes in nucleosome position at transcriptional start sites of specific genes in Zea mays mediator of paramutation1 mutants.

Editorial Comment. Chromatin and DNA modifications play key roles in transcriptional regulation and are associated with changes in nucleosome positioning. The maize gene *mediator of paramutation1* (*mop1*) is a homologue of an RNA dependent RNA polymerase (RDR2) that is required for the biogenesis of small RNAs that direct heterochromatin formation. Many maize genes are mis-expressed in the *mop1-1* mutant. Here, Labonne et al compare nucleosome positions between a *mop1-1* mutant and a wild type control at the transcriptional start sites (TSSs) of about 400 maize genes in leaf, ear shoot, tassel, and seedling samples by hybridization of nucleosomal DNA to a high density microarray. In a subset of the tissues, three of the 400 genes had consistent differences in nucleosome positioning between mutant and wild type plants. Nucleosome positions differed both upstream and downstream of the TSSs. For two genes, positioned nucleosomes appeared to have been lost in the mutant plants, and for the third gene, a positioned nucleosome appeared to have been gained. Surprisingly, the nucleosome differences were not associated with differences in transcript abundance. This work suggests that MOP1 affects chromatin structure at a small proportion of maize gene TSSs, and that these modifications have an indirect, if any effect, on gene expression. The widespread transcriptional changes in *mop1-1* seem not to be associated with TSS nucleosome distribution and are perhaps due to MOP1 mediated silencing of repetitive DNA. *Lewis Lukens 2013*

Li JF et al 2013. Plant Cell 25:1507-1522. Comprehensive protein-based artificial microRNA screens for effective gene silencing in plants.

Editorial Comment. Plant genome sequencing has uncovered thousands of uncharacterized genes creating the challenging task of assigning functions to all the proteins encoded by these genes. Using reverse genetics to create organisms that lack the unknown protein is one way to assign function. While the various collections of maize mutant lines available to the community continue to expand, there is not always a mutant available or functional redundancy with a paralogue complicates the analysis of the gene of interest. Gene silencing methods that exploit endogenous RNA degradation or translational repression pathways such as RNAi, VIGS, and amiRNAs provide an alternative way to knock down protein levels, but it is not unusual for a silencing construct to fail to work effectively. This can be especially problematic for species such as maize where generating transgenic plants is costly and time consuming. Li et al (2013) describe a prescreening method to evaluate the efficiency of amiRNAs (artificial micro RNAs) designed in silico. They co-expressed an amiRNA along with an epitope-tagged full-length version of the target in Arabidopsis protoplasts. They then looked for loss of the epitope-tagged protein via Western. They screened multiple amiRNAs (usually four) that target the same gene identifiying the most effective amiRNA. In their test cases, they found that the amiRNAi that was the most effective at knocking down protein levels in protoplasts was also the most effective in plants. In addition to prescreening amiRNAs for knocking out an single protein, this technique can be used to test how effective a single amiRNA is at targeting multiple parologues or to test the effectiveness of multiple amiRNAs arranged polycitronically or tandemly. Based on their experience with the amiRNAi screens, the authors also provide some new rules for more effective amiRNA design. While much of the validation of this technique was carried out in Arabidopsis, the authors demonstrate that prescreening amiRNAs in protoplasts works in a wide variety of plants including maize. *Amanda Wright 2013*

Li L et al 2013. PLoS Genetics 9:e1003202. Mendelian and non-mendelian regulation of gene expression in maize.

Editorial Comment. The Intermated B73 x Mo17 population (IBM) is a set of recombinant inbred lines that have been widely used in maize quantitative genetics studies for more than a decade. This paper reports on the analysis of gene expression levels (quantified using RNA-seq) in 105 lines selected from this population. These data allowed the identification of cis- and trans- regulatory variation effecting the expression of more than 19,000 maize genes. The authors also uncovered evidence of a number of other unexpected patterns of gene regulation including paramutation-like patterns of expression. In a number of cases the expression of non-syntenic genes (often associated with CACTA transposons) are correlated with reductions in the expression of homologous genes at ancestrally conserved syntenic locations. This suggests exotic sequences captured by transposons can interfere with the expression of the original gene, potentially acting as a source of novel regulatory function. The ability to look up the mapped locations of trans-eQTLs regulating specific genes using the included supplementary data may be of interest to the greatest number of individual researchers. *James Schnable 2013*

Liu S et al 2013. PLoS Genetics 9:e1003790. Genome-wide analysis of ZmDREB genes and their association with natural variation in drought tolerance at seedling stage of Zea mays L.

