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Chromosome Breaking *Ds* Sites in Maize, Revisited. Part I, Background, Methods, Description

--Neuffer, MG

This past year I received some supplemental funding (via NSF grant 0743804, with many thanks to Carolyn Lawrence and Mary Schaeffer for their help in this process) to update and revise information on my maize mutant collection that I had submitted to the Stock Center and to MaizeGDB back in 1995. A second objective was to provide information and stocks on a new dominant mutant collection I have generated in the past few years, with the generous support of Sarah Hake. In the process of organizing the data I decided it would be a good time to revisit a collection of *Ds* marker stocks that I had generated. The purpose of this Newsletter article is to combine published information (Neuffer 1994, 1995) about the *Ds* markers with the images and case descriptions already posted at MaizeGDB (http://www.maizegdb.org/), and to provide a general review of the uses and expression of these stocks. This text portion will be supplemented by information in Part II, where we use photographs of the stocks to illustrate our discussions of the proven cases. Part II is planned to be posted at Maize Gene Review (http://maizegenereview.org/) concurrently with this article, and the original data at MaizeGDB will be updated at the same time. I wish to thank Lou Butler for assistance in gathering the data, editing the text, preparing the artwork, and formatting this material for posting online. Thanks also are extended to Kelly Dawe and Hugo Dooner for reading the material and providing many helpful suggestions.

Background of Marker Stock Development:

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 (Table 1).

The transposable element *Ds*, discovered and analyzed in detail by McClintock (1951), is the responding element of the *Ac Ds* system. It has the unique property of being able to move about (transpose) in the genome when *Ac* is present. Thus, sites throughout the genome can be selected for further genetic analysis. At the resident site *Ds* can also suppress the function of an associated active gene and/or cause a break in the chromosome at that site, initiating the breakage-fusion bridge-cycle described by McClintock (1941; for diagram see http://profiles.nlm.nih.gov/LL/B/B/R/S/) of sequential chromosome breaks with associated losses and consequent gain of genetic material in the daughter cells of a mitotic or meiotic division. Mutations induced by *Ds* at the gene site are observable when they interfere with the gene's function, producing a recessive null phenotype. This loss of genetic function in a heterozygote with *Ds* on the homolog with the dominant allele allows a recessive allele to be uncovered as a chimera of recessive tissue. In some cases *Ds* acts as both breaking and suppressing. If the breakage feature is associated with suppression of the resident gene, this can affect observation of the genes used to mark chromosome loss.

It is complicated to interpret results from *Ds* experiments. The state of activity, variations in *Ac* dosage, and other genetic modifiers can all lead to variation at the insertion or neighboring site. Moreover, the characteristics of each variation depend on the marker used, the relative position of *Ds* and the marker on the chromosome arm, and on other types of genetic modifiers.

Originally we intended to find a site between the marker and the centromere, so that the marker would be lost as an early *Ds* event. It became clear, however, that the size, frequency, and characteristics of sectors shown at each new *Ds* site were related to the position on the chromosome arm relative to the marker used. In fact, expression was related to the position of three components: *Ds*, the marker, and the centromere. This position could be determined by the type of loss pattern in the kernel. Location on the distal side of the arm from the centromere was observable as frequent, small sectors, associated with twin duplicate-deficiency spots. Proximal location was characterized by frequent large sectors along with normal tissue in single dots, or dots in chains or clusters within the large deficient sector. Location at the gene site was usually accompanied by suppression of the gene function to produce the equivalent of the recessive or null allele that was exceptionally unstable when *Ac* was present, producing frequent reversions to some level of mutant gene expression. These appeared as sectors, usually dotted, of normal tissue on the null kernel background or elongated dominant streaks of red on the pericarp and anthocyanin on normal green plant leaves.

