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Studies of Dotted, a Regulatory Element in Maize[1,2]

I. Inductions of Dotted by Chromatid Breaks

II. Phase Variation of Dotted

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Summary. Dotted (*Dt*) is the regulatory element of a two-unit controlling system in maize. *Dt* causes the inherited change from the recessive a_1 (colorless) to its dominant allele, A_1 (anthocyanin production), during the development of the stalk, leaves, and endosperm. The mutation events are observed as sectors of color in an anthocyaninless background.

One of the most puzzling, but perhaps significant, aspects of controlling elements in maize is that they originate in conjunction with chromosome or chromatid breaks. This fact invokes a requirement that either an existing regulatory mechanism is disturbed by the breakage or that a foreign element is incorporated before fusion of the broken chromatids.

Experimental crosses were made between *Dt* tester stocks and a pollen parent, a large proportion of whose chromosomes 9 were undergoing the chromatid type of bridge-breakage-fusion cycle. New *Dt*'s were induced in endosperm sectors of 250 of 154,422 kernels tested (1/600); among these, two germinal *Dt*'s (Dt_4^{crown} and Dt_5) were recovered, presumably due to chromatid breaks during meiosis or the first microspore division. Dt_5 produces a mutation pattern very similar to the original Dt_1 and is located 0.33 crossover units away from the yg_2 locus. This is close to the known location of Dt_1 (7 crossover units distal to the yg_2 locus) and is suggestive of a specific site for *Dt* inductive breaks. Dt_4^{crown}, on the other hand, is inherited independently of the yg_2 locus and does not support this contention. Dt_4^{crown} represents a new "state" causing a high concentration of fine dots in the crown of the kernel, with little or no dotting at the base.

The phase variation of Dt_4^{crown} is discussed together with the tissue-dependent expression of $Dt_1^{in\text{-}ac}$ (Dotted, inactive-active). $Dt_1^{in\text{-}ac}$ is a new "state" of Dt_1 and shows inactive (no a_1 to A_1 mutations) and active (a_1 to A_1 mutations) phases in the endosperm, whereas it is only in the active phase in the diploid scutellum. The observed phase variation was shown to be a property of the regulatory elements, *Dt*, responding to differences in the cellular environment.

Dotted (*Dt*) is believed to represent the regulatory element of a two-unit system of control in maize that affects the expression of A_1, a gene required for anthocyanin pigmentation. In a_1 stocks, by analogy with more thoroughly studied two-unit systems (McClintock, 1965), a controlling element at the A_1 locus prevents expression of the dominant phenotype; removal of this element by activity of *Dt* results in sectors of color (A_1 phenotype) represented by purple dots in the colorless endosperm and scutellum and by purple stripes in the brownish stalk and leaf tissue. Dt_1 first appeared in Black Mexican sweet corn (Rhoades, 1936), and in a later study (Rhoades, 1938), its interaction with the a_1 allele provided the first demonstration of the genetic control of mutability. Two other *Dt*'s, presumably of independent origin, found in South American maize varieties were designated Dt_2 and Dt_3 (Nuffer, 1955). Dt_1 was located in or adjacent to the terminal heterochromatic knob of the short arm of chromosome 9, seven crossover units from yg_2 (yellow green seedling) (Rhoades, 1945). It has been used extensively in genetic studies and has consistently behaved as a standard Mendelian marker of that locus.

A Dotted phenotype was experimentally induced in $a_1\ a_1$ stocks known to be *dt dt* by breakage of chromosomes 9 during a bridge-breakage-fusion cycle (McClintock, 1950 and 1951a). Of the 93,078 kernels examined for *Dt*-like control of a_1, 117 had one or more A_1 dots of color. These dots were usually restricted to sectors of the aleurone; a single kernel exhibited the *Dt* phenotype throughout the aleurone layer, but the plant grown from this kernel did not contain *Dt*. This indicated that the breakage event responsible for the origin of *Dt* had occurred during the second microspore division and that only the sperm uniting with the polar nuclei possessed the new *Dt*.

The recovery of germinal inductions of *Dt* would provide an opportunity to test whether the origin of *Dt* depends on a break in one particular region of the short arm of chromosome 9 or whether the position of the break is unimportant. Therefore, a larger-scale experiment was performed in a search for induced heritable *Dt* factors. Two new *Dt*'s were recovered and analyzed. One of the isolates exhibits the typical *Dt* phenotype, but the other represents a new ex-

[1] In appreciation of his help and guidance, I dedicate this article to Professor Marcus M. Rhoades on his birthday.

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pression of *Dt* ("state") having phases of activity and inactivity. The behaviour of the latter element is discussed together with a consideration of $Dt_1^{in\text{-}ac}$, a new allele of Dt_1 that shows similar fluctuations of activity.