Editorial Comment. Previous studies in maize and Arabidopsis reported that the DREB family of transcription factors are important for responding to various abiotic stresses including drought. Lui et al (2013) identified 18 DREB genes in the maize genome and showed that they are all up-regulated in seedlings grown under drought conditions. Taking advantage of the natural variation between maize lines, the authors used recently published SNP data to determine that 14 of the 18 DREB genes are polymorphic within a diversity panel containing 368 lines. They also evaluated all 368 lines for drought stress tolerance. After compiling the SNP information and drought phenotypes, an association analysis determined that polymorphisms in one gene, *ZMDREB2.7*, correlated strongly with drought resistance. Supporting this finding, transforming Arabidopsis with *ZmDREB2.7* resulted in drought resistance plants. Additionally, after crossing drought sensitive and drought resistant lines, segregation of sensitive/resistant alleles in the F2 population correlated with the drought resistance phenotype of the plants. This gene can now be targeted in breeding efforts to generate more drought-tolerant plants. *Amanda Wright 2013*

Lu Y et al 2014. Plant Reproduction 27:19-20. Genetic and cellular analysis of cross-incompatibility in Zea mays.

Editorial Comment. In Mexico, the ancestral home of maize, there is great interest in gene flow among populations of ancestral teosinte, landrace maize, and imported hybrid varieties. Here, Lu and colleagues remind us that, in addition to ecological factors and cultural practice, plant biology plays a key role also in this process. Three genetic systems have been well described that confer cross-incompatibility between domesticated maize and wild teosinte. While the molecular mechanisms underlying these systems remain elusive, Lu and colleagues provide further data that extend our knowledge of the genetic basis of the *teosinte crossing barrier1* (*tcb1*) locus. Cross-incompatibility barriers consist of two functions, female and male: the female function confers the silk-barrier to incompatible pollen; the male function determines the competence of pollen to fertilize silks expressing the female function. While a rare haplotype that confers male function only has been identified in natural teosinte populations, Lu and colleagues, through detailed analysis of recombinants, separate genetically the female and male functions of a *tcb1*, suggesting a possible complex locus. Further, the authors characterize arrest of pollen-tube growth in incompatible interactions. Intriguingly, the morphology of arrested pollen-tubes is different between *tcb1* and other known cross-incompatibility systems, suggesting that various mechanisms may be involved. Finally, the authors report the loss of cross-incompatibility in lineages carrying active *Mutator* transposons. The potential availability of transposon-tagged alleles of *tbc1* has clear implications to the molecular identification of the gene or genes that constitute the *tbc1* locus. As the authors point out, cloning of the genes involved will go a long way to finally untangling the history and mechanism of one of the great stories of maize population biology. *Ruairidh Sawers 2013*

Maron LG et al 2013. Proc Natl Acad Sci USA 110:5241-5246. Aluminum tolerance in maize is associated with higher MATE1 gene copy number.

Editorial Comment. Although copy number variation (CNV) between maize inbred lines is extensive, increasing or decreasing the copy number of a specific gene has rarely been linked to a phenotype. Maron et al discovered that maize lines containing three copies of *mate1* have an increased aluminum tolerance relative to maize lines with only one copy. MATE1 is a transporter that moves citrate out of roots cells and into the rhizosphere where the citrate can complex with Al preventing the uptake of Al into root cells. *mate1* had previously been correlated with a QTL detected in a mapping population created by crossing an Al sensitive to an Al resistant line, but the molecular mechanism of the *mate1* associated resistance was unclear. A functional study showed that MATE1 proteins from the resistant and sensitive lines transport citrate equivalently ruling out amino acid changes as the cause of the Al tolerance. FISH suggested that there may be more than one copy of *mate1* at the end of chromosome six and this was confirmed by qPCR and by sequencing the region. The increase in *mate1* copy number results in an increase in expression of *mate1*. Sequencing showed that a highly similar 30 kb genomic region, which contains only the *mate1*

gene and multiple retrotransposons, is triplicated in the Al tolerant line. The lack of sequence diversity between the repeats suggests that the triplication was a recent event possibly occurring since domestication. After screening a diverse panel of 167 inbred and teosinte lines, the *mate1* triplication was identified in two additional inbreds, which are also Al tolerant. The three lines that contain the *mate1* triplication originated in a region of South America with acidic soils. Acidic soils increase the toxicity of Al and acidic soil tolerance is a desired trait in South American breeding programs. *Amanda Wright 2013*

Mascheretti, I et al 2013. Plant Cell 25:404-420. The WD40 repeat proteins NFC101 and NFC102 regulate different aspects of maize development through chromatin modification.

