Theoretical and Applied Genetics 48, 119-130 (1976)

Studies of Dotted, a Regulatory Element in Maize
III. Transpositions of Dotted and Its Stability at Various Locations *

E.B. Doerschug

Department of Genetics, Iowa State University of Science and Technology, Ames, Iowa (USA)

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

Since its discovery over 40 years ago, Dt has always been found in the terminal knob of the short arm of chromosome 9. This is puzzling because controlling and regulatory elements in general are not located permanently, but change positions (transpose) within the chromosomal complement. To resolve this seeming discrepancy, transpositions were looked for in a homozygous a Dt stock. Because the frequency of aleurone mutations is exponentially related to Dt dosage, a Dt transposition would result in a greatly increased number of dots if the egg or sperm nucleus contained both the transposed Dt and the Dt remaining on chromosome 9. A total of 6 transposed Dt's (Dt-T) were recovered in this manner.

Dt-TA was found linked to the gene Y ("yellow endosperm") of chromosome 6. Dt-TB no longer showed linkage with yg2 of chromosome 9, but remains unlocated (the original Dt in this stock is separated from yg2 by 6 or 7 cross-over units.). The remaining transpositions (C-F) assorted independently of Dt on chromosome 9.

The transposed Dt's had the same effect as Dt on the frequency and timing of aleurone mutations. An increase in transposition frequency and losses of Dt-T's was characteristic of several of the transposed Dt's. Dt-T's B-F transposed so frequently that testcross ratios of 7:1 (three Dt's) and 15:1 (four Dt's) were observed. No secondary transpositions or losses of Dt-TA were detected. Thus, Dt-TA resembles the original Dt with regard to its transposition frequency and stability.

Introduction

Controlling-regulatory elements in maize have been the subject of intense study in several laboratories for more than 20 years (see Fincham and Sastry 1974, for a recent review). These elements regulate the expression of genes that are directly responsible for a particular phenotype. In some systems, an element at the gene site effectively changes the expression of the gene (autonomous control). Other systems consist of two elements, one at the gene site and a second at another location (nonautonomous control). In the latter instance, gene activity is initiated by action of the independently located element on the gene-associated element. (According to Peterson's 1970a nomenclature, the gene-associated element is called a controlling element, whereas the independently located entity is a regulatory element. This practice will be used here for clarity.) Several significant features of these systems have emerged: 1) Changes of the controlled gene usually are from the inactive recessive to the fully active dominant. With autonomous control, the regulatory-controlling element complex leaves the gene site; in the nonautonomous type, the controlling element alone need move away to allow gene expression. 2) These changes normally are stable and are inherited as such in subsequent cell and plant generations. 3) Controlling and regulatory element in general are not located permanently but change positions within the chromosomal complement. 4) As a consequence of this behavior, many genes (probably all) are susceptible to the control of the element(s) of a particular system.

The present study is concerned with the nonautonomous "Dotted" (Dt) system, the first in which the control of one gene by another was demonstrated (Rhoades 1938). Subsequently, more detailed analysis of other systems have introduced unsuspected complexities and have indicated that differences in behavior of the various systems are common. Typically Dt causes heritable changes of the a gene (colorless aleurone) to its active allele, A

* Journal Paper No. J-8333 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1880.

(purple aleurone), producing a dotted effect on the kernel. The A locus is on chromosme 3 and, when in combination with the controlling element of this system, usually is noted as a-dt. The regulatory element, Dt, however, since its discovery in the 1930's (Rhoades 1936), always has been found in the terminal knob of the short arm of chromosome 9. This suggests a possible difference between Dt and other regulatory elements in maize (e.g., Ac, Activator; Spm, Supressor-Mutator; Md, Modulator; En, Enhancer); i.e., that Dt does not transpose, but remains fixed in position. Acoording to Rhoades (1945) the location of Dt is seven crossover units distal to the yg2 ("yellow green") locus, which resides in the ultimate chromomere. The major goals of this investigation were to determine: 1) if Dt is stationary or moves from one chromosomal location to another and 2) whether such movements, if they occur, affect the stability or modify the activity of Dt.

Materials and Methods

Detection and analysis of Dt transpositions

The investigation of transpositions of Dt ("Dotted") was suggested by Dr. Marcus M. Rhoades, who supplied the a a Dt Dt stock used in this study (Fig.1). The A gene is concerned with anthocyanin formation and is located on chromosome 3. The standard recessive allele a, which normally prevents anthocyanin coloration, responds to Dt on chromosome 9 to form purple A sectors on a colorless background (Rhoades 1938). Because of absence of complementary plant color factors in the stock supplied for these tests, the a Dt phenotype was expressed only in the aleurone layers of the kernels. Kernels with exceptionally high numbers of dots, believed to possess a transposed Dt in addition to the standard Dt, were selected for analysis.

Two criteria were used as evidence of transpositions of Dt from its usual location on chromosome 9: 1) a segregation of 3 dotted: 1 dotless kernels in a backcross, or 15 dotted: 1 dotless in an F_2 population the expected ratios for the independent segregation of duplicate factors; and 2) loss of linkage between Dt and yg2 ("yellow green") loci which are normally separated by 7 recombinational units (Rhoades 1945). Plants suspected of having a transposed Dt were crossed to an A a-m, *dt Yg2 dt yg2* stock (obtained from Dr. Gerald Neuffer, University of Missouri) and either self-pollinated or testcrossed to a-m a-m, *dt yg2 dt yg2* plants. The a-m allele mutates 406 times as frequently as does a (Nuffer 1961) and greatly facilitated the classification of dotted and dotless kernels in these crosses. The dotted-green progeny *(Dt Yg2)* from testcrossed and self-pollinated ears were grown and testcrossed again to obtain offspring segregating for only the transposed Dt or for Dt on chromosome 9. Chi-square analysis of the factor segregations and for linkage was carried out according to standard methods (see Mather 1951).