Materials and Methods

Genetic stocks: 1. Duplication 9/Deficient 9 (Dp 9/Df 9). An $A_1\ A_2\ C\ R$ (dominant factors required for colored aleurone) stock possessing a chromosome 9 carrying a reverse tandem duplication of the entire short arm and a homologue deficient for the terminal portion of the short arm including the *C* locus was provided by Dr. Barbara McClintock (Fig. 1). A deficient second stock carrying a similar deficient chromosome 9 was supplied by Dr. Marcus M. Rhoades. The duplication and deficient chromosomes 9 were marked by *wx* (waxy endosperm), and no *Dt* was present. An a_1 allele was introduced into the duplication 9 stock by crossing an $A_1\ A_1\ dt\ dt$ pollen parent heterozygous for the duplication and deficiency to $a_1\ a_1$ *dt dt* egg parents heterozygous for normal (*C Wx*) and terminally deficient (*−wx*) chromosomes 9. Fully colored (*C*), waxy (*wx*) kernels were grown, and the resulting $A_1\ a_1$ plants were used to pollinate $a_1\ dt$ (or $a^m - 1\ dt$) egg parents; other $A_1\ a_1$ heterozygotes were self-pollinated, $a_1\ a_1$ progeny grown, and individuals selected to pollinate additional $a_1\ dt$ (or $a^m - 1\ dt$) plants.

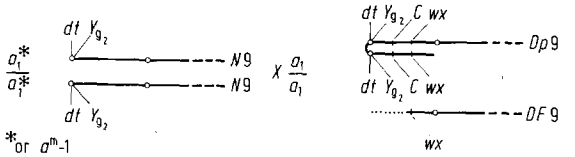


Fig. 1. Diagram of the cross used to study the effects of the bridge-breakage-fusion cycle on the induction of *Dt*. Symbols: a_1 and a^m–1, colorless aleurone, respond to *Dt* by mutating to A_1; Yg_2, normal allele of *yg* (yellow green seedling); *C*, colored aleurone; *wx*, waxy endosperm; N 9, normal chromosome 9; Dp 9, reverse tandem duplication of the short arm of chromosome 9; Df 9, terminally deficient chromosome 9 including *C*. There is a short piece of chromatin between the two heterochromatic knobs of the duplicated short arm of Dp 9

2. $a_1\ dt$ testers: The standard $a_1\ dt$ stock was supplied by Dr. Marcus Rhoades. An $a^m - 1\ a^m - 1\ dt\ dt$ stock was given to me by Dr. Gerald Neuffer. Both the a_1 and $a^m - 1$ alleles (chromosome 3) respond to *Dt* by producing mutant colored sectors ("dots") in an otherwise colorless background in the aleurone of the maize kernel. These alleles differ in their response to *Dt* in that $a^m - 1$ mutates to A_1 at a much higher frequency than does a_1 (Nuffer, 1961), thus giving a greater assurance of detecting a newly induced *Dt*.

3. $a_1\ dt\ yg_2$ tester: This tester was used in linkage studies of the induced *Dt*'s. Yellow green (yg_2) is seven crossover units proximal to Dt_1 in the short arm of chromosome 9 (Rhoades, 1945). The stock was provided by Dr. Neuffer.

4. *c sh wx* (colorless aleurone, shrunken endosperm, waxy endosperm) tester. Plants homozygous for these genes were used as female parent in crosses with putative Dp 9/Df 9 test plants, which were simultaneously used as pollen parents in crosses to $a_1\ dt$ and $a^m - 1\ dt$ testers. Confirmation of this chromosome 9 constitution was obtained genetically when about 70 per cent *C-c* variegated kernels were recovered from the cross of a Dp 9/Df 9 pollen parent to a *c sh wx* egg parent. Plants having the duplicated 9 heterozygous with a normal 9 gave a much lower frequency of variegated kernels.

5. $a_1^s\ Dt_1$ and $a_1^s\ dt$ testers: These stocks were supplied by the Maize Genetics Cooperation at the University of Illinois, Urbana. The a_1^s allele of a_1 does not respond to *Dt* (Rhoades, 1941).

Cytological examination of chromosomes: The chromosome 9 constitution of certain plants was determined cytologically where noted in "Results". Sporocytes were fixed in a 3 parts 95 per cent ethanol: 1 part glacial acetic acid mixture and, after two days, were stored in a freezer. The chromosomes were examined at pachynema in acetocarmine squashes.

Results

1. *The Induction of two new Dt's by Chromatid Breaks in Chromosome 9*

The genetic cross diagrammed in Figure 1 was used to study the induction of *Dt*'s by the chromatid type of bridge-breakage-fusion cycle. The meiotic events involving the Duplication 9 (Dp 9) and Deficient 9 (Df 9) homologues that lead to production of a bridge-breakage-fusion cycle in the mitotic divisions of the gametophyte and endosperm have been described (McClintock, 1951a). Pollen grains containing the deficient chromosome 9 are not functional; those bearing a 9 with the intact duplication compete unfavorably with pollen possessing a chromosome 9 with less redundancy derived by breakage of a dicentric bridge after crossing over in the duplicated region. Thus, high percentages of functional pollen grains contain a chromosome 9 undergoing the bridge-breakage-fusion cycle; after fertilization, the cycle continues during the nuclear divisions of endosperm development, but ceases in the embryo. A photograph (Fig. 2) of a Dp 9/Df 9 bivalent at pachynema