Editorial Comment. Chromatin remodeling is an essential process required for the initiation and progression of many developmental programs in plants and animals. Changes in the chromatin landscape allow some genes to accessed and expressed while restricting and suppressing others. In this study, Mascheretti et al explore the role of the chromatin modification factors nucleosome remodeling factor complex component101 (NFC101) and NFC102 in floral transition in maize. Analysis of transgenic maize lines that simultaneously down regulated NFC101 and NFC102 showed that these chromatin remodeling factors act to suppress the expression of *indeterminate1* (id1) and *centroradialis8 (zcn8),* two key activators of floral transition. However, modification of the ID1 locus appears to involve the function of Rpd3-type histone deacetylases (HDACs), whereas modification of the ZCN8 locus is HDAC independent. Disruption of the NFC101/NFC102 pathway also resulted in a wide range of developmental defects as well as ectopic tissue specific expression of transposable element repeat nonpolyadenylated RNA. This suggests that NFC101 and NFC102 act though distinct chromatin remodeling mechanisms that regulate multiple pathways during plant development. *Thomas Slewinski 2013*

Massman J et al 2013. Crop Sci 53:58-66. genomewide selection versus marker-assisted recurrent selection to improve grain yield and stover-quality traits for cellulosic ethanol in maize.

Editorial Comment. Selection of plants that exhibit certain favorable traits has been largely responsible for steady improvements in agricultural productivity. Marker assisted selection (MAS) and more recently genomic selection (GS) methods utilize plant genotypic information to inform the selection process. Two key benefits of selection based on genotype instead of phenotype are that young plants can be assayed and expensive trait measurements can be avoided. Marker assisted selection involves identifying a set markers that are significantly associated with a trait and using information from these markers to guide selection. Genomic selection also uses marker genotypes to predict performance but simultaneously estimates the effects of all markers. Here, Massman et al compare the effectiveness of MAS and GS in improving maize hybrid yield and stover-quality traits important for cellulosic ethanol production. They first performed one round of phenotypic selection on stover and stover + yield indices calculated from observed traits. They then performed two cycles of MAS and GS, selecting on predicted index values. GS resulted in larger index gains than did MAS over the three cycles of selection. Interestingly, however, individual stover and yield trait values largely did not improve in populations that had undergone GS or MAS relative to the initial population obtained through phenotypic selection. This result may have been due to the selection on indices rather than on individual traits. Although a number of studies have demonstrated the superiority of GS relative to MAS in simulations and cross-validation studies, this work provides an interesting empirical comparison of the methods and highlights the promise of GS. *Lewis Lukens 2013*

Mauro-Herrera M et al 2013 G3 3:283-95. Genetic control and comparative genomic analysis of flowering time in setaria (poaceae).

Editorial Comment. Studies of quantitative traits are widely used across many species to examine traits ranging from morphology, secondary metabolite production, stress resistance, and yield (as well as many others). The identification of QTLs allows researchers to study the sources of existing natural variation and acts as a complement to studies of mendialian mutations. However, until recently, and unlike mendelian genetic studies, comparisons of the loci underlying quantitative variation among different species have been rare. Mauro-Herrara et al demonstrate a straightforward approach to interspecies comparisons of QTL studies. In the paper the authors using a population of recombinant inbred lines to identify a set of major QTL responsible for the variation in flowering time between domesticated Setaria italica and its wild ancestor Setaria viridis. They then employed a synteny-based approach to compare their results to previous studies of variation in flowering time in maize and sorghum. While several QTLs are conserved among these species (which diverged ~25 million years ago) the authors are able to conclude that the major flowering time QTL identified on maize chromosome 10 is maize-specific. *James Schnable 2013*

McCarty DR et al 2013. PLoS One 8:e77172. Mu-seq: Sequence-based mapping and identification of transposon induced mutations.

Editorial Comment. Insertional mutagenesis with the Robertson's *Mutator* transposon has been important for both forward and reverse genetics in maize. The availability of a large collection of lines with insertions within many different genes is a key resource for gene function investigations. Families within the *Uniform-MU* population contain novel and stable *Mu* transposon insertions. There are currently over 45,500 germinal Mu insertions in over 8,236 maize lines. In this report, McCarty et al describe a novel method for identifying *Mu* insertions within *Uniform-*