Methods and stock preparation:

The original *Ds1* site described by McClintock (1951) was located proximal to *Wx1* on the short arm of chromosome 9. My stock of this original *Ds1* material has been termed *Ds-9S1*. Dr. Jerry Kermicle generously provided two additional stocks that were presumed to carry the *Ds1* site on the long arm of chromosome 10 near the *g1* locus; these were used to generate the remaining *Ds* stocks. One stock (*P1-vv, Ds-10L2 R1-sc* with *Ds* 10 cM proximal to *g1*) carried *Ds* on the long arm of chromosome 10, proximal to the centromere from the *R1-sc* allele at the *R1* locus and the other stock was *P1-vv*, *Ds-10L4 R1-sc* with *Ds* in the same general region. The pollen source was *P1-vv*/*P1-wr*, *Ds-10L2 R1-sc*/*Ds-10L2 R1-sc* or *R1-r* and homozygous dominant for all the markers in the tester stocks used except chromosome 2S (see below). Heterozygous *P1-vv* was used in order to retain the early large sector properties of 1 dose of *Ac*, thus increasing the likelihood of gametic events. The probability of capturing duplicate events from a single large tassel sector was minimal since cases were used from trials in two or more seasons and by selecting for visually different characteristics. All the genes used as marker stocks were present as dominant alleles in the pollen stock, except for chromosome 2S, where a special pollen stock was prepared using the dominant aleurone color allele *B1:Peru*. Since *B1:Peru* and *R1-sc* are duplicate factors, we used a *b1 r1* tester. Changes of aleurone color *B* function would appear as a color change on ears with purple kernels.

The *Ac* used was the one associated with *P1-vv* and in many of the *Ds* cases *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.

Vigorous testers homozygous for the appropriate recessive aleurone, endosperm, and seedling markers were prepared. If the tester mutant was lethal (*dek1*, *w3*, *o5*) normal kernels from a segregating F2 were used. Of these, 2/3 would be heterozygous (1/2 correct gametes), and 1/3 would be discarded as homozygous normal. For each tester, 100 or more plants were used as female parent. These were grown in an isolated, open-pollinated, detasseled plot according to the method of Stadler (1946). The ears were marked for each of the 16 chromsome arms that carried a usable marker. They were examined for two types of single kernel events: (1) Transposition of *Ds* near the marker and the centromere. Depending on the particular tester stock, mosaicism or sectoring for purple vs. colorless or bronze aleurone, and normal vs. shrunken, brittle or collapsed endosperm would reflect chromosome breaking activity in the endosperm, and many would be expected to have the same activity in the embryo thereby transmitting it to the next generation. (2) Transposition of *Ds* to the marker site which would suppress the dominant allele. This would result in a recessive mutant case with dominant revertant sectors due to the presence of *Ac*. Those without dots and revertant sectors are due to the absence of *Ac* because of chance segregation in gamete formation. They were considered potentially valid recessive mutable cases that could potentially show their mutability when recombined with *Ac*.

Single kernel cases were planted and observed for any variations from normal plant phenotype. Any kind of sectoring was especially noted as potentially indicating *Ds* activity in the plant. Plants were selfed and backcrossed to their respective tester for confirmation of *Ds* activity. Stable recessive cases were crossed to an *Ac* stock to test revertability.

Seedling markers were used in cases where endosperm markers were not available for that chromosome. All kernels were planted in sand benches and examined for seedlings of two kinds: 1) whole seedling mutant cases with multiple recessive sectors (*Ac*) and without sectors (no *Ac*); and 2) normal green seedlings with chimeras of recessive tissue for the marker used. Observed cases were transplanted to pots and were grown to maturity. The mutant seedlings for lethal phenotypes died, except when there was adequate revertant tissue to support plant growth. Those seedlings that survived to maturity were selfed and backcrossed to the recessive tester to confirm.

Many putative cases were observed in most of the tester stocks included in the crosses. Any that survived were grown to maturity and tested. The precision of observations varied greatly due to variation in the mutant. The aleurone color stocks gave excellent mosaic kernels with colorless or bronze sectors on part of the endosperm or aleurone layer (which is a part of the endosperm), and those with shrunken or brittle endosperm were also fairly good. However, the collapsed and opaque endosperm cases were difficult to recognize and many ambiguous cases were tested, most of which failed confirmation. Losses of other kernel cases to normal field conditions forced us to abandon any efforts at quantitative measurement of the frequency of events involving markers on individual arms.

The Non-transmitting cases:

There were 79 kernel cases which failed to transmit; these were thought to be misinterpretations or nontransmission. However, recent reconsideration of these cases led to another interpretation. The non-transmitting cases should have been included, because we now know that losses occur as a consequence of nuclear division separating unequal products. We originally looked only for those cases where losses were apparent or where the product was associated with a mutant phenotype. The other product of the event would be a duplication or some other variation that often did not have an immediate phenotype. These cases should have been examined for unusual phenomenon and/or delayed expression. Photographs were taken of all of these cases and data from these apparent non-corresponding cases will be revisited to consider what evidence we still have and what that evidence points to. This information will be presented in a later publication.