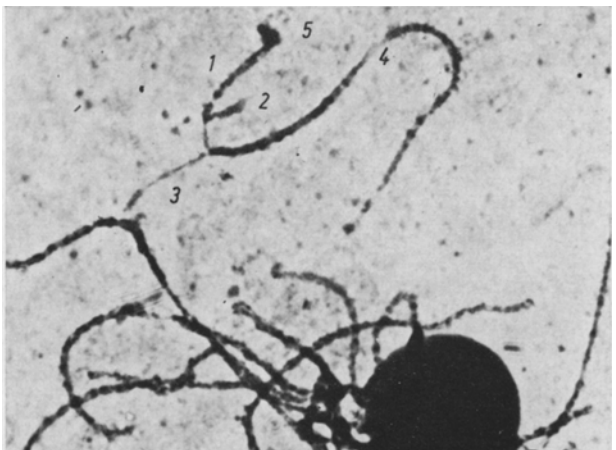


Fig. 2. Photograph of a duplication / deficient bivalent chromosome 9 at pachynema of meiosis. In this configuration the duplicated portion is paired with the homologous proximal portion of the same chromosome (1) except for the end which shows a non-homologous fold back (2). The deficient 9 is unpaired with its homologue throughout most of the short arm (3). The long arms of the duplication 9 and deficient 9 are paired normally (4). Note the pairing of the two knobs (5) (See Fig. 1)

shows one of the several pairing configurations observed in these plants. Among 11 pollen parents in which the chromosome 9 constitution was cytologically confirmed, (1129—1133, Table 1), nine contained a chromosome 9 with the reverse tandem duplication of the complete short arm, whereas two (1130-3 and 1131-5) had a less extensive duplication. The homologue in all instances was a chromosome 9 deficient for the terminal segment of the short arm. Most of the functional pollen grains in the Dp 9/Df 9 heterozygotes possessed a chromosome 9 that had undergone the bridge-breakage-fusion cycle. When these plants were used as pollen parents on a recessive *c* sh_1 *wx* tester, about 70 per cent of the resulting kernels were variegated (with the exception of plant 1132-2, which had a lower frequency). The chromosome 9 constitution of 13 of the remaining 16 pollen parents (Table 1) was not tested, but subsequent results indicate that they were Dp 9/Df 9.

Table 1. *Origin of Dt by the bridge-breakage-fusion cycle. Pollen parents of Dp 9/Df 9 dt dt* a_1 a_1 *or Dp 9/Df 9 dt dt* A_1 a_1 *constitution were crossed with* a^m — 1 a^m — 1 *dt dt or* a_1 a_1 *dt dt female parents. The* a_1 a_1 *or* a_1 a^m — 1 *kernels were scored for sectors of dotted aleurone. The chromosome 9 constitution of the pollen parent was determined cytologically (where noted) and by crossing with c* sh_1 *wx female parents*

Pollen parent	Chromosome 9 constitution	Frequency of kernels with C-c variegation when ♂ parent crossed with *c* sh_1 *wx* ♀*	♂ parent crossed with a_1 *dt* or a^m — 1 *dt* ♀	
			No. of kernels with 1 or more dots	Total No. of a_1 kernels
1445-8	—	—	4	2420
1445-12	—	—	3	1320
1447-1	—	—	1	1760
1447-8	—	high	2	770
1447-9	—	—	1	990
1447-11	—	high	1	660
1448-7	—	high	1	550
1449-4	—	—	—	1650
1449	—	—	2	1650
1449-7	—	—	1	1320
1450-1	—	—	2	990
1450-7	—	—	1	880
1451-2	—	—	4	1540
1451-3	—	—	1	1980
1451-7	—	—	1	770
1452	—	—	2	1100
1129-4	Dp/df	high	16	11,650
1129-5	Dp/df	high	0	400
1129-6	Dp/df	high	32	13,932
1130-1	Dp/df	high	26	13,275
1130-3	2/3 Dp/df	high	4	1,100
1130-4	Dp/df	—	44	18,250
1131-1	Dp/df	—	16	7,750
1131-3	Dp/df	high	46	10,250
1131-5	1/2 Dp/df	high	1	1,750
1132-2	Dp/df	medium	12	20,800
1133-6	Dp/df	—	26	34,775
		Total	250	154,422

* low = less than 25%, medium = about 50%, high = about 70% of the kernels (by inspection only).

Of 154,422 a_1 (or a^m — 1) kernels, 250 had sectors of *Dt*-like mutability (Fig. 3). The slightly higher rate of *Dt* induction compared with that observed by McClintock (1951b; 117/93,078) is attributed to the use of the highly mutable a^m — 1 allele as the indicator of *Dt* activity in these experiments. Three kernels had dots throughout the aleurone. It is unlikely that these three were the result of contaminant *Dt* pollen because the crosses were made in an isolation plot containing only *dt* plants.