The transposed Dt's were labelled Dt-TA, Dt-TB, etc., in order of their isolation. Previously described "Dotted" loci have been designated Dt (chromosome 9), Dt2 (chromosome 6), and Dt3 (chromosome 7) (Nuffer 1955).

Results

Detection of Dt transpositions

A search was made for transpositions of Dt in a homozygous a Dt stock that had been maintained for several generations by self-pollinating or sib-crossing. The mutation frequency of the a gene was uniformly high in this stock (Fig.1). Because the frequency of aleurone mutations is exponentially related to Dt dosage (Rhoades 1938, 1941), a Dt transposition would result in a greatly increased number of dots if the egg or sperm nucleus contained both the transposed Dt and the Dt remaining on chromosome 9. The endosperm of maize kernels is triploid; thus, the normal Dt dosage of 3 could be increased to 4 if transposition took place in the pollen parent, or to 5 if it occurred in the egg parent (Fig.2).

1255 a a Dt Dt plants were self-pollinated and the progeny ears were examined. Several kernels had significantly greater mutation rates of the a gene than did the rest of the kernels (Fig.3). Individuals in a stock established from these exceptional kernels were crossed as pollen parent (P_1) to A a-m *dt Yg2 dt yg2* plants. The F_1's, a-m a and heterozygous for whatever Dt's might be present, were testcrossed (as egg parent) by a homozygous a-m *dt yg2* stock or self-pollinated.

Such tests of original high dot isolate A revealed two ears segregating two Dt's, whereas 40 ears segregated one (Table 1). Similarly, only six ears of a total of 67 segregated two Dt's in backcrosses or self-pollinations involving isolate B. The χ^2 values (Table 2) are consistent with the hypothesis that two unlinked Dt's were segregating. The deviations of B-53 and B-60 from a 15:1 ratio do not detract from the conclusion because ears clearly are segregating Dt at more than one location.

Original isolates A and B most likely represent transpositions of the Dt gene from its mapped position on chromosome 9 to an unlinked site. The transposed Dt genes will be referred to as Dt-TA and Dt-TB.

The small number of ears in Table 1 with segregations of both Dt and Dt-TA or Dt-TB is puzzling. Half of the F_1 plants should have segregated two Dt's if Dt-T were heterozygous (and Dt homozygous) in the dotted P_1, and all should have segregated two Dt's if

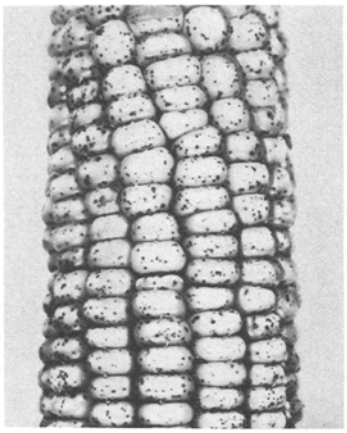

Fig.1. A self-pollinated ear of the a a Dt Dt stock used to isolate transpositions and losses of Dt

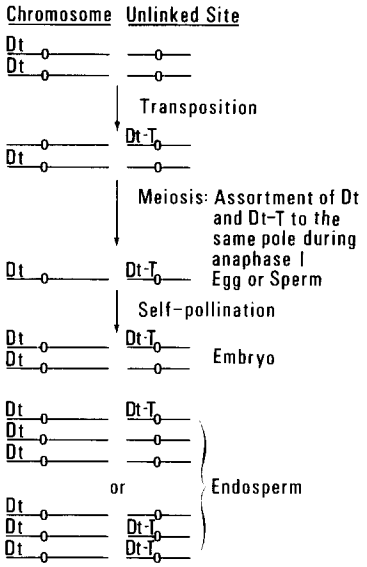

Fig.2. Method by which Dt dosage is increased in a a Dt Dt plants after its transposition from chromosome 9 to an unlinked site

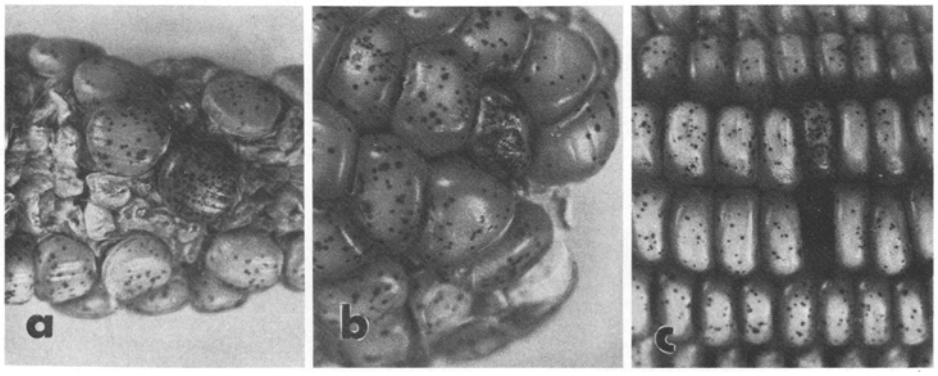

Fig.3. Single highly dotted kernels shown on the parent ears. Subsequent crosses demonstrated that the increased dotting frequencies were due to transpositions of Dt to other locations. a) Dt-TB, b) Dt-TE, c) Dt-TF

the P_1 plants were Dt Dt, Dt-T Dt-T. The gene yg2 ("yellow green"), closely linked to Dt, segregated in 55 of the 101 plants that segregated only one Dt. In all these, there was close linkage between the two genes. Therefore, the dotted P_1 plants were homozygous Dt. The unexpected low recovery of the transposed Dt's could have been due to unequal transmission of the Dt-T-bearing chromosome and its homologue in the pollen parent (P_1) or to instability of the Dt-T gene itself. These alternatives cannot be distinguished without closely linked markers.

Analysis of Dt-TA

Linkage of Dt-TA with Y ("yellow endosperm") on chromosome 6 was suggested by the nonrandom association of Dt and Y phenotypes on a 3 Dt:1 dt testcross ear (Table 1). The relationship was unclear, however, because of the presence of the independently segregating Dt. Dotted (Dt) kernels with yellow endosperm (Y) were selected, and the resulting plants were crossed again to an a *dt yg2 y* tester to obtain progeny lacking Dt.