MU families using targeted high-throughput sequencing. They start by assigning each of 576 families a position on a 24 x 24 grid. They extract two DNA samples on the opposite sides of plants from the 576 families. One DNA sample is pooled with a set of 23 families along a grid row, and the other DNA sample is pooled with a set of 23 families along the column. An analysis of *Mu* insertions within the 24 row and 24 column samples can identify the precise family in which an insertion has occurred by using the intersection of the two axes in which an insertion is detected. A problem with this approach is that Mu elements are numerous within the maize genome, and it can be tricky to identify novel insertions among ancestral insertions. Here, McCarty et al selectively amplify flanking sequence from only the terminal inverted repeats of *Mutator* transposons that are most active in plants with autonomous *MuDR* elements. With about 100 million 100 bp sequence reads, McCarty et al's method identifies 4,723 novel, germinal transposon insertions- insertions detected both in one column sample and in one row sample. The method distinguishes distinct Mu insertions at the same locus when the insertion sites differ by as little as a single nucleotide. Interestingly, 13,218 insertions have substantial sequence support but were found in a single column or row sample, suggesting somatic insertions of *Mu*. These single axis reads are greatly over-represented in certain samples suggesting that about 1% of the families have *Mu* activity. *Mu* activity remains despite selection against activity based on *bz1-mum9* expression, perhaps because of epigenetic phenomena. *Lewis Lukens 2013*

Meihls LN et al 2013. Plant Cell 25:2341-2355. Natural variation in maize aphid resistance is associated with 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside methyltransferase activity.

Editorial Comment. In this paper, Meihls and colleagues report the identification and cloning of the first aphid susceptibility QTL in maize. Using the natural variation present in the NAM population, the authors were able to detect a large effect, almost mendelian, aphid susceptibility QTL on Chromosome 1. Susceptibility was associated with the absence of a DOPIA transposon insertion into one of three tandem nearly identical O-methytransferase genes, *bx10a*, *b* and *c*. The insertion into *bx10c* was further associated with higher levels of DIMBOA-Glu, a benzoxazinoid defense compound that is known to induce callose formation in the plant and is toxic to aphids, thus resulting in plants that are more resistant. Susceptible lines that have a functional copy of *bx10c* gene convert more DIMBOA-Glu to HDIMBOA-Glu, which is more toxic to aphids, but does not induce callose deposition, and presumably other subsequent defense responses, as strongly as DIMBOA-Glu. This study highlights the trade-offs between the defense-inducing properties and direct toxicity to insects conferred by the variants of maize benzoxazinoids. *Thomas Slewinski 2013*

Regulski M et al 2013. Genome Res 23:1651-62. The maize methylome influences mRNA splice sites and reveals widespread paramutation-like switches guided by small RNA.

Editorial Comment. Cytosine methylation is an epigenetic modification of DNA that can affect gene expression and occurs in three sequence contexts: CG, CHG (where H is A, C, or T), and CHH. In this work, Regulski et al present a genome-wide map of cytosine methylation from maize inbreds B73 and MO17 in all three contexts by deep sequencing sodium bisulfite treated DNA. (Sodium bisulfite converts unmethylated cytosines to thymines.) They compared methylation patterns with sRNA and mRNA abundances as well as with DNA attributes. As expected, they found evidence of dense methylation in the pericentromeric regions of each chromosome, strong correlation between 24nt sRNA levels and CHH methylation, and low levels of cytosine methylation in exonic DNA relative to 5' and 3' DNA. A number of discoveries were especially interesting. They identify a spike of cytosine methylation at splice junctions. Acceptor sites with a high level of CHG methylation were less efficiently spliced than acceptor sites with low methylation levels, and among those genes that are alternatively spliced and have acceptor sites with different levels of methylation, the large majority of genes used the acceptor site with the reduced CHG methylation. They also identify highly differentially methylated regions between B73 and MO17. Methylation differences are correlated with expression levels of nearby genes, suggesting that these differences may contribute to trait differences between the two inbred lines. Most of the parental methylation patterns were found to be inherited along with parental alleles across nine recombinant inbred lines derived from the two parents, but about 0.9 Mb of the 6 MB of differentially methylated regions switched methylation states. A higher frequency of regions became hypermethylated than hypomethylated; hypermethylation occurs at a higher frequency within MO17 alleles than B73 alleles; and the same regions switch epigenetic states more than expected by chance. The mechanism that directs these epigenetic inheritance patterns is still unknown. Overall, Regulski et al suggest a fascinating possibility- that paramutation-like methylation changes lead to significant changes in gene regulation at an unexpectedly high frequency. *Lewis Lukins 2013*

Rodriguez VM et al 2013. J Exp Bot 64:3657-3667. Genetic regulation of cold-induced albinism in the maize inbred line A661.