Plants were grown from the three fully dotted kernels and tested for the presence of a heritable *Dt*. Two of the plants proved heterozygous for *Dt* and transmitted the character in a 1:1 ratio, both as ear and pollen parent. These new isolates were designated Dt_4 and Dt_5 (Fig. 4) because the symbols Dt_2 and Dt_3 were assigned to *Dt* genes found in South American maize (Nuffer, 1955). Both were crossed to an a_1 *dt* yg_2 tester, and the F_1's were backcrossed to the same stock. The testcross segregations are shown in Table 2.

The newly induced Dt_5 is clearly very close to the yg_2 locus, there being only 0.33% crossovers between them (Table 2). The completely colored kernels are

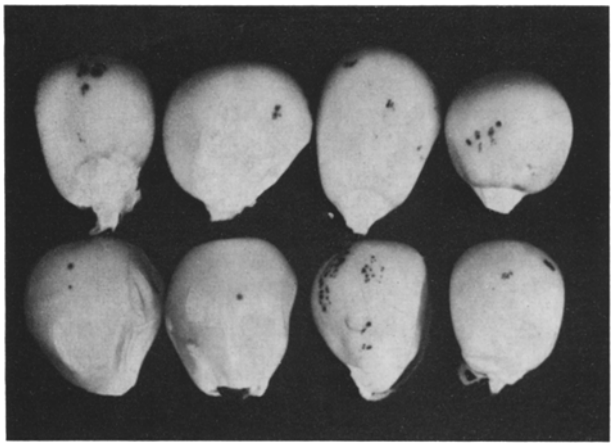


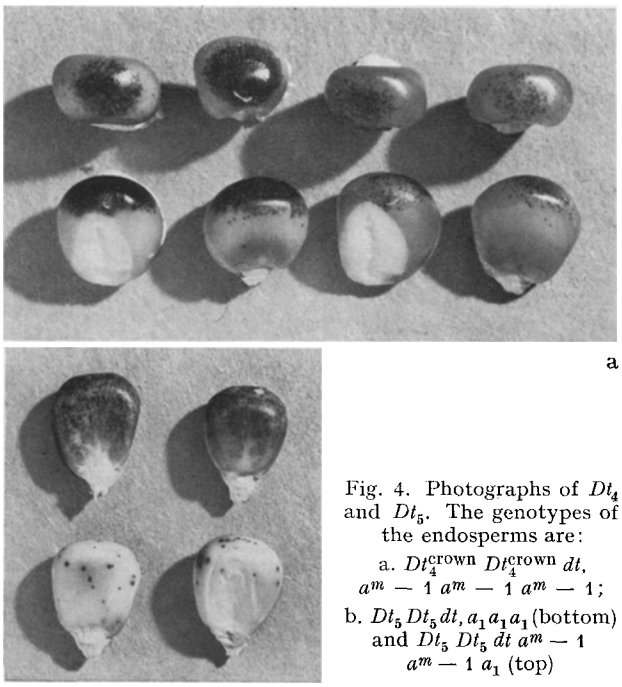
Fig. 3. Inductions of *Dt* by the bridge-breakage-fusion cycle. The first two kernels of the lower row were from the cross, $a_1a_1dtdt \times a_1a_1dtdt$ Dp 9/Df 9; the remainder were from the cross, a^m-1 a^m-1 *dt dt* × a_1 a_1 *dt dt* Dp 9/Df 9. One or more sectors of *Dt*-like mutability can be observed in each kernel

Table 2. *Linkage of Dt_4 and Dt_5 to yg_2 on chromosome 9. Testcross progeny from F_1 plants of $\frac{(Dt_4 \text{ or } Dt_5)}{dt}\frac{Yg_2}{yg_2}$ constitution pollinated by $\frac{dt}{dt}\frac{yg_2}{yg_2}$ plants*

	Ear	*Dt Yg*	*Dt yg*	Colored *Yg*	Colored *yg*	*dt Yg*	*dt yg*	Total
Dt_4:	1	91	96	14	13	105	91	410
	2	78	80	8	8	98	94	366
	3	77	91	5	7	88	84	352
	4	68	74	17	12	104	96	371
	5	71	76	13	17	105	68	350
	6	82	91	9	6	106	105	399
	7	49	45	13	7	72	69	255
	8	102	94	0	0	76	77	349
Totals		618	647	79	70	754	784	2852
Dt_5:	1	122	0	20	1	1	149	293
	2	113	1	14	0	0	132	260
	3	135	0	42	2	1	153	333
	4	173	1	11	0	1	195	381
	5	170	0	7	0	0	157	334
	6	128	0	0	0	1	147	276
Totals		841	2	94	3	4	933	1877
Corrected totals*		937.77	2.23	—	—	4	933	1877

Percentage crossing over in Dt_5-yg_2 region $= \frac{6.23}{1877} = 0.33$ map units

* assumes that all fully colored kernels are derived from Dt_5 activity.