Table 3, cross 1, shows the total numbers of progeny from 13 ears in which Dt-TA was linked to Y of chromosome 6 and showed no association with Yg2 of chromosome 9. The linkage χ^2 values of Dt-TA and Y (Table 4) for each ear (not shown) and for total data (χ^2 total = 291.519) were highly significant. On the other hand, the χ^2 values testing linkage of Dt-TA and Yg2 on the same ears were not significant, indicating inde-

Table 1. Summary of progeny from F_1 plants of $\frac{a}{a}\frac{Dt}{dt}\frac{Dt\text{-}T}{dt}$ constitution that were self-pollinated or testcrossed (× a-m dt). Listed are the number of plants that segregated 1:1 (one Dt segregating) and 3:1 (two Dt's segregating) for the testcrosses or 3:1 (one Dt segregating) and 15:1 (two Dt's segregating) for the self pollinations

	Testcrosses		Self-pollinations	
	1:1 (1 Dt)	3:1 (2 Dt)	3:1 (1 Dt)	15:1 (2 Dt)
Dt-TA	22	1	18	1
Dt-TB	22	3	39	3

Table 2. Analysis of ears deviating significantly from the expected ratios for the segregation of one Dt: χ^2 test of the hypothesis that two independently segregating Dt's were present in some F_1 plants. Segregation of 2 Dt's at independent locations yields ratios of 3 Dt: 1 dt in a testcross or 15 Dt: 1 dt in an F_2 population

					χ^2 for a Dt:dt segregation of:			
Reference Number	Cross*	Dt	dt	Total	3:1 (2 Dt's)	S	15:1 (2 Dt's)	S
Ta-1	t	331	127	458	1.820	n. s.		
TA-24	s	481	39	520			1.387	n. s.
TB-5	t	267	98	365	0.666	n. s.		
TB-13	t	197	76	273	1.173	n. s.		
TB-25	t	354	121	475	0.057	n. s.		
TB-44	s	566	28	594			2.392	n. s.
TB-53	s	405	40	445			5.697	P=.025-.010
TB-60	s	484	16	500			7.938	P=.005-.001

* t = testcross; s = self-pollination
Tested at the five per cent level of significance

pendent assortment of these two genes. The recombination data (Table 3) place Dt-TA in chromosome 6, about 39 recombination units from y.

Six sibling plants of those listed in Table 3, cross 1, are characterized by linkage of Dt with Yg2 of chromosome 9 (cross 2). No association of Dt with Y of chromosome 6 was found. Highly significant linkage χ^2's were obtained for joint segregation of Dt and Yg2 (Table 4, number 2).

Thirty-five of a total of 50 plants in which Dt- was segregating 1:1 showed linkage of Dt and Y. An excess of plants with Dt-TA was expected because Y kernels were selected for testing. The result is in contrast to the low recovery of Dt-TA in the F_1 generation (Table 1). This discrepancy may have occurred, however, because Dt-TA was introduced by the pollen parent, whereas plants giving rise to the progenies in Tables 3 and 4 were crossed as the egg parent. Competition of Dt-TA and dt-TA pollen grains due to a linked gametophyte factor could account for the low transmission of Dt-TA in the pollen parent. Competition between megaspores does not occur.

Analysis of Dt-TB

The data of Table 3, cross 3 together with the linkage χ^2 values of Table 4, no. 3 show that Dt-TB was segregating independently of both Yg2 in chromosome 9 and Y in chromosome 6. This clearly demonstrates the transposition of Dt from its position on chromosome 9 to some other location in the chromosomal complement. Unlike Dt-TA, however, Dt-TB was not linked to Y of chromosome 6.

Confirmation of Dt location on chromosome nine

Table 3, cross 4 and accompanying χ^2 Table 4, no. 4 record the joint segregation of Dt and Yg in sibling ears with no Dt-TB. The highly significant linkage χ^2 values reflect the tight linkage of Dt and Yg2. The combined data of Table 3 crosses 2 and 4, giving a total population of 6,468 plants, place Dt 6.0 crossover units from Yg2. This agrees well with the value of 7.1 found by Rhoades (1945).

Table 3. Analysis of Dt-TA and Dt-TB. Testcross data of $\frac{Dt}{dt}\ \frac{Yg2}{yg2}\ \frac{Y}{y}$ plants from progeny segregating two independent Dt's. The total numbers of progeny are listed only for ears that segregated 1 Dt: 1 dt. In these cases a Dt was linked with Yg2 of chromosome 9, Y of chromosome 6, or assorted independently of either of these two genes. χ^2 analysis of the data is found in Table 4.

No.	Cross	No. ears	Dt Yg	Dt yg	dt Yg	dt yg	Total	Dt Y	Dt y	dt Y	dt y	Total
1.	$\frac{\text{Dt-TA}\ Y}{dt\quad y}\ \frac{Yg2}{yg2}$ ♀ × dt y yg2 ♂	13	1328	1352	1341	1313	5334	1744	1094	1080	1711	1529
2.	$\frac{Dt\ Yg2}{dt\ yg2}\ \frac{Y}{y}$ ♀ × dt y yg2 ♂ (siblings of those in No. 1)	6	958	68	66	1006	2092	532	513	547	543	2135
3.	$\frac{\text{Dt-TB}}{dt}\ \frac{Yg2}{yg2}\ \frac{Y}{y}$ ♀ × dt y yg2 ♂	8	695	633	749	754	2831	648	707	710	816	2881
4.	$\frac{Dt\ Yg2}{dt\ yg2}\ \frac{Y}{y}$ ♀ × dt y yg2 ♂ (siblings of those in No. 3)	16	2067	123	135	2051	4376					

Analysis of Dt-TC, Dt-TD, Dt-TE, and Dt-TF

Four other kernels with high mutation rates of a_1 to A_1 were tested and found to result from Dt transpositions. Plants heterozygous for one or more Dt's were crossed as egg and pollen parents to the a-m dt stock of Neuffer to introduce an allele that is highly mutable in the presence of Dt: a a Dt? dt Dt-T? dt × a-m a-m dt dt.

