

Outline of topics discussed.

Introduction.

Part I. The c-m1 and standard Ds loci compared. Page 4

1. The c-m1 locus: General considerations. Page 4
2. Stabilization of the mutated c-m1 locus. Page 13
3. Evidence for the conclusion that no Ds locus was present at the standard location in the original c-m1 carrying chromosome. Page 15
4. The standard Ds locus: Two contrasting types of mutation behavior. Page 18
5. The relation between the two types of behavior of c-m1 and of the standard Ds locus. Page 20
6. Comparison of rates of mutation of c-m1 and of the standard Ds. Page 22
7. The Ac control of mutation during development. Page 23
8. The mode of origin of mutable loci. Page 26
9. Possible mechanism responsible for mutable phenomena. Page 31

Part II. The c-m2 locus. Page 42

1. Classification and description of types of mutations occurring at the c-m2 locus. Page 42
2. The origin of the c-m2 locus. Page 52
3. Illustrations of the phenotypic changes that are associated with c-m2 mutations.

Appendix - Extra Tables. Not explained in text.

Introduction

The c^{m-1} locus (hereafter written c-m1 for speed in typing) appears to have arisen as the consequence of a change in location of Ds: removal from its former position and insertion into (or close to) a normal C locus. In this new position, it behaves just as it did in its former position. The c to C mutations appear to be associated with its behavior in this new position, as I shall try to point out. In this new location, more has been learned about Ds behavior than could have been learned from Ds in its "standard" location. The position in chromosome 9 where Ds was first discovered will be called the "standard" location. A position of reference is needed because Ds does change its location. In its standard position, it is known that Ds changes its mutation pattern from one general type of behavior to another, i.e., the "early" loss type and the "few-late" loss type of behavior (for details of these two general types, see below). Previously, the behavior of the Ds locus in the "few-late" state had been difficult to analyze and interpret. In its new position at the C locus, various types of behavior of Ds are subject to analysis, as will be indicated.

Secondly, chromosome breakage of a type not previously recognized may be responsible for the mutation phenomena associated with mutable loci. The type of breakage and subsequent fusion of broken ends that will occur in any one "state" of a mutable locus may be controlled by the particular organization of the inserted locus responsible for this breakage phenomenon, at the time of its insertion into a new location. This breakage process need not lead to the simple

criss-cross type of fusions as previously suspected, nor to the formation of U-shaped dicentric chromatids already known to arise from mutations occurring in one "state" of the Ds locus. It is probably something quite different. This breakage mechanism will be outlined later. This mechanism should sometimes alter the organization of the inserted locus so that new types of organizations would be formed. These altered organizations will give rise to new "states" of the mutable locus, that is, different rates of production of visible mutations, of stabilization of loci, and of the types of fusions that will occur following breakage. The state of a mutable locus is something more than a quantitative change at a locus. Starting with any one organization that gives a particular series of breakage-fusions events, the types of change to new organizations giving an altered series of events may follow rather definite sequences.

Thirdly, stabilization of a mutating locus may follow as a consequence of loss, during a mitotic cycle, of the inserted locus responsible for the mutation phenomena or of its active component. With some organizations of the inserted locus, each mutation of the mutating locus, with some exceptions, will result in stabilization of the mutating locus. With other organizations, some of the mutations will not result in elimination of the active locus. In these cases, continued mutations will occur. This mutating locus may be a dominant, an intermediate allele or a recessive but mutations will continue.

Fourthly, I suspect that many loci, such as the C locus and the A₁ and A₂ loci are compound loci involving genic action associated

with successive or related steps in the conversion of substrate to product. The type of mutations occurring following insertion of a "Ds" locus into such loci will depend upon (1) the position of insertion, (2) the organization of the inserted locus at the time of its insertion and (3) the changes in organization of the Ds locus that will occur subsequently.

If the accumulating data has been interpreted correctly and continues to give further supporting evidence, it will be necessary to change the symbol "Ds" to something more expressive of its basic action. The observed dissociation phenomenon (dicentric chromatid formation) is only one of the consequences of mutations of Ds that occur. The various types of Ds mutation behavior are probably expressions of one basic type of event. This is the important event underlying all expressions of mutability of mutable loci. The newer evidence has made it possible to consider this event more specifically even though there is much that is purely speculative. Nevertheless, the expanded knowledge allows for better directed experimentation and observation.

Fifthly, there are probably a number of unstable dominant loci in my material. These will eventually throw unstable recessives. The wx-m1 locus arose from an unstable dominant locus, designated Wx-m. This unstable dominant locus has now been isolated. It will throw more unstable recessives. One such recessive, a new wx-m, is growing in the greenhouse but many more should be detected following suitable crosses now that the unstable dominant that throws unstable recessives has been isolated. The instability of dominants and the unstable recessives they throw are probably all expressions of the one kind of event, i.e., chromosome breakage. Mutable expressions go in a

cycle from unstable dominant to unstable recessive to unstable dominant, etc., or to various quantitative levels between full dominant and full recessive. (Full dominant is a relative concept. Super dominants can be isolated.) Stabilization may occur at any quantitatively expressed level, however.

Part I. The c-m1 and standard Ds loci compared.