a

b

Fig. 4. Photographs of Dt_4 and Dt_5. The genotypes of the endosperms are: a. $Dt_4^{crown}\ Dt_4^{crown}\ dt$, $a^m - 1\ a^m - 1\ a^m - 1$; b. $Dt_5\ Dt_5\ dt, a_1 a_1 a_1$ (bottom) and $Dt_5\ Dt_5\ dt\ a^m - 1$ $a^m - 1\ a_1$ (top)

presumed due to Dt_5 activity before fertilization; therefore, the three individuals in the apparent crossover class (colored-yellow green) could be accounted for, at least partly, by a_1 to A_1 mutations before meiosis, with subsequent segregation of the A_1-bearing chromosome 3 to the same pole as the *dt* yg_2-bearing chromosome 9. The relatively low proportion of this type (3/97) indicates that most a_1 to A_1 mutations resulting in fully colored kernels occur during the microspore divisions following meiosis. Because the colored, yellow-green type could not be unambiguously assigned to either a crossover or noncrossover class, the proportion found in the dotted classes was applied to the colored classes, and the totals for the *Dt Yg* and *Dt yg* columns were corrected to include the colored kernels before the percentage of crossing over was calculated. The data do not differentiate between a proximal or distal location of Dt_5 relative to the yg_2 locus.

Unlike Dt_5, Dt_4 segregates independently of the yg_2 locus (Table 2); either it is located on the long arm of chromosome 9, having greater than 50 per cent recombination with yg_2, or it is situated on another chromosome.

2. *Phase Variation of the Regulatory Element Dt*

Dt_4 differs from Dt_1 in its transmission, being inherited independently of the yg_2 locus, and in its phenotype, exhibiting an entirely new "state" of *Dt* activity. The dots are confined to the crown of the kernel and are not usually found in the remainder of the aleurone (Fig. 4). The fine-dot pattern is very dense, often giving the appearance of one mutant sector unless viewed with magnification. The Dt_4^{crown} phenotype occurs whether the responding allele is the standard a_1 or $a^m - 1$ and has been inherited coin-

cidently with Dt_4 activity for three generations (Dt_4 was heterozygous in each generation). This state is similar to En^{crown} (Enhancer) (Peterson, 1966), where activity is restricted to a certain part of the aleurone; i.e. mutability is affected by the cellular environment.

Another example of altered *Dt* activity was observed during the study of Dt_1^{TB}, a *Dt* that arose by transposition of Dt_1 to a location inherited independently of yg_2 (Doerschug, unpublished). Kernels sectored for dotted expression were first observed among the backcross progeny of a female $Dt_1\ Yg_2/dt\ yg_2$, Dt_1^{TB}/dt^{TB}, $a^m - 1/a^m - 1$ plant. Several kernels had no dots at all in the aleurones, but were heavily dotted in the scutella. Whereas three-fourths of the kernels were expected to have fully dotted aleurones, the ear contained 172 kernels with uniformly dotted aleurones, 117 completely dotless types, and 96 with dots in sectors of the aleurone and with or without dotted scutella. Plants were grown from the latter class of kernels and crossed with tester strains to determine the cause of altered dotted expression in these kernels.

Self-pollinations of plants grown from sectored kernels produced kernels with colorless aleurones, some of which had well-defined dotted sectors (Fig. 5) the dotting in the scutella, when it occurred, was uniform (Fig. 6). (Color in the scutellum requires additional genes, which were not being followed here.) A very few kernels had dots throughout the aleurone. A plant arising from a kernel with *Dt* activity confined to sectors of the aleurone was selected for intensive study and used as pollen parent in a variety of crosses. (The allele specifying reduced *Dt* activity was subsequently called $Dt_1^{in\text{-}ac}$, Dotted inactive-active.) All the kernels on an ear from a cross between this plant and an $a_1^s\ a_1^s$, $Dt_1\ Dt_1$ stock had a large number of dots uniformly distributed over the aleurone (Fig. 7). Since a_1^s does not respond to *Dt*, the only source of a mutable a_1 allele was in the plant being tested; thus, the presence of two normally mutable $a^m - 1$ genes in the pollen parent was established.

The activity of $Dt_1^{in\text{-}ac}$ was studied by crossing the test plant to an $a^m - 1\ a^m - 1$, *dt dt* female parent. The resultant ear showed that $Dt_1^{in\text{-}ac}$ was inactive in most of the aleurones, but occasionally became active, producing sectors of a_1 mutability (Fig. 8). The same behavior was observed in a cross to an $a_1^s\ a_1^s$, *dt dt* female parent.

An independent modifier of *Dt* activity could not have caused the sectoring since the Dt_1 from the $a_1^s\ Dt_1$ stock was not affected. A modifier linked to $Dt_1^{in\text{-}ac}$ whose influence is restricted to genes in the same homologue might be postulated, but it seems more likely that induction of $Dt_1^{in\text{-}ac}$ activity and inactivity was autonomous.