Table 5 summarizes the segregations of the Dt's in these crosses. The presence of 3 Dt: 1 dt ratios in each is evidence for the segregation of two independent Dt's.

A detailed analysis of those plants segregating more than 3 Dt: 1 dt as the pollen and (or) egg parent is presented in Table 6. Plant no. 3, involving Dt-TC, segregated 7 Dt: 1 dt whether used as pollen or egg parent. Two independent transposed Dt's evidently were present in addition to Dt. Plant 4 segregated 3 Dt: 1 dt when used as egg parent and 7 Dt: 1 dt in the reciprocal cross. A second transposition may have occurred in a sector of the plant including the tassel. Evidence for more than one transposed Dt was found with Dt-TD in plants nos. 5 and 6 and with Dt-TF in plant no. 8. Plant no. 7 segregated 15 Dt: 1 dt in the testcross, the expected ratio for 4 independent Dt genes. The ratios obtained on several ears were intermediate between expected ratios (nos. 1, 2, 5, 6, 8), whereas segregations from several of the reciprocal crosses were 3:1 or 7:1. Transpositions evidently were occurring frequently; intermediate ratios could result from transpositions in sectors of the ear or tassel and (or) transpositions producing linkage between two Dt's.

Secondary transpositions of Dt-T's or additional transposition of the original Dt led to a search for multiple transpositions among discarded progenies segregating for Dt-TA and Dt-TB. No segregations greater than 3:1 were found for Dt-TA crosses, but two ears involving Dt-TB contained kernels in the proportion 7 Dt: 1 dt.

Stability of the Dt gene

More than 1200 self-pollinations of stocks homozygous for a and Dt were made to detect transpositions. Some of the resultant ears contained a very low percentage of dotless kernels, whereas in others, there was a ratio of 3 dotted to 1 dotless. Dotless kernels were not expected on the basis of random distribution of dots inasmuch as the frequency of aleurone mutations in these stocks was uniformly high (Fig. 1). Nevertheless, almost 9 % (34 + 41/861) of self-pollinated ears in a Dt Dt stock had dotless kernels (Table 7). Presumably, dotless kernels originated from changes in either or both parental contributions to the endosperm. If the alteration involved one parent only, the immediate kernel phenotype would be dotted, followed by a segregation of the dotted and dotless phenotypes in the next generation, or it would be dotless because of a lower mutation rate with decreased dosage of Dt or a. A change in both the egg and the pollen parent would give rise directly to a dotless kernel.

The a allele frequently changes in certain stocks to a stable form (a-s) unable to mutate to A_1 (or only with a low frequency) in the presence of Dt (Rhoades

Table 4. χ^2 analysis of the data in Table 3. Values were calculated for Dt:dt, Yg2:yg2, and Y:y factor pair segregations and for the joint segregation of Dt with either Yg2 or Y. A χ^2 - sum (the sum of the χ^2's, for individual ears -- the individual ears are not listed in Table 3 for the sake of brevity), the χ^2 total (the values listed in Table 4), and χ^2 - heterogeneity ($\chi^2_s - \chi^2_t$)

No.	*	χ^2 Dt:dt	χ^2 Y:y
1.	χ^2 - Sum	6.506	12.597
	χ^2 - Total	0.039(P=.9-.8)	0.064(P=.9-.8)
	χ^2 - Heterogeneity	6.467(P=.9-.8)	12.533(P=.5-.4)
2.	χ^2 - Sum	4.633	1.086
	χ^2 - Total	0.948(P=.8-.7)	0.248(P=.7-.6)
	χ^2 - Heterogeneity	3.685(P=.6-.5)	0.838(P=.9)
3.	χ^2 - Sum	15.515	16.065
	χ^2 - Total	10.150(P=.055-.001)	9.450(P=.005-.001)
	χ^2 - Heterogeneity	5.365(P=.7-.6)	6.615(P=.5-.4)
4.	χ^2 - Sum	12.080	17.988
	χ^2 - Total	0.003(P > .9)	0.179(P=.7-.6)
	χ^2 - Heterogeneity	12.077(P=.7-.6)	17.809(P=.3-.2)

* corresponds to the number in Table 3

Table 5. Summary of analysis of Dt-TC, Dt-TD, Dt-TE, and Dt-TF. Testcrosses were made of Dt plants from progenies that segregated two independent Dt's. The number of plants segregating 1,2, or more Dt's is listed for each isolate. Each plant was used as a pollen and egg parent unless indicated. χ^2 analysis was carried out for each cross

Transposition isolate	Number of plants that segregated		
	1 Dt:1 dt	3 Dt:1 dt	greater than 3 Dt:1 dt as ♀ or ♂ parent
Dt-TC	3	4	4
Dt-TD	3	7	3
Dt-TE	5 *	6 *	0
Dt-TF	5 *	7 *	1

* crossed as egg parent only

1945). Consequently, dotless kernels of separate origin were crossed to the testers listed in Table 8 to determine the cause of the lack of mutability. Crosses of seven of the eight dotless stocks with dt dt plants carrying the standard a allele or the highly mutable a-m found by Nuffer (1961) produced only dotless kernels (families 2-8): similar crosses involving the remaining stock (family no.1) resulted in a low frequency of dotted kernels. When crossed with a-s Dt (the a-s Dt stock has dotless aleurones inasmuch as a-s does not mutate in the presence of Dt), all the dotless stocks gave F_1 kernels with aleurone mutations, with the exception of family no.6, which produced ears with all dotless kernels, ears segregating for dotted and dotless, and ears with only dotted kernels. Thus, a dottable a allele was present in the dotless stocks, and the loss of mutability was attributed to loss of Dt activity. Additional crosses involving family no.6 substantiated the finding that both a mutable and a stable a allele were present. It is possible that the loss of Dt activity was coincident with a mutation of a to a-s. It is not known whether the families 2-8 have an inactive Dt allele or whether they had an actual physical loss of Dt. Numerous sibling crosses of the dotless stocks (Table 8) as well as self-pollinations and crosses with a-m dt have produced no reactivation of Dt. The chromosomes 9 of these families, when observed at pachynema, appeared to be normal and had the small terminal knob characteristic of Dt stocks. Several losses of Dt and one mutation of a to a-s accounted for the occurrence of dotless kernels on otherwise a a Dt Dt ears. The apparent discrepancy

