February 8, 1954

The mechanism of transposition of Ds; Origin and behavior of c^{m-1}

I. Review of previous discussion:

1. Transpositions of Ds to positions in short arm of chromosome 9 between the known markers:

a). Recovered cases -- positions of Ds non-random. Ph[illegible], IDs Sh Bz [illegible] (1)

b). The aberrant kernels with transpositions -- wrinkled endosperms; dead embryos.

c). The lack of germination of about ½ of the selections.

d). The dominant lethals or semi-lethals. -- To be discussed later.

3. The changed states of Ds: One case discussed: The Ds - f.l. type.

a). Origin in one step from regular Ds -- many breaks.

b). Occurs only when Ac present.

c). From time of occurrence, suggests one of the consequences of changes occurring to Ds.

d). Return to former state -- occurs only through successive steps. Must select carefully. Ds - f.l., a relatively stable state.

e). The meaning of this state will become apparent when the mutable c-ml, derived from insertion of Ds at C is discussed. It is not that changes are absent at Ds but that they do not lead to dicentric formations.

f). These states important in understanding the nature of mutation at the mutable loci.

→ Meet - Ds positions: ① at new position, none at standard position. ② Ds at both new position & at standard [illegible]

II. The mechanism responsible for the transpositions of Ds.

1. Means of appreciating what the mechanism may be came from study of several cases in which a duplication occurred at the time of transposition of Ds. These cases will be discussed.

2. First case -- A kernel showing breaks between I and Sh as well as breaks eliminating I Sh Bz and Wx were seen in a single kernel in cross of:

Female C sh bz wx, ds ac x male $\frac{\text{I Sh Bz Wx Ds}}{\text{C sh bz wx ds}}$ $\frac{\text{Ac}}{\text{ac}}$

The ear:

I kernels		C kernels	
I Sh Wx, non-var.	59	C sh bz wx	128
I Sh Wx, var.	57 - Breaks to right of Wx		
I sh bz wx non-var.	5	C Sh Bz Wx non-var.	2
I sh bz wx, var.	0	C Sh Bz Wx var.	5 - breaks [illegible]
I Sh bz wx var.	0	C sh Bz Wx non-var.	1
		C sh Bz wx, var.	0

I Sh Bz(and few bz) wx non-var. 52 C sh bz Wx non-var. 31
I Sh Bz wx var. 0 C sh bz Wx var. 22

Odds: 1 I Sh Wx kernel with pattern of var. suggesting breaks between I and Sh as well as to right of Wx.

wx
I/kernel with C Bz areas. Spontaneous breakage type; not investigated

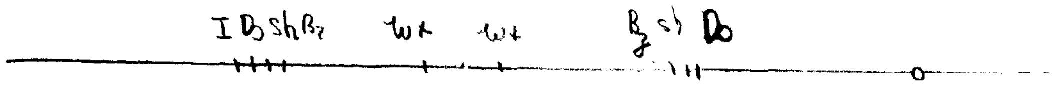
3. Plant grown from odd kernel in greenhouse. Matured very early. Had to be self-pollinated and could not be crossed -- no plants ready.

4. The kernel types on ear -- 13 different classes. Obviously something unusual about the I Sh Bz Wx carrying chromosomes:

178 with I : 96 with C
168 with Wx : 106 with wx.

Variegation patterns -- several types. Suggested duplication present.

5. The 13 different types of kernels grown in field in following summer. Cytological and genetical analysis made of the plants in each of the sub-cultures. Crosses made with each. Tests continued the following summer. The results showed that the male gamete contributed by the parent carrying I Sh Bz Wx Ds / C sh bz wx ds in the first cross carried a chromosome 9 with a duplication as follows:

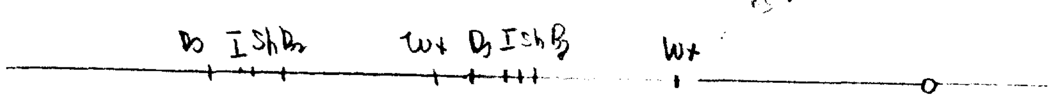


IV. Case II duplication:

1. Found in cross the subsequent summer. Cross the same type as in above case:

C sh bz wx ds ac female x $\frac{\text{I Sh Bz Wx Ds}}{\text{C sh bz wx ds}}$ $\frac{\text{Ac}}{\text{ac}}$

2. Analysis of this case showed: Duplication in tandem order. Ds to left of I.



V. Analysis of the possible origin of duplication and transposition of Ds.

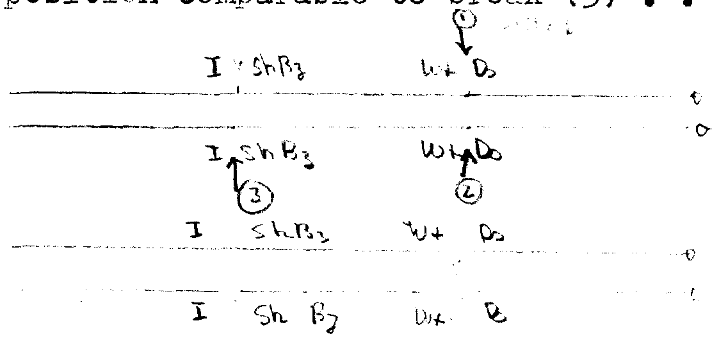
1. Required conditions in both cases:

a). Duplication arose from breaks in sister chromatids: In the I Sh Bz Wx Ds chromatid. This shown by composition of markers in each duplicated segment.