Since the tested plant was homozygous for the highly mutable $a^m - 1$, sectors of $Dt_1^{in\text{-}ac}$ reactivation were clearly defined. The reactivation sectors on

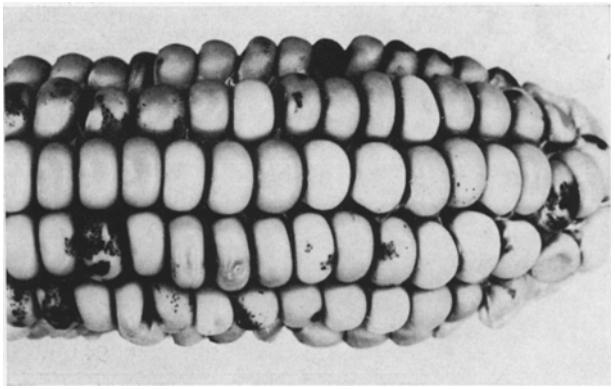


Fig. 5. Ear from the self-pollination of a $Dt^{in\text{-}ac}\ dt\ a^m - 1\ a^m - 1$ plant

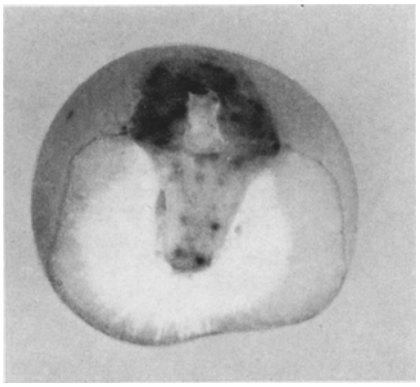


Fig. 6. Cross section through a kernel of the constitution $a^m - 1\ a^m - 1\ a^m - 1\ dt\ dt\ Dt_1^{in\text{-}ac}$. The exposed scutellum is dotted throughout. A single dot in the endosperm is visible at the left

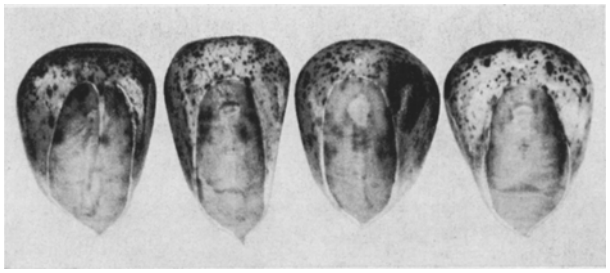


Fig. 7. Kernels from the cross $a_1^s\ a_1^s\ Dt_1\ Dt_1$ ♀ × $Dt_1^{in\text{-}ac}\ dt\ a^m - 1\ a^m - 1$ ♂

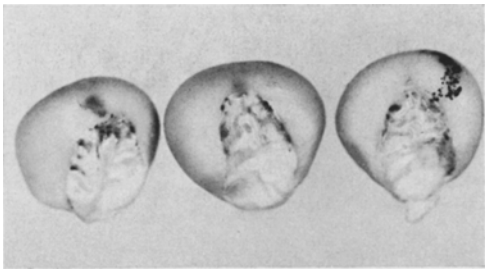


Fig. 8. Kernels from the cross $a^m - 1\ a^m - 1\ dt\ dt$ ♀ × $Dt_1^{in\text{-}ac}\ a^m - 1\ a^m - 1$ ♂

Table 3. *Tissue dependence of* $Dt^{in\text{-}ac}$ *phase reversals. Analysies of the crosses* $a_1^s\ a_1^s\ dt\ dt$ *(1191)* ♀ *or* $a^m - 1\ a^m - 1\ dt\ dt$ *(1183)* ♀ × $a^m - 1\ a^m - 1\ Dt^{in\text{-}ac}\ dt$ *(1179-1181)* ♂. *Both parents of Nos. 2—5 were probably heterozygous for a scutellar color factor*

No.		Number of kernels			
		Dotted scutella; dotless endosperms	Dotted scutella; sectors of mutability in endosperm	Dotless scutella; dotless endosperms	Dotless scutella; sectors of mutability in endosperm
1	1191 × 1181-1	129	22	154	0
2	1183-8 × 1181-1	84	35	185	1
3	1183-10 × 1180-1	99	13	177	0
4	1183-3 × 1180-3	114	13	195	0
5	1191 × 1179-1	128	48	256	3

self-pollinated ears were larger than on outcrossed ears, where only one $Dt_1^{in\text{-}ac}$ was present in the triploid endosperm (compare Fig. 5 and Fig. 8). It is possible that $Dt_1^{in\text{-}ac}$ reactivation in these kernels was dose-dependent; i.e., the inactive $Dt_1^{in\text{-}ac}$'s acted synergistically to reactivate other inactive alleles in the same kernel. According to this explanation, the combined action of the $Dt_1^{in\text{-}ac}$'s caused earlier reactivation, resulting in larger sectors of $Dt_1^{in\text{-}ac}$ activity.