χ^2 Yg2:yg2	χ^2 Dt-Y Linkage	χ^2 Dt-Yg2 Linkage	Degrees of Freedom
13.275	316.605	21.589	13
0.003(P > .9)	291.519(P < .0005)	0.507(P=.5-.4)	1
13.272(P=.4-.3)	25.086(P=.025-.010)	21.082(P=.050-.025)	12
4.941	2.091	1613.421	6
1.499(P=.3-.2)	0.105(P=.8-.7)	1611.327(P < .0005)	1
3.442(P=.7-.6)	1.986(P=.9-.8)	2.094(P=.9-.8)	5
11.940	9.073	7.015	8
1.148(P=.3-.2)	0.767(P=.4-.3)	1.586(P=.3-.2)	1
10.792(P=.2-.1)	8.306(P=.4-.3)	5.429(P=.7-.6)	7
17.988		3412.715	16
0.179(P=.7-.6)		3404.844(P < .001)	1

Table 6. χ^2 analysis of segregation of Dt greater than 3 Dt:1 dt among those listed in Table 5

No.	Dotted/dt ♀ × dt/dt ♂ and reciprocal	Dt	dt	Total	χ^2 for a Dt:dt segregation of: 1:1	S	3:1	S	7:1	S	15:1	S
1	Dt-TC-1	322	69	391	163.706	***	11.273	***	9.471	**		
	reciprocal	163	53	216	56.010	***	0.025	n.s.				
2	Dt-TC-2	385	94	479	176.787	***	7.383	**	22.228	***		
	reciprocal	132	41	173	47.867	***	0.156	n.s.				
3	Dt-TC-3	340	44	384	228.167	***	37.556	***	0.381	n.s.		
	reciprocal	177	29	206	106.330	***	13.107	***	0.469	n.s.		
4	Dt-TC-4	226	73	299	78.291	***	0.055	n.s.				
	reciprocal	190	34	224	108.643	***	11.524	***	1.469	n.s.		
5	Dt-TD-1	388	51	439	258.699	***	41.932	***	0.313	n.s.	21.584	***
	reciprocal	191	17	208	145.558	***	31.410	***	3.560	n.s.	1.313	n.s.
6	Dt-TD-2	340	44	384	228.167	***	37.556	***	0.381	n.s.	2.639	n.s.
	reciprocal	260	61	321	123.368	***	6.157	*	2.639	n.s.		
7	Dt-TD-3	402	23	425	337.979	***	86.972	***	19.523	***	0.510	n.s.
	reciprocal	156	10	166	128.410	***	31.880	***	6.365	*	0.014	n.s.
8	Dt-TF-1	328	66	396	174.223	***	14.298	***	6.511	*		
	reciprocal	254	38	292	159.781	***	22.374	***	0.070	n.s.		

* Five percent level of significance
** One percent level of significance
*** One tenth percent level of significance

between this result and those of Rhoades (1941) and Peterson (1953), who attributed the dotless kernels to mutations, of a to a-s, possibly is the result of sampling errors inherent in small populations. It may be also that the stocks used contained different states of the a and(or) Dt alleles where changes to the inactive a-s and losses of Dt occurred at different rates.

A number of crosses of the type a a Dt Dt ♀ × a a dt dt ♂ were made. Four ears in a total of 40 had dotless kernels that were randomly distributed and not in clusters. The proportion of dotless kernels was large on some ears, but never accounted for half the kernels. Dotless kernels also were observed on ears of the reciprocal crosses. Thus, in these plants, independent losses of Dt (or loss of Dt activity) must have occurred after divergence of the cell lines forming individual ovules in the female parent. In the pollen parent, however, one cannot distinguish be-

Table 7. Number of ears bearing dotless kernels which arose from self-pollinations of homozygous a Dt stocks

a a Dt Dt family	Number of plants segregating 3 Dt: 1 dt	Number of plants giving a low frequency of dt kernels (1-10 %)	Number of plants with all dotted kernels	Total number of plants
63-1, selfed	7	12	147	166
63-2, selfed	3	0	85	88
63-3, selfed	12	8	201	221
63-4, selfed	3	4	42	49
63-5, selfed	0	0	84	84
63-6, selfed	5	9	157	171
63-7, selfed	6	8	68	82
	36	41	784	861

Table 8. Phenotypes of kernels resulting from crosses between dotless types of different origin and tester strains. The dotless types were derived from the selfed progeny of homozygous Dt stocks

No.	Dotless families	Phenotype of kernels × a* sh2 dt	× a-m *dt a	× a-s* Dt	× sib (No. of crosses)
1	741	a few kernels dotted	a few kernels dotted	all kernels dotted	------
2	743	------	dotless	dotted	dotless (6)
3	744	dotless	dotless	dotted	dotless (4)
4	745	dotless	dotless	dotted	dotless (3)
5	747	dotless	dotless	dotted	dotless (7)
6	748	dotless	dotless	dotless or dotted +	dotless (2)
7	749	dotless	dotless	dotted	------
8	1255	dotless	dotless	dotted	dotless (2)

*a, the standard allele that responds to Dt
a-m, a highly mutable a allele described by Nuffer (1961)
a-s, an a allele that does not respond to Dt
+ The ears testing individual sib plants were completely dotted, completely dotless, or segregated 1:1

tween frequent independent losses of Dt and a single event involving a sector of the tassel.