The c-m1 locus; general considerations

For the details, the c-m1 case may be considered first. A brief review of the behavior of this locus will give the essential facts necessary to an understanding of the discussion. The c-m1 locus arose in the cross of a ^{male = 376114} yg c sh Bz wx ds ac female plant by a male plant that was Yg C Sh Bz wx Ds / Yg C Sh Bz wx Ds, Ac ac. ^{plant # 201} Ten crosses of this male parent were made to c sh ds ac female plants. The ratios were: 2077 C, non-variegated (ac ac ac) : 1199 C to c variegated, early losses (Ac ac ac) : 317 C to c then classified as having the few-late type of Ds action (mainly Ac ac ac) : 1 c to C variegated kernel. This last kernel was obviously not the regular C to c variegation expected as the consequence of Ds action. It showed the opposite type of variegation, from c to C. A plant was grown from this aberrant kernel in the summer of 1947 (plant 4204). The plant was variegated. It showed streaks of yg. This variegation was not unexpected because it was known that both chromosomes 9 in the male parent carried Ds. If Ac were present, just such yg streaks should appear. It was only necessary to determine that Ac was also present in this plant; the later tests proved that plant 4204 was Ac ac. The constitution of this plant was then believed to be YG c sh wx ds / Yg c-m1 Sh wx Ds, Ac ac. The data and analysis

given below shows that the location of Ds is not as given. Instead, it is probably at, within or close to the C locus. In this plant (4204) no evidence has been obtained for a Ds in the "standard" location.

← *Summer 1947*
Plant 4204 was crossed to:

- (1) yg c sh wx ds ac
- (2) C sh bz wx ds ac
- (3) C Sh wx Ds / C Sh wx Ds, ac ac
- (4) pyd c Sh wx ds / yg c Sh wx ds, ac ac.

From cross (1), it was clear that the male parent possessed two chromosomes 9, one with Yg c-ml Sh wx and one with yg c sh wx. (For kernel counts, see Table 1, Appendix.)

From cross (2) it was clear that a Ds locus was likewise present in the c-ml Sh wx chromosome. ~~About half~~ ^{many} of the Sh kernels (the Ac containing kernels) were C Bz-C bz variegated. (See Table 2, Appendix.) There were few such kernels in the sh class. (This latter observation should have made me suspicious about the location of Ds but I failed to register a reaction. At the time, I did not know that Ds could change its location. Realization of this did not enter my consciousness until late this spring, following the harvest of the greenhouse crop.

From cross (3) it was likewise clear that a Ds locus was present in the c-ml Sh wx chromosome. Some of the kernels were C to c variegated following Ds mutations in the C Ds chromosome contributed by the female parent; in some of these kernels, the c areas showed c to C ^{or c to C} mutations due to c to C mutations occurring at the c-ml locus in the chromosomes 9 contributed by the male parent (4204). These latter kernels most always showed homozygous deficient tissues of the type

occurring when three Ds loci are present, one in each chromosome 9. The combined evidence indicated quite definitely that a Ds locus was present in the c-m1 carrying chromosome of plant 4204.

From cross (1) it was obvious that the expressed mutation rates of c-m1 are not alike in all the kernels. The majority of the kernels show relatively few c to C mutations. A small number of Sh kernels, however, had very many c to C mutations. I assumed from this that the kernels showing only a few c to C mutations were either (1) those having a c-m1 Sh wx Ds constitution and that the Ds mutations were eliminating the segment of the short arm carrying the c-m1 locus from many sectors of these kernels so that the c to C mutations could not occur in these sectors; or (2) that the state of the c-m1 locus was low, i.e., few c to C mutations were occurring even in those kernels with c-m1 and no Ds (those derived from cross-over chromatids). Again, it was assumed that those kernels with many c to C mutations probably possessed no Ds because Ds could have been removed from the chromosome as a consequence of crossing over between Sh and the assumed ^{position of the} Ds locus in the parent plant (4204). Because both chromosomes 9 in the parent carried wx, there was no immediate genetic determination of such a cross-over. The sh kernels showing c to C variegation obviously carried a cross-over chromatid. Barring double crossovers, which are rare, the c-m1 chromosome in these kernels should have no Ds locus. These kernels, however, did not show high rates of c to C mutation. That a changed location of Ds had occurred when c-m1 arose could not be clearly revealed from an analysis of the crosses made during the summer of 1947. That something was wrong soon became obvious. The nature of the discrepancies will be indicated.

The selection of kernels for tests in the greenhouse last winter centered on establishing the control of c-ml mutation phenomena by Ac. From this crop, it was determined that Ac controlled the c-ml mutations; without Ac, no mutations occurred. It also showed that the time in development when mutations would occur depended upon the dosage of Ac. In this respect, it was quite similar to Ds in response. In the progeny derived from crosses of those plants that were grown from the highly c to C variegated kernels, this greenhouse crop also showed the exact correspondence in patterns of mutation to those already known for one type of Ds. You will remember seeing this similarity in comparing kernels of the constitution C ds / C ds / I Ds, Ac ac ac (and Ac Ac ac) with those kernels having c ds / c ds / c-ml, Ac ac ac (and Ac Ac ac). The two sets of kernels were indistinguishable, respectively, in color pattern of variegation.

The summer planting (1948) included a selected number of Wx kernels showing c to C variegation from cross (4) above. The Yg plants arising from these kernels were all variegated for pyd. ^{or for M9} This was not anticipated for I had expected a few of these plants to have a c-ml chromosome with no Ds because of crossing-over between c-ml and wx in the male parent. It did not bother me too much as the sample was small. In order to be sure to eliminate Ds from a c-ml carrying chromosome, some of these plants were crosses (1) to c sh wx ds ac, (2) to C sh bz wx ds ac, and (3) to plants having the rearranged chromosome 9 with c sh Wx and a female-transmissible deficient chromosome 9 carrying Sh and wx but deficient for the C locus. There were other reasons for these crosses but mainly it was to observe the effects of c-ml mutations when Ds was certainly removed from the chromosome. No Ds (standard location) should be

present in most of the c-m1 Wx chromatids. Only double crossovers would give the constitution c-m1 Wx Ds and these are rare.