Observations:

Usually *Ds* showed a chromosome breaking property, but cases of suppression, either with or without breakage, were also observed. *Ds* losses appeared to occur much sooner in the kernel (larger sectors) than in the seedling (smaller sectors). This observation, however, could merely reflect the observed relative maturity of the respective tissues. Determining whether a mutable allele due to suppression was also a chromosome breaker related largely to the properties of the marker gene. For example, identifying twin spots required different dosage levels of gene expression. As we accumulated chromosome breaking *Ds* cases for arms using aleurone color markers we observed two major types of aleurone color mosaics: large mutant sectors indicated early breakage events, while smaller sectors indicated later events. These were distinct from timing changes in *Ds* events resulting from changes in *Ac* dosage, because our material usually had only one dose of *Ac* from the male parent and therefore should have had only large sectors. This was not always true, as other factors such as genetic modifiers and an unidentified independently segregating *Ac*, which occasionally appeared in the stocks used.

Several types of *Ds* cases were observed. There was a high frequency of large sectors. According to a personal communication from Dr. Jerry Kermicle, the original *Ds-10L2 R1-sc* case similarly displayed a high number of large sectors. The kernels appear more colorless than colored due to the large amount of tissue with lost gene function. The *Ds* site was shown to be proximal (between the marker and the centromere). We also observed an unusual mosaic pattern, which we originally thought was cases of parental colored tissue with repeated subsequent loss of color. However, these were actually found to be chains, clusters and/or

islands of colored tissue (Figures 1, 2) which we interpreted to be retention of the functional gene in acentric fragments. These fragments were carried along in daughter cells as clones from the initial break. The clusters, chains and islands were as a rule more intensely pigmented for those markers having a dilution dosage effect (i.e., *C2*, *R1-scm*, *B1:Peru*), to be expected because the acentric fragment from a proximal break would carry duplications for the gene being followed. These observed islands of normal tissue within a sector of mutant tissue were similar to those seen many years ago by L.J. Stadler and also by me, his student. We were performing experiments involving radiation-induced color losses and were unable to interpret the meaning of the spots. Stadler called these islands "recovery spots" (Figure 2). We observed that the frequency and size of these recovery spots depended on the chromosome arm and the proximity of the marker, and therefore the *Ds* site to the centromere. It appears that short acentric fragments are not retained or "recovered" as frequently as long ones.

Other *Ds* cases displayed a relatively high frequency of medium and small sectors, and a low frequency of large sectors. For genes with a dosage effect on aleurone expression (for example, *Ds-4L6* with a *C2* marker, and *Ds-1S3*), the mosaic kernels were dilute (depending on the dosage threshold for gene expression of the gene studied) with a few large sectors and many small colorless sectors or patches scattered randomly over the aleurone layer. Considering that only one *Ac* was present, these were rather late events. The small patches ranged from about 1/32 of the kernel surface to those composed of only a few aleurone cells. Large sectors could cover from 1/4 to 1/3 of the aleurone surface, but these were rare.

Frequently, the colorless patches were angular and sharply outlined, and were edged by a smaller and intensely pigmented sector. The smaller sector was clearly on the dilute side of the border rather than on the colorless side. These spots of colorless and intense pigmentation arising from a dilute background were termed twin spots (Figure 3). McClintock (1941) explained these spots to represent a deficiency and duplication for the marker gene which occurred as a result from a break in the chromosome distal to the marker. The broken ends then rejoined, forming a bridge at mitotic anaphase with two copies of this marker. Then, a second non-median break occurred at telophase such that both copies of the gene went to one daughter cell and none to the other.

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Table 1:Transposition sites for chromosome breaking *Ds* stocks.