Associations of "changes in state" with Dt transpositions

McClintock (1951) found that a "change in state" of an element often was associated with its transposition. Differences in either the physical attachment or in the chromosomal environment affected the activity of the element. Consequently, kernels carrying the transposed Dt's were examined for changes in Dt activity. The effect of Dt-TA and Dt-TB upon the mutability of a remained unaltered.

Because transpositions and losses of Dt were known to occur, a comparison of Dt stability at its standard location and at other positions was made by χ^2 analysis of Dt:dt ratios in backcrosses involving Dt, Dt-TA, and Dt-TB. Table 4 lists the χ^2 values for Dt:dt segregations when Dt was linked to Yg2 of chromosome 9. The six ears of Table 3 and 4, nos.2, segregated 1026 Dt to 1066 dt kernels (χ^2 = 0.765, P = .4-.3), and the 16 ears of Tables 3 and 4, nos.4 segregated 2190 Dt to 2186 dt kernels (χ^2 = 0.003, P = 0.9-0.8). Both agree with the expected 1:1 ratio of Mendelian genes.

Dt-TA on chromosome 6 segregated in Mendelian fashion. Thirteen ears yielded 2838 Dt on 2791 dt kernels, with a χ^2 value of 0.127 (P = 0.8-0.7) (Tables

3 and 4, nos 1). The unlinked Dt-TB however, significantly deviated from the 1:1 ratio expected in a test-cross. There were 1355 Dt and 1526 dt kernels found on eight ears (Tables 3 and 4, nos.4). The Dt-induced mutation rate was sufficiently high to make misclassification an unlikely cause of this deviation. Although seven of the eight ears did not have significant deviation from 1 Dt: 1 dt, the dt class was in excess in each instance producing the highly significant total deviation with a χ^2 value of 10.150 (P = 0.005-0.001). Upon transposition from chromosome 9 to a new location, Dt-TB had evidently undergone a change in stability.

Consistent with these apparent losses of Dt-TB was the small proportion of ears segregating 1:1 for Dt-TB:dt (from which the eight ears of Table 3, no.3 were taken). Only 10 of a total of 77 ears segregated Dt-TB, whereas 67 segregated 1:1 for Dt when there should have been equal numbers of each type. The low recovery was similar to that of the previous generation (see "Detection of transpositions"). Gametophyte factors cannot be invoked, however, because, in the later backcrosses, the Dt-containing plants were used as egg parents. It is more likely that losses of Dt-TB were occurring during the development of the sporophyte.

A further indication that "changes in state" were associated with the transposition of Dt was the frequent recovery of backcross ears segregating three and four Dt's among Dt-TB, Dt-TC, Dt-TD, and Dt-TF families (see section discussing these transpositions). As mentioned before, however, secondary transpositions of the Dt-T and additional transpositions of Dt could not be distinguished in these instances.

Discussion

Transpositions

Were it not that the elements of other mutable systems in maize changed position in the chromosomes, there would have been no reason to suspect that Dt might move from its known position in the short arm of chromosome 9. Dt had been maintained for more than 30 years in genetic stocks and had been used in diverse linkage studies as a consistent marker of the terminus of that arm. In this respect, it resembled the typical gene. When sufficiently rigorous means of detection were employed, however, transpositions of Dt were readily isolated. Dt need not be confined to one location for it to effectively regulate a activity. No difference was observed in the response of a to Dt at any of its new locations, although such a change was commonly associated with transpositions of Ac (McClintock 1951). Selection of kernels with an increased number of a mutations as possible cases of transposition may have resulted in detection of Dt-T's that affected a mutability in much the same manner as did Dt; thus, transposed Dt's producing altered a responses may have been overlooked.

Six primary transpositions of Dt were isolated in the initial study. Although their effects on a mutability were the same, differences in the behavior of the Dt-T's were obvious. Dt-TA showed 39 per cent recombination with Y of chromosome 6. Dt2, also on chromosome 6, was reported to have 26 per cent recombination with Y (Nuffer 1955). The difference in recombination values does not allow the conclusion that Dt2 and Dt-TA are at distinct sites because the frequency of crossing over can vary considerably in dissimilar genetic backgrounds. Therefore, it is possible that Dt-TA and Dt2 are allelic and that Dt transposes preferentially to certain sites on the chromosomes.

Essentially all the transpositions of a regulatory element can be detected when a controlling element-regulatory element complex is initially present at the locus showing instability. Studies of such autonomously controlled loci have been made by Greenblatt (1968, 1974) of *Modulator* and by Peterson (1970a) of *Enhancer*. These regulatory elements were shown to transpose preferentially to sites on the same chromosome. Furthermore, there was a decided preference for positions close to the initial location of the elements. A significant proportion of the transpositions, however, did move to unlinked sites (35 % of transpositions of *Modulator* and 75 % of transpositions of *Enhancer*). The method of detection for Dt transpositions used here selected for transfer of Dt to unlinked locations; transposition to closely linked sites would have been overlooked. Therefore, no direct comparisons may be made in this regard.

Inasmuch as Y is approximately 17 map units from rgd ("ragged seedling"), which is at or near one end of the short arm of chromosome 6, Dt-TA must lie in the long arm distal to Y and close to the locus of su_2 ("sugary endosperm"). At this position, Dt-TA was

as stable as Dt. χ^2 analysis of its segregation at either position in testcrosses revealed no significant deviation from 1:1.

Testcrosses of Dt-TB (unknown location), however, produced highly significant deviations from 1:1. A large excess of dt kernels was found. It is not known whether the excess was due to losses of Dt-TB activity or to actual physical deletions of the element. If the latter were responsible, an increased transposition rate of Dt-TB might be expected. Indeed, two secondary transpositions were found among siblings of the testcrossed plants showing irregular segregation of Dt-TB. The occurrence of secondary transpositions in plants with Dt-TC, Dt-TD, or Dt-TF suggested that limited instability may be common in some locations while Dt's on chromosomes 9 and 6 show greater stability. Differences in stability of Dt at different locations are similar to those found for the regulatory element of *Suppressor-mutator* (Spm) by McClintock (1957). From a position near Wx on chromosome 9, Spm transposed in most plants, whereas, when it was located on chromosome 6 near Y, there was evidence for only one transposition in a total of 44 ears.