Editorial Comment. As the geographical range of maize expands, it is increasingly subject to low temperatures both early and late in the growing period. Thus, there is great interest in uncovering natural variation for cold tolerance and the genes that contribute to it. Seedling leaves of the inbred line A661 have a dramatic reduction of chlorophyll content when the plant is exposed to a chilling temperature below 15C. This work revealed that the chlorophyll

reduction was unlikely due to chlorophyll degradation in A661 nor due to a non-functional chlorophyll biosynthesis pathway. The authors suggest that at cool temperatures, A619 chlorophyll is not stably integrated into photosynthetic antennae, remains free in the chloroplast, and activates the expression of an early light inducible protein gene that prevents reactive oxygen species accumulation. The cold induced albinism is likely caused by a number of alleles. The strongest QTL on chromosome 2 explains a 14% of the phenotypic variation. The identity of this gene and other contributing genetic factors will be of significant interest. *Lewis Lukens 2013*

Sekhon RS et al 2012. Plant Physiol 159:1730-1744. Transcriptional and metabolic analysis of senescence induced by preventing pollination in maize.

Editorial Comment. Sink-source interactions underpin all aspects of growth and yielding capacities in crop plants. Initiation of leaf (source) senescence is coordinated with final phases of grain (sink) filling. This process recycles resources in the leaf and directs them toward the grain. In this study, the authors analyzed natural and sugar-induced (by pollination prevention) senescence in leaves and internodes using transcriptional and metabolic analysis. The authors found that similar senescence processes in leaves are later recapitulated in the internodes, supporting the hypothesis that leaf senescence is developmentally coordinated with other plant organs. Interestingly, in the non-pollinated plants, metabolic changes in leaves, such as increased carbohydrate content, can be detected a week after anthesis but global transcriptional reprogramming does not occur until 24 to 30 days after anthesis, revealing a substantial buffering capacity in the senescence process. However, differences between natural and sugar induced senescence were detected. A subset of senescence-associated genes (SAGs), ethylene, JA and salicylic acic biosynthetic genes were up-regulated in natural senescence, but unaltered in the sugar-induced senescence. This suggests there are some key differences between the two senescence induction pathways. Investigations such as this will help to identify new gene targets that control agronomically important traits where accurately timed senescence is critical - including stay green, days to harvest, and harvest index. *Thomas Slewinski 2013*

Shen M et al 2013. PLoS One 8:e57667. Leveraging non-targeted metabolite profiling via statistical genomics.

Editorial Comment. Metabolomic assays have great power to clarify biological processes, but identifying the compounds that correspond to thousands of mass spectra profiles and elucidating the relationships among the compounds are challenges. In this manuscript, Shen et al creatively utilize network analyses to characterize the maize seed metabolome. They collect over 8,500 mass spectra profiles from cooked, whole kernals from 210 diverse maize accessions and generate a metabolite network. Modularity is a common property of biological networks. Shen et al extract groups of metabolites, modules, with abundance levels that are highly correlated across the lines. The modules identified functionally related mass spectra, and the principle of guilt by association helped in the identification of unannotated mass spectra. The 210 accessions have been densely genotyped, and the authors also mapped loci that explain differences in modules across inbred lines. For example, SNPs associated with variants of an alpha-zein seed storage gene correlate with a module including a MS peak likely derived from the protein. This work's novel approaches provide important insights into the organization, diversity and genetic control of the seed metabolome. L*ewis Lukens 2013*

Strigens A et al 2013. Plant Cell Environ 36:1871-1887. Association mapping for chilling tolerance in elite flint and dent maize inbred lines evaluated in growth chamber and field experiments.

Editorial Comment. Chilling and cold temperature sensitivity limit the cultivation of maize in the Northern and Southern hemispheres, and breeding for chilling and cold tolerant varieties would increase yields in these areas. Here, Strigens et al investigate physiological and developmental traits in a large set of inbred lines from North American dent, European dent, and European flint germplasm collections. They assay plants in several field locations and in a controlled environment with a mild chilling stress. To identify alleles that explain genotypic variation, they associate SNPs with genotypes' trait values. Estimates of genotypic variance are significant for all traits measured in field and chamber. For most chamber traits, genetic variation is significantly higher in plants grown in ~14C than in plants grown in the control temperature, ~25C. Flints are considered more cold tolerant than dents, but growth of dents is not unusually affected by the chilling temperatures. Genotypic values for biomass related traits measured in the chamber are moderately correlated with field growth rates. The inbred lines have high linkage disequilibrium, and Strigens et al find a number of SNPs associated with photosynthesis and developmental traits. Many QTL correspond to candidate genes. One difficulty with genetic analyses of quantitative traits such as chilling tolerance is G x E. A genotype's performance in a chamber may not correlate well with the field, and a genotype's performance across different field trials may not correlate. This article discusses a number of interesting methods for data analysis. One nice example is the principle components analysis of extensive climactic information gathered from the multiple field sites. Components capture key environmental differences among the locations, and the authors correlate the allelic effects of QTL in each environment with the components from the PCA. Thus, they identify alleles that may be expressed in certain types of environments. *Lewis Lukens 2013*

Thurber CS et al 2013. Genome Biology 14:R68. Retrospective genomic analysis of sorghum adaptation to temperate-zone grain production.