One ear from the cross $a_1^s\ a_1^s$, *dt dt* × $a^m - 1\ a^m - 1$, *dt* $Dt_1^{in\text{-}ac}$ gave a 1:1 ratio for kernels with dotted and colorless scutella (Table 3, No. 1). Kernels in the two classes were scored for aleurone sectors showing $Dt_1^{in\text{-}ac}$ activity; sectors of $Dt_1^{in\text{-}ac}$ reactivation were found only on kernels with dotted scutella. The 3:5 ratios for kernels with dotted:dotless scutella on the four remaining ears (Table 3, Nos. 2—5) were attributed to the segregation of a scutellar color factor assumed to be heterozygous in both parents. Nevertheless, sectors of $Dt_1^{in\text{-}ac}$ reactivation were found mainly on kernels with dotted scutella. The dotless scutella of the four exceptional kernels (last column, Nos. 2 and 5) were not unexpected since, presumably, a scutellar factor was segregating in addition to $Dt_1^{in\text{-}ac}$. It was concluded that inactivations of $Dt_1^{in\text{-}ac}$ followed by occasional reactivations were tissue-dependent, occurring in the aleurone, but not in the scutellum. Thus, kernels with colorless scutella (with proper scutellar factor constitution) were assumed to be of *dt dt dt* constitution.

Linkage tests show independent inheritance of yg_2 and $Dt_1^{in\text{-}ac}$, suggesting that $Dt_1^{in\text{-}ac}$ was derived from Dt_1^{TB} rather than Dt_1. No tests of Dt_1^{TB} and $Dt_1^{in\text{-}ac}$ allelism have been made, however.

Discussion

One of the most puzzling, but perhaps significant, aspects of controlling elements is that they originate in conjunction with chromosome or chromatid breaks (McClintock, 1950, 1951a; Bianchi et al., 1969). This fact invokes a requirement that either an existing regulatory mechanism is disturbed by the breakage or that a foreign element is incorporated before fusion of the broken chromatids.

We have reported here that 250 of 154,422 kernels (or about one in 600) exhibited some form of dotting when the regulatory element *Dt* was induced as a consequence of a bridge-breakage-fusion cycle in chromosome 9. Most of these *Dt*'s, however, were unavailable for genetic analysis since they arose during endosperm development and were not included in the embryo. The chromatid breaks responsible for the two germinal *Dt*'s presumably occurred during the anaphases of meiosis or the first microspore division. Thus, both sperm nuclei contained a newly induced *Dt* and, consequently, *Dt* was transmitted to the embryo as well as to the endosperm. The frequency of germinally induced *Dt*'s was about 1 per 77,000 gametes.

The phenotypes produced by the new *Dt*'s are of interest. Dt_4^{crown} represents a new "state" previously unknown for this element. Kernels of the genotype Dt_4^{crown} *dt*, $a^m - 1\ a^m - 1$ have a concentration of fine dots in the crown area of the aleurone, with very few or no dots elsewhere (Fig. 4). A high frequency of germinal mutations of a_1 to A_1 occurs. Dt_5, on the other hand, produces a mutation pattern very similar to that of the original Dt_1, whether with the standard a_1 or $a^m - 1$ allele.

The data are equivocal with regard to the position of the break in chromosome 9 at the time of *Dt* induction. Linkage tests show that Dt_5 is very close to the yg_2 locus, there being only 0.33 percent crossing over between them (Table 2). This compares with 7 per cent crossing over between Dt_1 and yg_2 (Rhoades, 1945; Doerschug, unpublished). According to the design of the experiment, the primary selection was for the "Dotted" phenotype. It should be clear, however, that there also was strong selection for a chromosome 9 having a complete short arm with or without a partial duplication of that arm; pollen bearing a deficient 9 would not compete, and pollen having a shorter duplication would be less handicapped in competition than those with an extensive amount. Thus, if the break position were unimportant and bridge-breakage occurred randomly, it is more likely that a chromosome 9 with a short duplication would have been found. The Mendelian segre-

gation of Dt_5 in the original plant and in later generations, as well as the regularity of Yg_2, yg_2 segregations, attest to the absence of a deficiency or duplication in the derived chromosome 9.

These data suggest that a break near or in the proximal knob of the Duplication chromosome 9 produced a normal chromosome 9 with the induced Dt_5 at the same position or very close to Dt_1. It is possible that the two *Dt*'s are at the same location and that the lower recombination of Dt_5 with yg_2 is due to interference of crossing over by a small structural rearrangement in this region.

Dt_4 is inherited independently of yg_2. Unfortunately, chromosome 9 was not examined cytologically in the original plant, so its structure remains uncertain. Its independent location is not entirely unexpected. Transpositions of controlling elements occur frequently when they are in certain "states" (McClintock, 1956, 1965), and such "states" of *Dt* have been found (Doerschug, unpublished). A transposition of *Dt* to another location could have occurred during or immediately after its origin. Alternatively, the broken chromatid ends could have interacted with another chromosome to produce a fusion, exchange, break, etc., as previously has been observed during the chromosome type of bridge-breakage-fusion cycle (McClintock, 1950, 1951a). Thus, the induction of Dt_4 does not distinguish whether the site of origin was at a particular site on chromosome 9 or whether it involved another chromosome as well.