The results from cross (1) were strikingly different from expectation. Nearly all the Wx kernels that showed c to C variegation were also heavily Wx to wx variegated; this Wx to wx variegation resembled the usual Ds type variegation. In these kernels, the rate of c to C variegation was low, except for sectors in some of the kernels. A few of the Wx kernels, however, were heavily variegated for c to C. These kernels showed only a few wx areas and they usually were quite small. A tabulation of the classes of kernels resulting from cross (1) may interest you for they tell the full story much better than words (table 1). Because the male parent was heterozygous for Ac, only half of the kernels having a c-m1 locus could show c to C variegation. Also, the kernels that received the stable c locus (from the parental c Sh Wx chromosome) will not show c to C variegation with or without Ac. You will note that only 14 of the 990 c Wx kernels (no visible c to C mutations) showed Wx -wx variegation whereas all of the 140 c to C variegated kernels that were Wx likewise showed Wx to wx variegation. The presence of detectible sh sectors in some of these heavily Wx-wx variegated kernels made it clear that loss of a segment of the short arm of chromosome 9 was responsible, or often associated with, the appearance of the wx sectors. Some kind of Ds-like action was occurring in these kernels with a c-m1 Wx chromosome. This Ds action was not associated with a standard Ds locus in the c Sh Wx chromosome of the male parent. If a Ds locus had been present in this chromosome, many of the c Wx kernels should have been Wx - wx variegated but they were not. Secondly, all the Wx kernels showing c to C variegation likewise

Table 1

$$c \text{ sh } wx \text{ ds } ac \text{ } \varphi \text{ } x \frac{cm-1 \text{ Sh } wx}{c \text{ sh } wx} \delta$$

Cross	c → C		c → C			Non-Var.	
	wx	c wx	c wx	wx → wx	wx → wx	C wx	C wx
4350-5 x 4434D-3	46	90	141	27	3	1	1
4346-18 x "	40	80	146	18	1	0 ?	0
4349-2 x	34	83	148	22	0	0	1
Totals	120	253	435	67	4 ↓	1	2

Table 1 (continued) (extended)

Cross	c → C	c wx	c wx	c → C	c	C wx	C wx
	wx			wx → wx	wx → wx		
4346-16 x 4434D-5	27	57	71	8	2 ?	0	2
4349-31 x " "	29	82	134	16	3	1	3
4350-18 x " "	10	29	54	6	1	0	1
" -26 x " "	37	99	148	28	0	2*	2
" -34 x " "	34	100	134	15	4 +	0	3
Totals	137	367	541	73	10 ✓	3	11

* 1 var. for C-c but peculiar. Late losses, wx-wx.

+ 2 have defective embryo.

1 These are probably c → C, potentially. Tests of ^{row 2} non-var. c^{m-1}, ac carrying kernels have shown var. in the next generation.

showed Wx to wx variegation. These represent the kernels receiving a crossover chromatid that brought c-ml and Wx onto the same chromosome. Since all of these chromosomes show this Ds-like action, a Ds-like locus must have been present in the c-ml Sh wx chromosome of the male parent. Its location must be in, close to or to the left of the c-ml locus. Thirdly, of the 16 C kernels arising from germinal mutation of c-ml, only one showed any C to c variegation and this was a peculiar type. (It will be pointed out later that just such new variegations are to be expected. They represent changed location or changed organizations of the inserted locus responsible for mutable phenomena.) Unless all such C kernels have an ac ac ac constitution, a Ds type variegation should be present in some of them, on the assumption that a Ds is in some position other than the c-ml locus. Fourthly, examination of the C areas in both the c to C, Wx - wx kernels and the c to C wx kernels were likewise instructive. In these kernels, with some expected exceptions, the C areas were not variegated for C to c. In the c to C, Wx - wx kernels, the C areas were Wx, not Wx -wx variegated as would be expected if Ds were present to the left of the C locus. Obviously, then, when a mutation to C occurred, the Ds-like action in this chromosome ceased (with some exceptions, to be considered later).

The combined observations made me suspect that the Ds action was associated with the presence of Ds in the c-ml locus and that the Ds locus was usually lost from the chromosome when a mutation to C occurred. The following conclusions were formulated: that (1) the c-ml locus arose from a C Sh wx Ds chromosome following removal of the Ds locus from its standard location and insertion into the normal C locus; (2) when a mutation of c-ml to C occurs, Ds disappears; (3) the

insertion of Ds into the C locus brought about an inhibition in the expression of the genes at the C locus; consequently the modified C locus resembles c in phenotypic expression. (4) When this Ds locus is removed from the chromosome, the normal genic action of the C locus can reappear. (5) Two contrasting types of Ds action ^{can} be recognized when Ds is in this new position: (a) one giving the typical dicentric chromatid through breakage and fusion at this new location and (b) one giving the c to C mutations that usually result in no visible alteration of the chromosome.