Symbol	Marker	Position
Ds-1S1	*Dek1*	distal
Ds-1S2	*Dek1*	probably distal
Ds-1S3	*Dek1*	distal
Ds-1S4	*Dek1*	proximal
Ds-1L1	*Bz2*	proximal
Ds-1L2	*Bz2*	at the *Bz2* (*bz2-m3*) locus
Ds-1L3	*Bz2*	at the *Bz2* (*bz2-m3*) locus
Ds-1L6	*Bz2*	at the *Bz2* (*bz2-m*) locus
Ds-2S1	*B1:Peru*	distal
Ds-2S2	*B1:Peru*	unknown
Ds-2S3	*B1:Peru*	at the *B1:Peru* (*b1-m1*) locus
Ds-2S4	*B1:Peru*	at the *B1:Peru* (*b1-md2*) locus
Ds-2L1	*W3*	unknown
Ds-3L1	*A1 Sh2*	proximal
Ds-3L2	*A1 Sh2*	proximal
Ds-4S1	*Bt2*	unknown
Ds-4S2	*Bt2*	unknown
Ds-4L1	*C2*	distal
Ds-4L3	*C2*	at the *C2* locus
Ds-4L4	*C2*	distal
Ds-4L5	*C2*	distal
Ds-4L6	*C2*	distal
Ds-4L7	*C2*	distal
Ds-5S1	*A2*	proximal
Ds-5S2	*A2*	proximal
Ds-5L1	*Bt1*	distal to *Bt1* and proximal to *Pr1*
Ds-7L1	*O5*	distal
Ds-7L2	*O5*	proximal?
Ds-7L3	*O5*	proximal
Ds-8L1	*Pro1*	unknown
Ds-9S1	*C1*-I	proximal?
Ds-9L2	*Dek13*	unknown
Ds-10L2	*R1-sc*	proximal
Ds-10L4	*R1-sc*	proximal
Ds-10L5	*R1-sc*	*Ac* at the *R1* locus
Ds-10L6	*R1-sc*	*Ac* at the *R1* locus

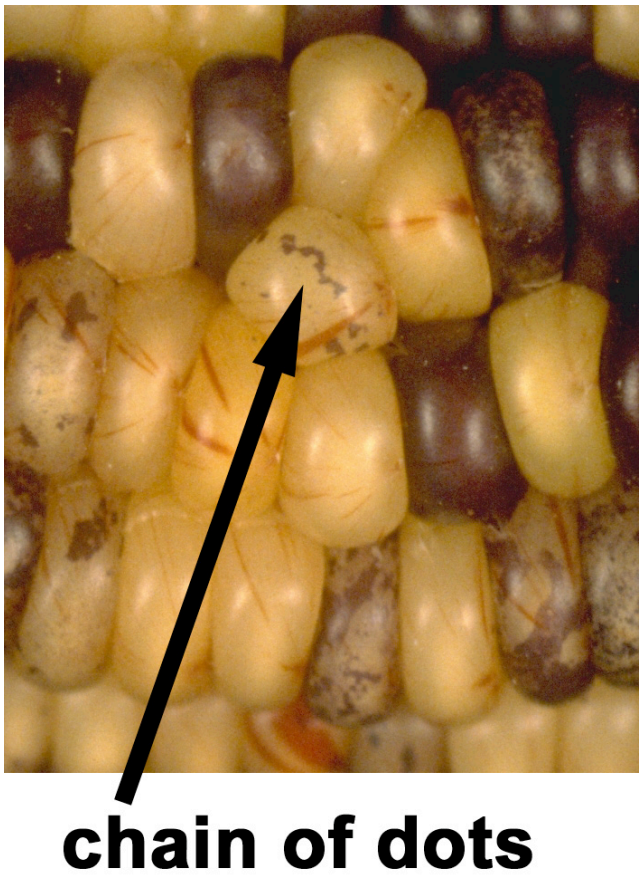

Figure 1: A good example of chains of dots.