Editorial Comment. Sorghum is crop species that is a close relative of maize and, like maize, was originally domesticated in the tropics but now widely grown in temperate environments. These similar life stories mean than much of the genetic diversity of both species is found in tropical environments that do grow well (or at all) in temperate environments like the American midwest. This paper takes advantage of a research program that has been increasing the genetic diversity available to temperate sorghum breeders by crossing NILs of diverse tropical sorghum landraces using a temperate adapted line as the donor parent and selecting for temperate adaptation in each round of backcrossing. The authors compared 580 "exotic progenitor" lines to temperate adapted lines derived from each progenitor using genotyping-by-sequencing. They identify fixed introgressions across all the temperate adapted NILs of known temperate adaptation loci including loci that regulate flowing time and dwarfism. Intriguingly they also identify regions of the genome where donor parent sequences have become fixed only within NILs derived from specific subpopulations of tropical germplasm. Overall this paper is another demonstration of how cheap genotyping is allowing biologists to study the genetic causes behind the variation breeders are selecting for. *James Schnable, 2013*

Udy, DB et al 2012. Plant Physiol 160:1420-1431. Effects of reduced chloroplast gene copy number on chloroplast gene expression in maize.

Editorial Comment. In early maize leaf development, chloroplastic DNA (cpDNA) accumulates to high levels. One reasonable explanation for this phenomenon is that a high cpDNA copy number generates abundant RNA that in turn facilitates the massive biogenesis of photosynthetic enzyme complexes. Here, Udy et al discover that mutations in a nuclear encoded, predicted organellar DNA polymerase are responsible for a number of *white2* (*w2*) maize mutants with pale green to white leaves. Plants homozygous for a weak *w2* allele have very pale yellow green leaves. Plants homozygous for a strong *w2* allele are albino. The weak and strong mutant alleles cause about a 5 fold and 100 fold decrease in chloroplast DNA copy number, respectively. Udy et al find that in early leaf development, a number of chloroplast encoded RNAs are reduced roughly proportionally to the DNA reduction. Thus, DNA copy number does appear to limit the abundance of a number of RNA transcripts. Nonetheless, other genes' transcripts are less sensitive to the loss of plastid DNA, and *rpoB* RNA is elevated despite DNA loss. The authors also investigate the abundance of photosynthetic enzyme complex subunits within the weak, *w2* mutant. The reduction of protein abundances within the mutant exceeds the reduction in chloroplast RNA abundance, indicating that different chloroplast RNAs interact synergistically to affect protein levels. Overall, this work paints an intriguing picture of how chloroplast DNA copy number affects chloroplast RNA, photosynthetic enzyme complexes, and leaf appearance. *Lewis Lukens 2013*

Vigeland MD et al 2013. New Phytol 199:1060-1068. Evidence for adaptive evolution of low-temperature stress response genes in a Pooideae grass ancestor.

Editorial Comment. Local adaptation is a potent driver of diversity. The grass family (*Poaceae*), consisting of ~10,000 species, first evolved ~70mya in a warm climate, but has since dispersed and adapted, such that today grasses are found from tropical forests to freezing Arctic and Antarctic ecosystems. Among the present day grasses, the *Pooideae* sub-family (a species rich group, including many important crop species) has been particularly successful in adaptation to cold climates. Here, Vigeland and colleagues investigate both the timing and the mechanism of adaptive evolution to low-temperature in the grasses using a phylogenomics approach. The authors make use of the complete genomes of maize, sorghum, rice and *Brachypodium*, and partial sequence of wheat, barley, *Lolium* and *Festuca*. After determining orthology relationships among gene models identified from the various species, they produce a phylogenetic tree for each group of orthologs. Subsequently, they identify those trees that contain "low-temperature" genes, as defined a priori on the basis of transcriptomic analyses. Examining low-temperature trees to determine rates of molecular evolution, and for evidence of positive selection, the authors conclude that adaptation to cold-stress occurred early in the evolution of the *Pooideae*, and, as such, may have played a key role in expanding the niche-range and in the development of many of our most important crops. *Ruairidh Sawers, 2013*

Wills D et al 2013. PLoS Genetics 9:e1003604. From many, one: Genetic control of prolificacy during maize domestication.