For an understanding of the events underlying mutability phenomena, conclusions (4) and (5) above are most important. They bring up the question: Does Ds in its standard location show these same two contrasting types of behavior? A positive answer to this question is indicated. Two dissimilar types of behavior of Ds in its standard location have long been recognized. The extreme of one type gives many dicentric chromatids as the visible consequence of Ds mutations. The other extreme gives the "few-late" pattern of chromosome segment losses, i. e., few dicentric-forming mutations occur. The relationship between these two contrasting types of ~~Ds~~ mutation behavior of Ds in its standard location will be indicated after some of the supporting evidence for the conclusions (1) to (4) are given. Cross (2) -- C sh bz wx ds ac female by c-ml Sh Bz wx / c Sh Bz Wx, Ac ac male -- will be mentioned first.

On the ears resulting from cross (2), a number of kernels were variegated for Bz -bz. In those that had the Wx locus, the C bz areas most always were variegated for Wx -wx, i.e., Bz was lost before Wx. This would be expected if dicentric chromatid formation resulted from

Ds mutations at the C locus. (If Ds were in its standard position, the bz areas would be wx.) The results from one such cross are as follows:

C sh bz wx ds ac (Plant 4684-7)		x	c-ml Sh Bz wx / c Sh Bz Wx, Ac ac (Plant 4434D-5)	
C Bz Wx non-var.	C Bz - C bz Wx -wx		C Bz* wx not obviously var.	C Bz -C bz wx
124	12		89	41

The kernels resulting from crosses of type (3) were likewise useful because the classes are those expected on the basis of the conclusions given above. One such cross gave:

<p>Def. $\frac{c\ sh\ Wx}{SH\ wx}$</p>	x	c-ml Sh wx / c Sh Wx, Ac ac
(Plant 4642-6)		(Plant 4434D-5)

- 3 C Sh Wx non-variegated.
- 1 C Sh wx non-variegated
- 11 c to C lightly variegated; Wx-wx variegated; homozygous deficient tissues (from dicentric chromatids following Ds mutations).
- 19 c to C lightly variegated; wx. Homozygous deficient tissues present (from dicentric chromatid formation resulting from Ds mutations).
- 5 c to C, heavily variegated; wx. No obvious homozygous deficient tissues. (Such tissues would not be detected if dicentric chromatid formation occurred late.)
- 60 c Sh wx; no homozygous deficient tissue.

 * Unless early losses of Bz occur, it is often not possible to detect C Bz-C bz variegation. Bz substance from the surrounding Bz areas diffuses into the bz areas giving them the Bz phenotype. A few of the kernels in this ~~class~~ ^{class} may belong in the C Bz-C bz class.

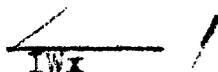
43 c to C. ~~variegated~~ mostly lightly variegated; Wx; no homozygous deficient tissue

240 c Sh Wx; non-variegated; no homozygous deficient tissue.

The plant arising from the original kernel showing c to C variegation (plant 4204) is now ^{believed} ~~known~~ to have had the constitution yg c sh Bz wx ds / Yg c-ml Ds Sh Bz wx ds, Ac ac. Before the transposition of Ds to the C locus was realized, the selection of sh kernels that showed c \rightarrow C variegation in the cross of this plant to c sh wx ds ac plants, was expected to give kernels having a c-ml locus but no Ds locus. Such c^{m-1} carrying chromosomes represent cross-over chromatids and if Ds had been present at its standard location in the c-ml Sh wx chromosome of plant 4204, they should have no Ds locus. Plants were grown from some of these c \rightarrow C sh kernels. Crosses of these plants were made to (1) C sh bz wx ds ac plants and to (2) plants having a deficient chromosome 9 (the deficiency included the C locus) Sh wx and a rearranged chromosome 9 with c sh Wx (as well as to c sh ds ac plants). Following cross (1), C Bz - C bz variegated kernels appeared on the ear. Following cross (2), homozygous deficient tissue appeared in the c \rightarrow C Sh wx kernels. Obviously, the Ds action had not been removed from the c-ml chromosome by crossing-over between c-ml and Sh.

Because the transposed position of Ds in the c-ml chromosome was not suspected this past summer, the most favorable types of crosses to illustrate a new position of Ds were not systematically made. Nevertheless, some additional crosses, made for other purposes, have been helpful because they are consistent with the above interpretation. An illustration of one of such case is given in the cross of a c-ml sh wx / c-ml sh wx, Ac ac male plant to an ac ac female plant that had

IWx



a terminal deficient chromosome 9 with C Sh wx. This

cross gave:

90 I Wx kernels

3 C Wx normal

34 C wx normal; no homozygous deficient tissue

20 C wx, with large amounts of homozygous deficient tissue.

The homozygous deficient tissue in the 20 C wx kernels arises as the consequence of dicentric ^{chromatid} formation associated with Ds mutations at the c-ml locus.

There are several points that require further consideration.

- (1) Why does a stable C locus arise when a c to C mutation occurs? What happens to the Ds locus during such an event?
- (2) What is the relation between the two visibly different types of events that can occur at the c-ml locus; (a) dicentric chromatid formation and (b) the c to C mutations that do not alter the chromosome morphology?
- (3) Does Ds in its standard location show these same two main types of behavior?
- (4) What interrelations exist between these two contrasting behaviors of Ds?
- (5) What is the evidence for the conclusions that no Ds locus is present at the standard location in the original c-ml Sh wx chromosome? Stabilization of the C locus following a c to C mutation of c-ml will be considered first.

Stabilization of the mutated c-ml locus.