The first step of a Dt transposition would be its removal from the original chromosomal site. If it were then incorporated elsewhere in the complement, a transposition would have occurred. If, however, it were not incorporated elsewhere, a loss of Dt would be observed. Thus, the losses of Dt (activity) and the occurrence of transposed Dt's are both likely to be consequences of its initial removal from the chromosome.

The discovery of Dt at new locations, the correlation of the instability of one of these, Dt-TB, with secondary transpositions, the high recovery rate of dt kernels in homozygous Dt stocks, and the occurrence in the same progeny of transposed Dt's and of chromosomes 9 that lost Dt all point to a causal relationship of transpositions of Dt and its instability at the previous locations.

Significance of transpositions

The discovery of the regulation of one gene (a) by another (Dt) was an important contribution to the development of genetic theory (Rhoades 1936, 1938, 1941, 1945), for it demonstrated one means by which gene activity could be controlled. After resumption of A activity through the action of Dt, a stable and heritable A allele was present that thereafter seemed to be unaffected by Dt. By analogy with similar control of genes by Ac-Ds, it was postulated that a controlling element at the A locus suppressed the activity of the dominant allele and that Dt caused resumption of A activity by inducing loss of the controlling element from the chromosomal locus (McClintock 1956b). The responding element presumably transposes from a in a manner similar to transpositions of Dt.

Thus, a unique feature of Dt, the inferred element at a, and other regulatory and controlling elements, is their ability to physically leave a chromosomal position and reassociate elsewhere. In this respect, the elements resemble various bacterial plasmids, proviruses, or pieces of transposable inserted DNA (Jacob and Monod 1961; McClintock 1961; Smith-Keary and Dawson 1964). The most striking similarity transposition has been discussed. Although the exact location of the transposed Dt's has not been determined in most instances, one situation was found in which four Dt's segregated independently of each other. Dt factors have been discovered on chromosome 6 (Dt2, Brazil) and chromosome 7 (Dt3, Peru) in two maize strains of 98 tested from Central and South America (Nuffer 1955) in addition to the original site on chromosome 9 (Dt) (Rhoades 1945). Two of the Dt's may well have arisen as transpositions of the third, although evidence of such an event is lacking. An additional site of Dt in the chromosomal complement may have been discovered in this study; Dt-TA represents a transposition of Dt from chromosome 9 to a position on chromosome 6 close to or coincident with the locus of Dt2. The transpositions detected here, as well as those found in studies of other controlling elements (McClintock 1951, 1956a, b, 1964; Van Schaik and Brink 1959; Greenblatt and Brink 1962; Greenblatt 1966, 1968, 1974; Peterson 1970a) attest to the fact that attachment sites are not restricted to specific chromosomes. Likewise, in bacteria, the sex factor, the multiple drug resistance factor, and bacteriophage that perform generalized transduction are able to occupy many chromosomal sites (Driskell-Zamenhof 1964). On the other hand, most temperate bacteriophage attach at unique positions. The best studied temperate phage, λ, was shown to occupy a distinct position linked to the cistron controlling phosphogalactotransferase.

Peterson (1970b) presents a thorough discussion of possible microbial counterparts to maize controlling elements. On the basis of Nelson's (1968) placement of four independent insertions of controlling elements at distinct sites within the wx locus, Peterson suggested that controlling elements are foreign genetic material inserted without site specificity into a gene, consequently interfering with transcription or translation. As described by Peterson, there are adequate analogous situations in bacteria; e.g., the Mu-1 gene inserted at the gal and other loci in *E. coli* (Taylor 1963) and three different insertions of various size into the gal operon (Jordan et al. 1968). In each of these situations the insertion of genetic material (450 to 1800 nucleotide pairs in the latter reference) causes suppression of gene activity, which is restored upon the removal of that exact piece of DNA from the gene. This explanation is perhaps more appropriate, at least at present, than considering controlling elements and their regulatory properties analogous to temperate phage. There is no direct evidence to support the latter possibility; furthermore, controlling elements have not been observed to replicate independently of the chromosome, nor to cause gross changes in the physiological activity of the cells in which they exist.

The physical relationship between controlling elements and the chromosome is puzzling and was the subject of another report (Doerschug 1973). The chromatid and chromosome breaks associated with the origin or controlling elements during the bridge-breakage-fusion cycle (McClintock 1950; Bianchi et al. 1969; Doerschug 1973) and the chromatid breaks that are the result of activity of the element Ds argue that some recombination-like mechanism is responsible for their insertion and excision (Campbell 1969). That elimination of elements evidently occurs in somatic cells does not detract from such a mechanism because the enzymatic events involved in meiotic recombination occur throughout the life cycle; synapsis simply increases the probability that a recombinational event will occur between homologous regions of two chromosomes (Howell 1971). In view of this and the mapping of controlling elements within the wx locus (Nelson 1968), the integration of controlling elements into the chromosome rather than a lateral attachment is favored (Greenblatt 1968). The size of controlling elements is unknown, although it has been argued (Peterson 1970b) that the fine structure analysis by Nelson (1968) shows no increase in recombinational distance when an element is inserted into the wx locus, and that, consequently, elements must be short. A large insertion of a portion of chromosome 9, however, resulted in no increase in recombinational distance between markers on either side of the added piece (Rhoades 1968). Thus, I would be hesitant to relate the lack of greater recombinational distance to the small size of the element.

The importance of controlling element systems has yet to be evaluated fully. A system involving frequently transposing regulatory elements seems too variable to play a major role in gene regulation and differentiation. Yet, the transient regulation of structural genes may represent a state in the evolution of stable controlling systems. It may be advantageous for transposable elements (initially nonspecific) to exist in cells, enabling rapid development of regulatory systems. After initiation of control, whether or not the system stabilizes, would depend on the selective forces. Thus, instability may result from the modification of a stable regulatory element and may reflect a previous condition of that element or may be due to the initiation of control of a gene by an uncommitted element.