Editorial Comment. There are many common morphological shifts that take plant during plant domestication. One of the most prominent alterations, especially in grain crops, is the reduction in number and increase in size of the inflorescence - known as prolificacy. Wills et al explore the underlying genetic basis of this trait in maize using a genome-wide QTL scan of teosinte-maize recombinant inbred lines to identify causative regions responsible for the shift from 100 or more small ears in teosinte, to one or two large ears in maize. The authors show this trait has a relatively simple genetic architecture that can mostly be attributed to one large-effect QTL located on the short arm of chromosome 1. The causative genomic region maps to the promoter of the *grassy tillers1* (*gt1*), a transcription factor that functions in axillary bud outgrowth suppression. The maize *prol1.1* region causes a gain of expression and function of *gt1* in the nodal plexus of the meristem which leads a reduction in ear-forming axillary branches-conditioning the domesticated trait. Importantly, this study highlights how simple and subtle changes in gene

expression can cause dramatic shifts in the productivity and harvestability in plants. *Tom Slewinski 2013*

Xu Z et al 2013. Crop Sci 53:735-745. The realized yield effect of genetically engineered crops: U.S. maize and soybean.

Editorial Comment. Patenting and growing genetically engineered (GE) crops, as well as labeling the food products that contain them, have been at the forefront of recent political and legislative decisions in the US and many other countries. But what are the realized yield effects of GE on agricultural production? This is the issue that truly underpins their widespread adoption and use in the first place. Xu et. al. investigate whether and to what extent GE crops have impacted realized yields by analyzing historical data from non-irrigated maize and soybean in the U.S. from 1964-2010. They found that GE technology did confer an increase in realized yields, as well as a strong trend in yield growth in both crops. GE varieties of maize had the largest increase in realized yields, with the most significant gains in the Central Corn Belt. However, data from GE varieties of soybean implicate a slight yield drag. Overall these data highlight an encouraging trajectory for GE technology, mainly because first generation transgenic traits were designed to protect the yield and yield potential of their hybrid background, not increase yield per se. Although, the trends forecasted from these data suggest that projected yield gains made by first generation transgenic traits will fall short of the accelerated demands humans will put on agriculture. Thus new and novel GE traits, outside the realm of protection technology, will most likely be needed to boost yields beyond currently projected production capacity. *Tom Slewinski, 2013*

Zhai J et al 2013. Plant Cell 25:2417-2428. Plant microRNAs display differential 3' truncation and tailing modifications that are ARGONAUTE1 dependent and conserved across species.

Editorial Comment. Small RNAs are key regulators of many plant processes. For example, most plant miRNAs are bound by ARGONAUTE1 (AGO1), a key component of the RNA Induced Silencing Complex (RISC), that uses miRNA as templates to specify mRNA targets. The Arabidopsis *HUA ENCHANCER1 (HEN1)* encodes a methyltransferase that methylates the 3' end of sRNAs, including miRNAs. In *hen1* mutant backgrounds, the abundance of microRNAs (miRNAs) is reduced, and unmethylated miRNAs undergo 3' modifications including truncation and oligo-uridylation (tailing) that is associated with their degradation. In this work, Zhai et al deeply sequence sRNAs from wild type and hen1 mutants from Arabidopsis, maize, and rice. They find that in hen1 mutants, 3' ends of miRNAs have a diversity of truncated and tailed forms. Interestingly, the patterns of truncation and tailing differ across miRNA families but are broadly conserved across species- points that are nicely illustrated with graphical representations of counts of miRNAs with different truncation and tailed attributes. They also discover that AGO1 can bind truncated and tailed miRNAs, and an *ago1* mutation suppresses the 3' modifications of miRNAs in hen1. This finding indicates that the 3' miRNA modifications occur when miRNAs are in association with AGO1. Although the 3' truncation and tailing of miRNAs is relatively infrequent, the authors observe some similar patterns in wild type plants, and the work fundamentally contributes to an understanding of miRNA function and regulation. *Lewis Lukins, 2013*

Zhang J et al 2013. PLoS Genetics 9:e1003691. Generation of tandem direct duplications by reversed-ends transposition of maize Ac elements.

Editorial Comment. Comparative analyses using chip hybridization and genome re-sequencing have revealed a great number of copy number variants (CNVs) present among maize lines and varieties. While clearly widespread, many questions remain unanswered regarding CNVs, both in maize and other organisms. Notably, how are they formed, and what is their functional significance? Zhang et al address the former question, with a specific focus on tandem duplication. It has been hypothesized previously that tandem repeats are typically formed by non-allelic homologous recombination. Here, the authors suggest a further mechanism based on alternative transposition events catalysed by the termini of adjacent transposons. Through an elegant use of the kernel marker P1, the authors select and characterize molecularly a number of novel reciprocal deletion/duplication events. The authors go on to identify three further duplications in the B73 reference genome that bear all the hallmarks of formation following alternative transposition. Given the frequency of events uncovered in the study, alternative transposition may represent a specialized yet active modulator of genome size. *Ruairidh Sawers 2013*

Zhang X et al 2012. Plant Cell 24:4577-4589. Identification of PAN2 by quantitative proteomics as a leucine-rich repeat-receptor-like kinase acting upstream of PAN1 to polarize cell division in maize.