On examining the variegated kernels arising from the first crosses (c sh wx ds ac x 4204), it was noted that the majority of the C areas arising from mutations of c-ml to C were not variegated for C to c, as could be expected. This expectation was based on the assumption that a Ds locus was present in its standard location in the c-ml

carrying chromosome of the male parent. If this had been true, 70 to 80 percent of the c to C variegated kernels should have had this Ds locus. Consequently, this absence of C to c variegation in the C areas was unexpected. I wondered at the time whether, in this case, a c to C mutation was associated with a change in Ac activity so that no further Ac controlled mutations would occur in the sector. The evidence for such a conclusion was not substantiated in other crosses. It was known that c to C mutations in one chromosome could occur following Ds mutations in the homologous chromosomes. This was revealed in kernels resulting from the cross of C wx Ds / C wx Ds ac ac female x 4204. It was obvious here that dicentric chromatid-forming Ds mutations could occur in the female chromosomes 9 early in development and that these could be followed in later cell generations by c to C mutations at the c-m1 locus in the chromosome 9 contributed by the male.

In the crosses of c sh wx ds ac x 4204, a number of germinal mutations of c to C appeared on the ears. None of these C kernels showed any C to c variegation of the Ds type. If a standard Ds locus were present in the C chromosome of these kernels, many of these C kernels should have shown the Ds activity by giving C to c variegation, for half of them should have an Ac ac ac constitution. 29 C, non-variegated kernels arising from these crosses, and from some similar ones made in the greenhouse last winter, were removed from the ears and plants grown from them in the field this past summer to determine (1) if an Ac locus were present, (2) if Ds were present and (3) if the C locus arising from a c-m1 mutation was stable in later generations. Crosses to c ds Ac Ac were made to test (2) and (3);

crosses to c sh wx ds ac were made to test for (2) and (1); and crosses of these plants by I wx Ds ac ac were made to test (1). In all cases, with the exception of the C kernels coming from one particular greenhouse plant (to be considered later) the C loci were completely stable in the presence of Ac. No Ds activity was observed.

It can be concluded, then, that a mutation of c-ml to C frequently produces a stable C locus and that dicentric chromatid-forming Ds activity, previously shown to occur when a c-ml locus is present, usually disappears as a consequence of the mutation phenomena resulting in a C phenotype from a c-ml locus.

Evidence for the conclusion that no Ds locus was present at the standard location in the original c-ml carrying chromosome

Conclusive proof that a Ds in the standard position would be capable of undergoing Ds mutations in C sectors arising from c-ml to C mutations and, therefore, that there was no Ds activity in the standard position in the original c-ml carrying chromosome, came from the following crosses. Plants were obtained with the constitution of chromosomes 9 as follows:

c-ml wx ac

c Wx Ds Ac

Ac was linked with Ds in the c Wx Ds chromosome (designated by Ds Ac). These plants were crossed to female c sh wx ds ac plants. A number of the ears have been examined but only 1 has been shelled and counted (table 2). The results from these crosses were obviously so alike that there has been no immediate pressure to obtain large numbers for a table. Because of the linkage of Ds and Ac, most of the c-ml Wx

Table 2

			4346-7	x			4664		
			c sh wx ds ac	9	x	c^{m-1}	wx ds ac		
						c	wx ds ac		
c wx	c	c - C*	c wx	c → C	C → c+	C			
non-var.	wx wx	wx → wx	non-var.	wx	wx → wx	wx		non-var.	
22	128	23	134	13	3	5			

* ^{Worm} C areas are C → c variegated.

+ Germinal C mutations. These kernels are C → c, wx → wx variegated.

chromosomes should have the constitution c-m1 Wx Ds Ac. As table 2 shows, all the c to C kernels with Wx likewise show Wx - wx variegation. In these kernels, the C areas are often variegated for C to c (Ds type). These c areas are wx. Also, in these crosses, all the C Wx kernels (germinal mutations) are C to c variegated with the c areas being wx:—typical Ds type.

There can be no question, then, that Ds mutations can occur in a c-m1 carrying chromosome following a c to C mutation if Ds is in its standard location. With Ds at the C locus (c-m1) a mutation of c to C usually eliminates the Ds locus or its action and the newly arising C producing locus is stabilized against further mutation phenomena. Mutations of c-m1 to C do not usually result in morphologically altered chromosomes 9. One obtains a normal chromosome 9 with a normal C locus action.* Nevertheless the Ds locus at c-m1 shows two main contrasting types of behavior (1) the production of dicentric chromatids and (2) the mutations giving rise to a C phenotype without alteration of the chromosome and usually with elimination of further Ds activity.

* The use of "normal C locus" must be qualified. Mutations at the c-m1 locus have given many apparently normal reacting C loci. There are a number of sub-levels and some super-levels as well as a few resembling the pink mutations (very rare) of c-m2 arising as the consequence of mutations of c-m1. These represent both quantitatively different reaction capacities as well as qualitatively different reaction capacities. According to my present interpretation, such quantitative levels of expression of C activity are to be expected as well as very much less frequent changes that will incorporate adjacent loci (the pink reaction). It would be more profitable to postpone this discussion until after ^athe mechanism ^{possibly} responsible for mutable phenomena has been discussed.

The standard Ds locus: Two contrasting types of behavior

As the last sentence above indicates, it is concluded that Ds activity is responsible not only for the dicentric chromatid formation at the c-m1 locus but also for the c to C mutations of this locus. This conclusion is based upon the striking resemblance between two contrasting types of Ds activity when Ds is in its standard location and the two contrasting types of events occurring at the c-m1 locus. The nature of the evidence for this general conclusion will be considered.