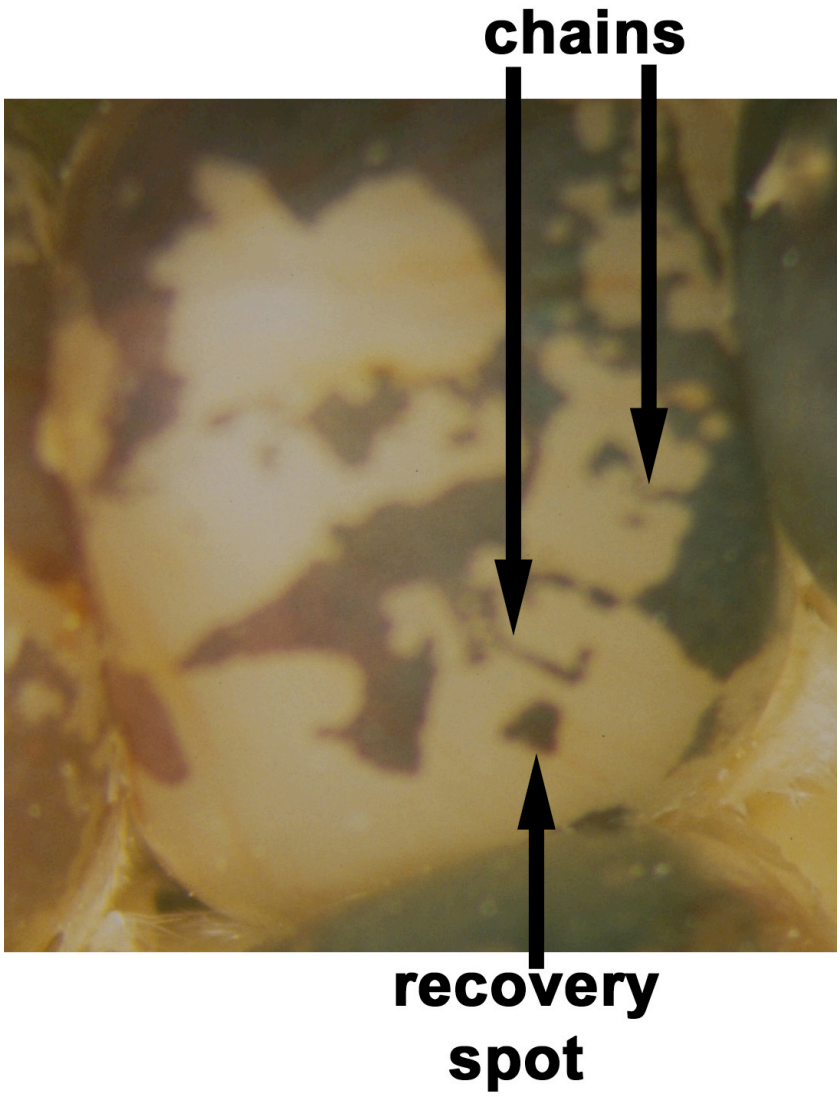

Figure 2: Two examples of chains and an example of a recovery spot.

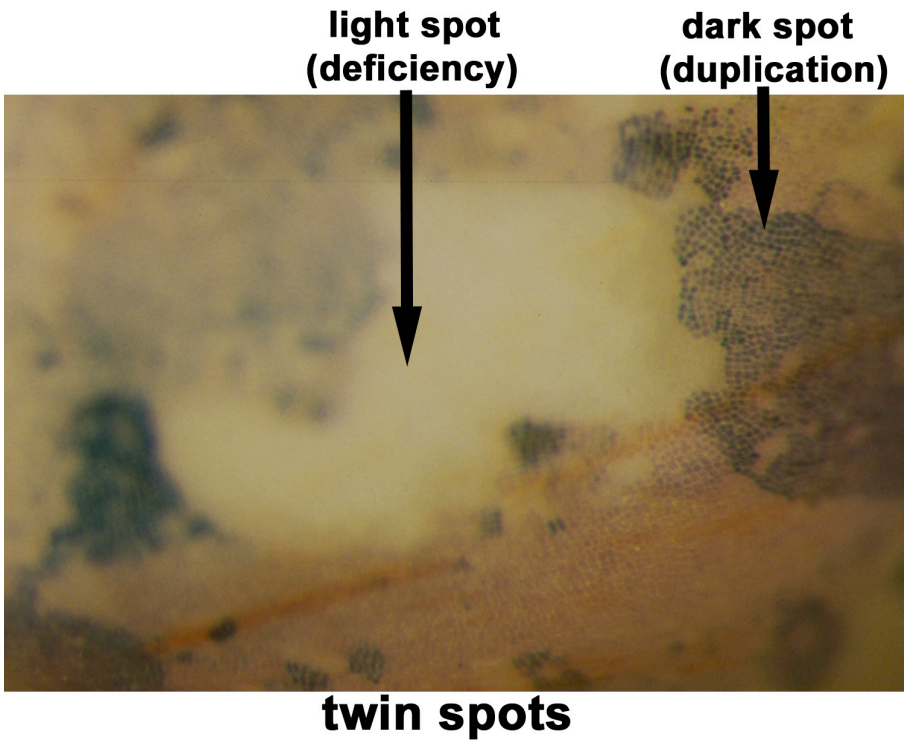

Figure 3, typical expression of twin spots.