Editorial Comment. Polarized and asymmetric cell divisions underlie many aspects of plant development. Unequal cell division can give rise to daughter cells with distinct cell identities that govern subsequent cell fates - as in the case of stomata and flanking subsidiary cell formation in the maize leaf epidermis. Pangloss1 (PAN1) was previously identified as a kinase-dead Leu-rich repeat-receptor-like kinase (LRR-RLK) involved in stomatal development by promoting the polarization of the subsidiary mother cell (SMC) division towards the adjacent guard mother cell (GMC). In this article, the authors describe the identification, cloning and genetic interactions of *pangloss2* (*pan2*), a second gene that functions in polarization and development of the SMC. Using a combination of quantitative proteomics and bulk segregate mapping, the authors identified PAN2 as a second kinase-dead LRR-RLK. Localization and interaction studies revealed that PAN2 is required for, and co-localizes with

PAN1, although the two proteins do not directly interact. Along with the synergistic interaction observed in the double mutants, the authors propose that PAN2 acts first in the cascade of events that leads to SMC formation and functions cooperatively or is partially redundant with PAN1 to perceive or amplify positional cues from the GMC. Importantly, this study also highlights how new technologies, such as quantitative proteomics, can be integrated into forward genetic approaches in order to elucidate gene identity and function. *Thomas Slewinski 2013*

Zhang YF et al 2013. PLoS One 8:e67369. The requirement of WHIRLY1 for embryogenesis is dependent on genetic background in maize.

Editorial Comment. In this paper, Zhang et al report the cloning of the *emb16* mutant and the discovery that *emb16* is allelic to a previously cloned mutant, *why1*. *why1* encodes a RNA/DNA binding protein needed for genome stability and ribosome assembly in plastids. *emb16* in the W22 background is embryo lethal while *why1-1* in a different genetic background results in albino seedlings. Extensive molecular analysis of expression and protein levels suggest that *emb16* and *why1-1* are both null mutations, however, the *emb16/why1-1* complementation tests produce in a mixture of embryonic lethality and albino seedling phenotypes. These findings indicate that differences in genetic backgrounds can result in alternate phenotypes when the WHY1 protein is missing. Mutants lacking two other proteins needed for plastid protein synthesis also show background dependent phenotypes suggesting that some genetic backgrounds either do not require robust plasmid protein synthesis for embryogenesis or have unknown compensatory mechanisms. There is no apparent paralogue of WHY1 in any genetic background removing the simpler explanation that a hidden paralogue is compensating for the loss of WHY1. Additionally, while originally reported to localize to chloroplast, this paper reports that WHY1 also localizes to the nucleus. This paper serves as a reminder about the complexities of the maize genome and its evolutionary past and the impact it can have on mutant analysis. *Amanda Wright 2013*

IX. SYMBOL INDEX

X. AUTHOR INDEX

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 verbatim, promptly. Be concise, not formal, but include specific data, tables, observations and methods. Notes that require extensive editing will be returned. Notes are best provided as pdf, but other files are acceptable. Send your notes as attachments or as the text of an email 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 with authors' initials --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 tab instead of using spaces, to ensure quality tabulations on the web. Mailing address:

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Nos. 44 through 50	MNL 50:157
Nos. 51 to date	Annual in each issue
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Nos. 12 through 35	Appendix to MNL 36, 1962 (copies available)
Nos. 36 through 53	MNL 53:153
Nos. 54 to date	Annual in each issue
Stock Catalogs	Each issue, updates only after No 78, and MaizeGDB
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**
Consensus Linkage Maps	Bins, Genetic, IBM2 Neighbors see **MaizeGDB**
Plastid Genetic Map	MNL 69:268; Maier RM et al 1995 J Mol Biol 251:614.
Mitochondrial Genetic Maps	MNL 70:133; 78:151; Clifton et al 2004 Plant Physiol 136: 3486.
Newly characterized genes	**MaizeGDB** *[new and updated gene records]*

Cooperators (that means you) need the Stock Center.

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(1) **Contact Carson Andorf 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.**
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(4) **Contribute to the maize gene review wiki.** Add your favorite gene to the maize gene wiki: http://maizegenereview.maizegdb.org/doku.php. These data will be linked to MaizeGDB with credit provided to contributors.
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