That the Ds locus in its standard position gives two main types of recognizable behavior, has been known for a long time. In an Ac ac ac constitution, Ds loci have been isolated that give many dicentric chromatids as the consequence of mutations of Ds. Kernels with appropriate genic constitutions reveal this clearly. Many of these mutations occur early in development. Such Ds loci have been designated "Ds-early". A contrasting type of Ds behavior results in only a few losses of segments of the short arm of chromosome 9 and they usually occur late. Such Ds loci have been designated "Ds-few-late". In the kernels with a few-late Ds locus, many aberrant types of chromosome breakage occur; the break~~ages~~^{ages} originate at positions other than the Ds locus. The significance of this will be discussed later.

To show that the few-late type of Ds behavior is associated with a particular state (organization) of the Ds locus and that it is not due to modifiers located elsewhere in the chromosome complement, the following crosses were made. Plants were obtained that had the constitution: I Sh Pz Wx Ds-early / I Sh Bz wx Ds-few late, Ac ac. These plants were crossed to female plants with C sh bz wx ds ac.

The types of variegated kernels, with respect to Wx and wx constitutions, were tabulated. The results of these crosses are given in table 3.

Crossing over between Wx and Ds is low, only 1 to 2 or 3%. The 10 I - C bz, wx kernels showing early losses probably arose from crossing over between wx and Ds. The 34 I - C bz, Wx - wx kernels, with the few-late loss pattern of Ds, represent cross-overs between Wx and Ds together with newly arising few-late Ds loci derived from early Ds loci. The few-late type of Ds locus arises rather frequently as a mutation of the early Ds locus but only rarely, ^(none observed as yet) does a Ds-early arise from a Ds-few-late locus. The evidence from table 3 strongly supports the conclusion that the early and the few-late types of variegation reflect two different "states" of the Ds locus. Other observations make this conclusion even stronger. One pertinent observation concerns the appearance of sectors showing the few-late type of Ds behavior in kernels that otherwise show the early type of Ds behavior. If the few-late type of Ds behavior arises rather frequently from the early type of Ds behavior, then such sectors should be seen.

The most instructive evidence for the presence of two such contrasting types of Ds behavior can be obtained from comparisons of these two types of behavior of Ds at the standard location with the same two types of behavior of Ds when at the c-m1 locus. I shall attempt now to point out the similarities.

Table 3

C sh bz wx ds ac ♀ x $\frac{I \text{ Sh Bz } \cancel{Wx} \text{ Ds-early}}{I \text{ Sh Bz } \cancel{Wx} \text{ Ds-few-late}}$ ac ac ♂

Cross	I Wx non-var.	I-C bz Wx Wx early losses	I-C bz Wx Wx "few-late"	I Wx non-var.	I-C bz Wx early losses	I-C bz Wx "few-late"	Odds*
4363-8 x 4679-2	42	46	4	39	0	33	4
4684-10 x "	60	58	1	73	0	51	1
4362-23 x 4679-3	126	93	15	117	6	114	3
4363-35 x 4679-5	77	72	1	63	2	87	4
4364-1 x 4679-5	120	92	13	120	2	79	5
Totals	425	361	34	412	10	364	17

* The "odds" are kernels showing transposed positions of Ds, that is, I Sh Ds Bz Wx or I Sh Bz Ds Wx

The relation between the two types of behavior of c-m1 and the two types of behavior of Ds at its standard location.

In the cross of a female c sh wx ds ac plant by a male c-m1 Sh wx c Sh Wx, Ac ac plant, the Wx kernels showing c to C variegation were of two distinct types. These were considered earlier but will be further considered here. One type was most frequent. It gave very few visible c to C mutations but many mutations leading to dicentric chromosomes. There were a few Wx kernels, however, with very many c to C mutations. In these kernels, few dicentric forming mutations occurred for very few wx spots were present. In general the pattern of these wx spots resemble in distribution and size the patterns given by the few-late Ds in its standard location. In both cases, losses of segments of the short arm of chromosome 9 occurred infrequently and late in development. With respect to types and frequencies of loss of segments of the short arm of chromosome 9, both can be classified as "Ds-few-late" states. Again, a similarity is found between those c-m1 Wx kernels showing low rates of c to C variegation but high rates of dicentric formation with the pattern produced by Ds-early. Furthermore, in some of these kernels, distinct sectors appear with high rates of c to C mutations. These sectors are Wx and have few if any wx spots in them.

To review: The kernels with high rates of c to C mutations give low rates of segmental losses in the short arm of chromosome 9. The pattern of these losses resemble the patterns of losses obtained from the few-late Ds in its standard location. The patterns of losses in the kernels with high rate of losses of segments of the short arm of the c-m1 chromosome, resemble the patterns of losses obtained from

the standard Ds-early locus. In the former kernels, the sectors with high rates of c to C mutations resemble in frequency and occurrence the sectors in the latter kernels that show low rates of segmental loss (Ds-few-late sectors). Again, c-m1 loci giving high rates of segmental loss throw c-m1 loci giving the low rates of segmental loss. Many of these, in turn, have a high rate of c to C mutation. It is concluded, then, that the state of Ds-early in the standard location and the state of c-m1 giving high segmental losses represent the same mutational state of Ds whereas the Ds-few-late in the standard position and the c-m1 with high rates of c to C mutations represent the same mutational state of Ds. From this, it is concluded