Most, if not all, of the mutable genes studied in maize have been unessential for plant development. Some argue that the behavior of elements controlling such genes may not be characteristic of regulators of genes necessary for cell and plant development. It is only because the mutable genes are unessential, however, that the control system could be tested genetically. That transposable elements can affect major genes was demonstrated by the fact that transpositions of Ds from a known location to new positions in the short arm of chromosome 9 often were associated with dominant lethality (McClintock 1956b).

Acknowledgement

I am grateful to Dr. Marcus M. Rhoades for suggesting the study of Dt and for his help during my studies. I especially thank Ms. Ellen Dempsey for her interest and attention to this research problem. The work was supported by the following: a predoctoral fellowship from the National Institute of Health and by the Iowa Agriculture and Home Economics Experiment Station.

Literature

Bianchi, A.; Salamini, F.; Parlavecchio, R: On the origin of controlling elements in maize. Genet. Agrar. 22, 335-343 (1969)

Campbell, Allan M.: Episomes. New York: Harper and Row 1969

Doerschug, E.B.: Studies of Dotted, a regulatory element in maize; I. Inductions of Dotted by chromatid breaks. II. Phase variation of *Dotted*. Theoret. Appl. Genet. 43, 182-189 (1973)

Driskell-Zamenhof, P.: Bacterial Episomes. The Bacteria, Vol. 5: 155-222. New York: Academic Press 1964

Fincham, J.R.S.; Sastry, G.R.K.: Controlling elements in maize. Annu. Rev. Genet. 8, 15-50 (1974)

Greenblatt, I.M.: Transposition and replication of *Modulator* in maize. Genetics 53, 361-369 (1966)

Greenblatt, I.M.: The mechanism of *Modulator* transposition in maize. Genetics 58, 585-597 (1968)

Greenblatt, I.M.: Movement of *Modulator* in maize: A test of an hypothesis. Genetics 77, 671-678 (1974)

Greenblatt, I.M.; Brink, R.A.: Twin mutations in medium variegated pericarp in maize. Genetics 47, 489-501 (1962)

Howell, S.H.: The appearance of DNA breakage and repair activities in the synchronous meiotic cycle of *Lilium*. J. Mol. Biol. 55, 357-378 (1971)

Jacob, F.; Monod, J.: On the regulation of gene activity. Cold Spring Harbor Symp. Quant. Biol. 26, 193-212 (1961)

Jordan, E.; Seadler, H.; Starlinger, P.: 0° and strong-polar mutations in the gal operon are insertions. Mol. Gen. Genet. 102, 353-363 (1968)

Mather, K.: The measurement of linkage in heredity. London: Methuen and Co. Ltd. 1951

McClintock, B.: The origin and behavior of mutable loci in maize. Proc. Natl. Acad. Sci. (U.S.A.) 36, 344-355 (1950)

McClintock, B.: Chromosome organization and genic expression. Cold Spring Harbor Symp. Quant. Biol. 16, 13-47 (1951)

McClintock, B.: Intranuclear systems controlling gene action and mutation. Brookhaven Symp. Biol. 8, 58-74 (1956a)

McClintock, B.: Controlling elements and the gene. Cold Spring Harbor Symp. Quant. Biol. 21, 197-216 (1956b)

McClintock, B.: Genetic and cytological studies of maize. Carnegie Inst. Wash. Year Book 56, 393-401 (1957)

McClintock, B.: Some parallels between gene control systems in maize and in bacteria. Am. Nat. 95, 265-277 (1961)

McClintock, B.: Aspects of gene regulation in maize. Carnegie Inst. Wash. Year Book 63, 592-602 (1964)

Nelson, Oliver E.: The *waxy* locus in maize. II. The location of the controlling element alleles. Genetics 60, 507-524 (1968)

Nuffer, M.G.: Dosage effect of multiple Dt loci on mutation of a in maize endosperm. Science 121, 399-400 (1955)

Nuffer, M.G.: Mutation studies at the A locus in maize. I. A mutable allele controlled by Dt. Genetics 46, 625-640 (1961)

Peterson, P.A.: Mutation of a to a-stable. Maize Genet. Coop. News Lett. 27, 61 (1953)

Peterson, P.A.: The En mutable system in Maize. III. Transposition associated with mutational events. Theoret. Appl. Genet. 40, 367-377 (1970a)

Peterson, P.A.: Controlling elements and mutable loci in maize: their relationship to bacterial episomes. Genetica 41, 33-56 (1970b)

Rhoades, M.M.: The effect of varying gene dosage on aleurone color in maize. J. Genet. 33, 347-354 (1936)

Rhoades, M.M.: Effect of the Dt gene in the mutability of the a-allele in maize. Genetics 23, 377-397 (1938)

Rhoades, M.M.: The genetic control of mutability in maize. Cold Spring Harbor Symp. Quant. Biol. 9, 138-144 (1941)

Rhoades, M.M.: On the genetic control of mutability in maize. Proc. Natl. Acad. Sci. (U.S.A.) 31, 91-95 (1945)

Rhoades, M.M.: Studies on the cytological basis of crossing over. Symposium: Replication and recombination of genetic material, ed. Peacock, W. J.; Brock, R.F. Australian Academy of Science, Canberra, 229-241 (1968)

Smith-Keary, P.F.; Dawson, G.W.P.: Episomic suppression of phenotype in *Salmonella*. Genet. Res. Camb. 5, 269-281 (1964)

Taylor, A.L.: Bacteriophage - induced mutation in *Escherichia coli*. Proc. Natl. Acad. Sci. (U.S.A.) 50, 1043-1051 (1963)

Van Schaik, N.W.; Brink, R.A.: Transpositions of *Modulator*, a component of the variegated pericarp allele in maize. Genetics 44, 725-738 (1959)

Received November 21, 1975
Communicated by R.W. Allard

Earle B. Doerschug
Asst. Professor of Genetics
Department of Genetics
Iowa State University of Science and Technology
Ames, Iowa 50011 (USA)