In summary, the results presented here leave open the possibility of a specific break point in chromosome 9 for *Dt* induction; but without additional germinal inductions, they are not convincing. In addition to enlarging the scale of the experiment, a test of the effect of mechanical breakage of other chromosomes would be pertinent to the problem of the origin of controlling elements since these and other studies (McClintock, 1950, 1951a; Bianchi et al., 1969) have relied exclusively on breakage of the short arm of chromosome 9 as a source of at least three independent regulatory-controlling-element systems.

The basic composition and structure of controlling elements is still open to question. They could arise by an alteration of existing regulatory mechanisms during breakage, or they could result from invasion of a foreign element after a chromatid or chromosome break. McClintock (1951a) originally suggested the involvement of heterochromatin in their origin because of parallels with position effect in *Drosophila*, the alterations of heterochromatic regions during the bridge-breakage-fusion cycle, and the location of Dt_1 in a heterochromatic knob. Subsequent studies of controlling elements, however, have not supported this theory. If *Dt*'s are produced by specific breaks in the terminal heterochromatic knob of chromosome 9, a reexamination of this concept is warranted. On the other hand, these systems may originate by the incorporation of foreign genetic systems, such as viruses or episomes (McClintock, 1965), and chromosome breakage may enhance this process (see Peterson, 1970, for a complete discussion and challenge of this concept). This suggestion has precedent in bacterial virus systems where chromosome breaks are required for incorporation of the virus genome (Campbell, 1969). It is interesting in this regard that chromosome breakage increases the incidence of transformation of normal to cancerous cells by viruses. It is not clear whether this is due to an increased rate of viral integration and subsequent control or to an activation of the already integrated "oncogene" (Huebner, 1969). According to the latter, most eukaryotic cells contain integrated, but latent, viral genomes. In the case of maize-controlling elements, breaks may trigger the activity of integrated foreign elements in a similar manner. Since at least two different controlling elements have originated by breakage of the same chromosome 9 (McClintock, 1950, 1951a; Bianchi et al., 1969; this report) as well as by X-rays and ultraviolet irradiation (Neuffer, 1966), it seems that, whether or not viral genomes are involved, it is most likely that controlling elements are the result of alterations of structures already present in the maize genome.

The "state" of Dt_4^{crown} produces a phenotype that is an example of phase variation of regulatory elements (Peterson, 1966). Dt_4^{crown} was active only in the crown of the kernel so that no mutations of a_1 to A_1 were produced in the basal portion of the kernel, even though a dottable $a^m - 1$ allele was present. Presumably, metabolic conditions in the cells of the two regions of the kernel differ, and activity or inactivity of Dt_4^{crown} is determined by the cellular environment.

The tissue dependence of $Dt_1^{in\text{-}ac}$ expression was of particular interest. Dotting of the scutellum was consistently uniform, even though fluctuations of $Dt_1^{in\text{-}ac}$ activity were occurring in the endosperm of the same kernel. The scuttellum and endosperm are both post-fertilization tissues. The former is a derivative of embryonic tissue, whereas the endosperm is formed by fertilization of the two polar nuclei of the embryo sac. It is possible that, in the restoration of the totipotency of cells that accompanies formation of the zygote, the potential for full $Dt_1^{in\text{-}ac}$ was restored. At some time in the development of the sporophyte or during fertilization of the polar nuclei, this potential was lost, followed by sporadic resumptions of activity. Schwartz (1960) suggested a similar interpretation to account for variation in the mutability of c^m, except that c^m was stabilized to a null level of expression in the embryonic and early sporophytic environment. Alternatively, $Dt_1^{in\text{-}ac}$ is fully active in all tissues except the endosperm, where it is initially inactive but sporadically reverts to the active phase. In either case, the active and inactive phases

were direct responses of $Dt_1^{in\text{-}ac}$ to differences in its cellular environment. The normal state of *Dt* is not affected by changes in the surrounding tissues.

Reversals of phases of activity were found for the regulatory element *Spm* (McClintock, 1961). Alternations of phase occurred during any period of the life cycle and were interpreted to be autonomously controlled by *Spm*. The active and inactive phases of En^{crown} or En^{flow}, however, were associated with specific areas of the aleurone (Peterson, 1966), as are the phases of *Dt* activity in Dt_4^{crown} described here. $Dt_1^{in\text{-}ac}$ has characteristics in common with both En^{crown} or En^{flow} and *Spm*. Random reversals of phase occurred in a single tissue (the endosperm), but such fluctuations were not a property of the scutellum where $Dt_1^{in\text{-}ac}$ was fully active. A parallel has been made between the phase variations of *Spm* and En^{crown} or En^{flow}, on the one hand, and flagellar phase variation in *Salmonella* on the other (McClintock, 1961, 1965; Peterson, 1966).

The significance of reversals of phase of regulatory elements is unclear. If the regulatory elements of maize represent normal elements that have become erratic, this phenomenon indicates still another complexity in the mechanism. However, it does seem that, whether one is concerned directly with the control of a structural gene or with the activity of its regulator, the phase of activity of the elements is modified by the cellular environment.

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