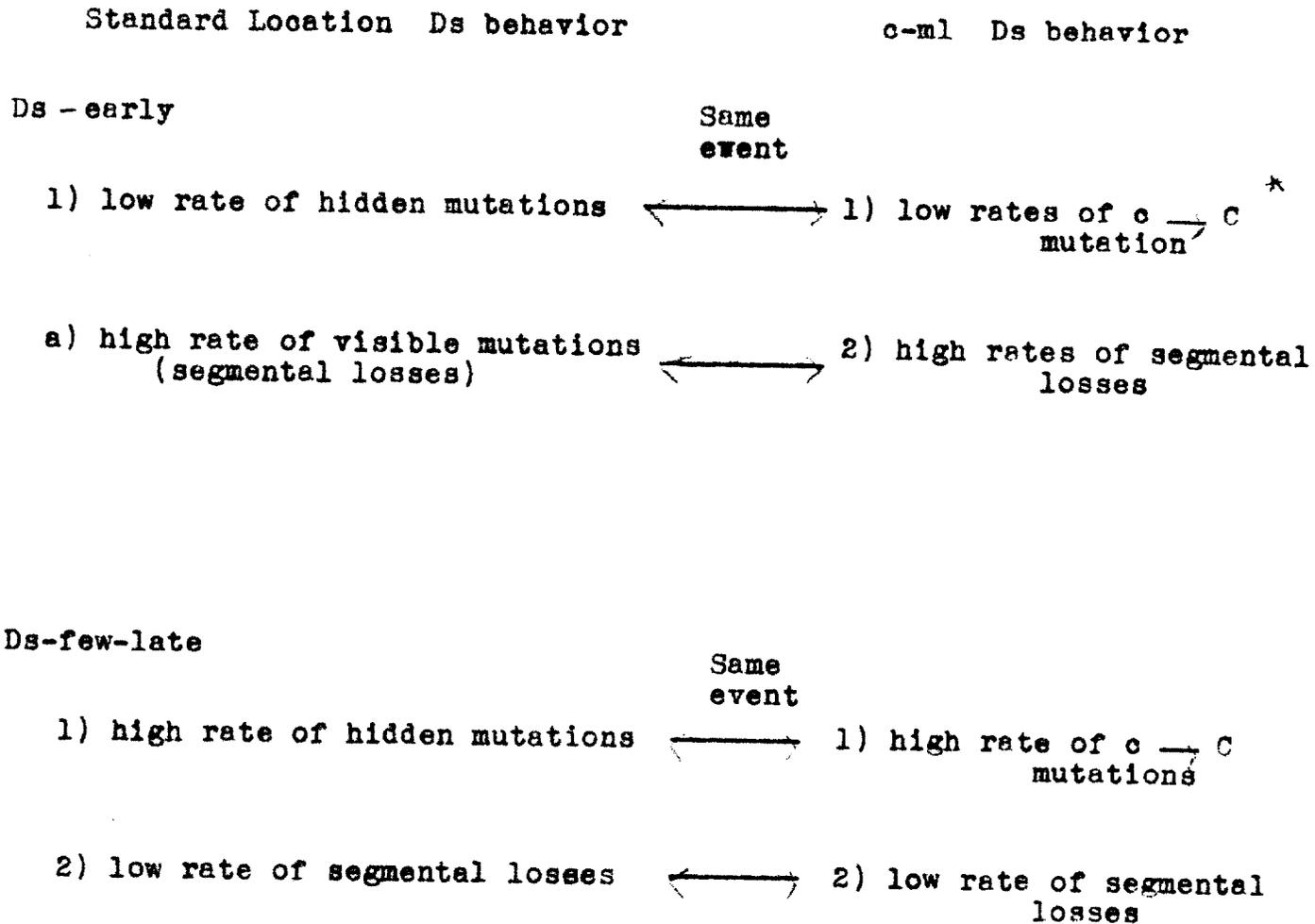
(See figure 1)

that the Ds-few-late, in its standard location, is undergoing many mutations ^{type} which are hidden because no visible phenotypic effects follow from such mutations. In order to see this type of mutation behavior of Ds, the Ds locus must be inserted into or close to a locus that will give a phenotypically detectible effect, following such a mutation. This occurs when Ds is in the c-m1 locus but does not occur when inserted in its standard location.

One more resemblance between c-m1 and Ds (standard location) should be mentioned. When kernels with high rates of c to C mutations of c-m1 are selected and plants grown from them, the high rate of mutation of c to C is retained in later generations. Again, in this respect, the high rate of c to C mutation pattern of c-m1 resembles the few-late type of Ds behavior (standard position) because in both cases, mutations back to the dicentric chromatid forming type of Ds behavior occur rarely. The "few-late" type of Ds behavior represents a relatively stable state of a Ds locus.

Figure 1

Comparison of Ds activity at the standard location and at c-m1



* See vol. 1, page 20. Varieties giving low c → c mutation rates but high direct loss will be recognized at another time.

Comparisons of rates of mutations of c-m1 and standard Ds.

It follows from these observations and conclusions that one is in a position to interpret the mutation rates in the kernels with C ds / C ds / I Ds-early, Ac ac ac (or Ac Ac ac, Ac Ac Ac) with those having c / c / c-m1, Ac ac ac (or Ac Ac ac, Ac Ac Ac). Because the color pattern produced by losses of I that occur as a consequence of dicentric forming Ds-early mutations are the same as the color pattern produced by c to C mutations, direct comparisons of mutation rates and patterns can be made. With these particular constitutions, the kernels showing high rates of c to C mutations and those having a Ds-early (standard location) may be indistinguishable in phenotype. Since c-m1 loci giving high rates of c to C mutation show relatively few segmental losses and since it has been concluded that the² loci have a few-late type Ds locus, it is further concluded that the rates of Ds mutation in both the early Ds state and the few-late Ds state are really the same. Ac controls the time and frequency of some event that occurs at a Ds locus whether or not the consequence of this event leads to a detectable change in phenotype. The mutations resulting in no detectable change in phenotype may be called "hidden" mutations. The presence of hidden mutations will remain a factor causing difficulty in the study of all mutable loci. Their presence is suspected in all the Ac controlled mutable loci. They are a factor that will interfere with any attempt to evaluate mutation rates of any mutable locus. Means of detecting and classifying the types of hidden mutations is absolutely necessary if an understanding of the factors involved in bringing about visible mutations is to be realized.

The Ac control of mutation during development

The similarity in rates of mutations at the c-m1 locus, when the $c \rightarrow C$ rates are high, to segmental losses rates at the standard Ds locus when these are high, has presented an opportunity to determine something about the nature of Ac action in particular cells during development. Why do mutations occur in some of the cells during development and not in others? If two Ac-controlled mutable loci are present in a cell, will a mutation occur at each locus at the same time in the same cell? How could this be determined?

Crosses of plants having c-m1 wx ds in chromosome 9 (high rate of $c \rightarrow C$ mutations) by plants having c (stable) wx Ds-early (standard location) in chromosome 9 should make this relationship analyzable. If, in the resulting kernels, the c-m1 locus in a c-m1 wx ds chromosome mutates to C, a C area will arise, the size and position depending upon when and where the c to C mutation occurred, that is, in what cell this occurred during development. If, in this same cell, a mutation occurred at the Ds (standard) locus in the c wx Ds chromosome, the wx locus will be deleted and the cells arising following this mutation will be wx. By examining the starch constitution of the cells underlying the C areas in the kernels resulting from such a cross, it should be possible to determine whether or not particular conditions, controlled by Ac, arise in certain cells and whether these conditions affect all Ac controlled mutable loci alike - that is, produce a mutation in all Ac-controlled mutable loci. If so, the C areas should have underlying wx starch constitutions and an exact correspondence in sectors should appear; or some relationship should be visible between the two types of mutations that will tell whether such a coincidence of mutations occurred, (that is, whether one-strand or two strands

are involved in the mutation process in a particular cell).

Fortunately, in some of these crosses, the frequency of coincident mutations of *c-m1* to *C* in a *c-m1* carrying chromosome and of *Ds* mutations in the *c Wx Ds* chromosome is remarkably high. A large proportion of the *C* areas have underlying *wx* starch. This is easily seen in the *Ac ac ac* constitutions but it is better observed in *Ac Ac ac* constitutions when a relatively early mutation occurs. In this latter case, the coincidence mutations are very frequent. There are a number of exceptions but such exceptions should be anticipated. This is because several types of hidden mutations occur and also because one of these changes the state of the locus. These newly arising states will give changed rates of visible mutations. Also, the mutation process itself frequently gives rise to exceptional consequences some of which are visible others of which are hidden, as will be shown later.

The high rates of coincident mutations, mentioned above, might lead one to conclude that the *Ac* control of mutability is related to some particular *Ac*-controlled condition that arises in particular cells during development. When this condition arises, it will affect a mutation at all *Ac* controlled mutable loci in this cell whether or not the mutation occurring at any one particular locus will result in a changed phenotypic expression. The *Ac* locus itself may mutate at the same time also. All of this may well be true but the difficulties in proving it are great. The crosses described above certainly suggest this conclusion regarding *Ac* control of mutable loci. Nevertheless, other crosses, originally thought to be similar, do not give such a striking coincident rate of visible mutations of these two *Ac* controlled mutable loci even though many coincident mutations did occur in the

development of the kernels on these ears. I am inclined to believe, from observing patterns and rates of visible mutations, that there can be a limited range of states of Ds loci, from ones giving few hidden mutations and many visible mutations to others giving many hidden mutations and few visible mutations. Combinations of c-m1 ds / c Wx Ds that are not reciprocally balanced in this respect would show a number of non-coincident visible mutations. This would not mean, however, that coincident mutations did not occur. Rather, it was not possible to see them.

A possible method is available for examining whether or not a range exists in the rates at which these two classes of mutation will occur. It involves various isolates of the c-m1 locus. Here, two of the types of mutations of a single Ds locus can be distinguished in c ds/ c ds / c-m1 Wx constitutions -- the c to C mutations and the segmental loss mutations. Also, some of the mutations leading to changes in state may be recognized. Selection of c-m1 loci giving intermediate frequencies between high c to C and low segmental losses vs. low c to C mutation and high segmental loss can be made. As yet, I do not have available for examination a selection of such c-m1 loci with the proper constitutions. However, a comparison of the behavior of the c-m1 locus derived from single plants in the crosses of these plants to both (1) c sh wx ds ac and (2) C sh bz ds ac certainly presents suggestive evidence for the presence of such intermediate states of Ds. The c to C rate of mutation following cross (1) has been compared with the C Bz - C bz variegation rate following cross (2). Medium rates of c to C variegation appearing in the kernels following cross (1) are correlated with medium rates of loss mutations in the kernels arising from cross (2). If this proves