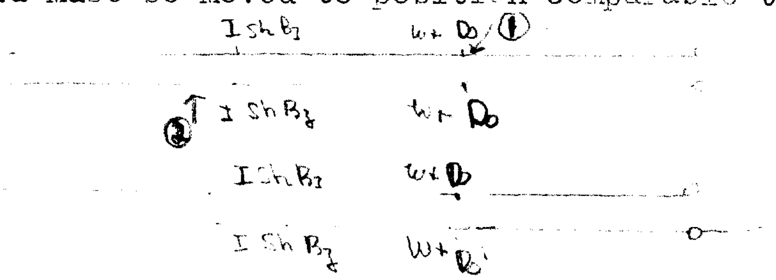
Dup. in Case I = inverted order with W+ Sh Bz

" " Case II = Tandem order with I Sh Bz W+.

b). Case I: Must have break in both sister chromatids to left of Ds, at least, to get duplication order (breaks 1 and 2), and one break in one chromatid (break 3). In this case, Ds inserted in the sister chromatid at the position comparable to break (3) ! !



c). Case II: Must have break in sister chromatids to right of Ds (breaks 1 ~~and 2~~) and to left of I in one chromatid (break 2). Ds in one chromatid must be moved to position comparable to break (2) in sister chromatid.



VI. How can all of this be accomplished by one type of event rather than the coincident event of transposition of one Ds to position corresponding with break in sister chromatid (break 3)? The evidence from the two cases suggest that the transpositi n phenomenon and the break positions are related and the expression of one type of initial situation associated with Ds action.

1. We know: Ds, as we have been following it, produces many dicentric chromatids at the locus where it may be. How does this occur?

2. Ds associated with the origin of translocations between chromosomes: These seen on a number of occasions. Therefore, Ds can produce the conditions leading to breakage and fusion.

3. A possible mechanism that accomplishes the transpositions and the chromosomal abnormalities: Associated with the state of Ds that produces dicentric formation. This important to keep in mind.

(Diagrams on board, pages 4a and 4b).

VI. The origin of c-ml.

1. In discussion of transposition of Ds, mentioned those cases where Ds inserted between markers. Will now discuss those cases where it is inserted at or adjacent to a known genetic factor. First case detected was that of insertion of Ds at the C locus.

2. The first detected case. Peculiar variegation pattern in cross of:

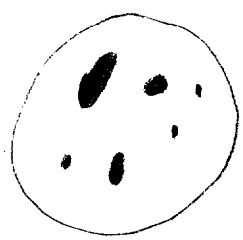
$$yg\ c\ sh\ wx\ ds\ ac\ \text{female} \times \frac{Yg\ C\ Sh\ wx\ Ds}{Yg\ C\ Sh\ wx\ Ds}\ \frac{Ac}{ac}\ \text{male.}$$

3. One male used in crosses to 10 different females of given constitution:

a). On ear, 1 : 1 ratio of CSh, non-var. to C to c var. with exception of single kernel. Photo of ear. Photo (2)

b). The pattern of variegation: of the usual type. Colored background with colorless areas. No color in the colorless Areas. Various types of patterns of losses of C in the different sectors on kernel.

c). Among a total of 3594, kernels, all as expected except one. The pattern of variegation on this kernel: colorless background with colored areas:



No colorless areas in Colored areas.

d). Plant grown from this kernel - culture 4204. Appearance of plant:

Many small streaks of yg. Ds-break type pattern: Concluded Ds in the Sh wx chromosome.

e). Plant crossed in various ways to test for subsequent appearance of peculiar variegation; for Ac; for Ds. The tests:

To c sh Bz Wx; C sh bz Wx; Self-pollinated; to and by Ac-tester.

to pyd c Wx / yg c Wx.

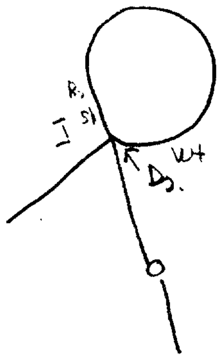
f). The appearance of the kernels in the croses to c sh: 9 ears.

23 full colored Sh		
509 colorless to colored var.	Sh:	725 colorless Sh
15 " " " "	sh:	1453 " sh

1449 kernels c → C. Photograph: c m-2 sh/sh. (3)

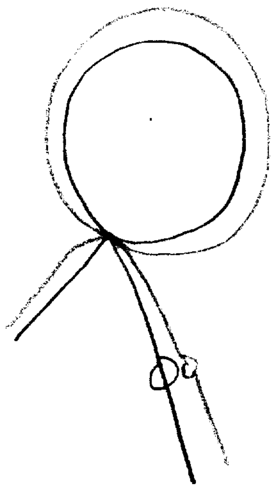
Case I

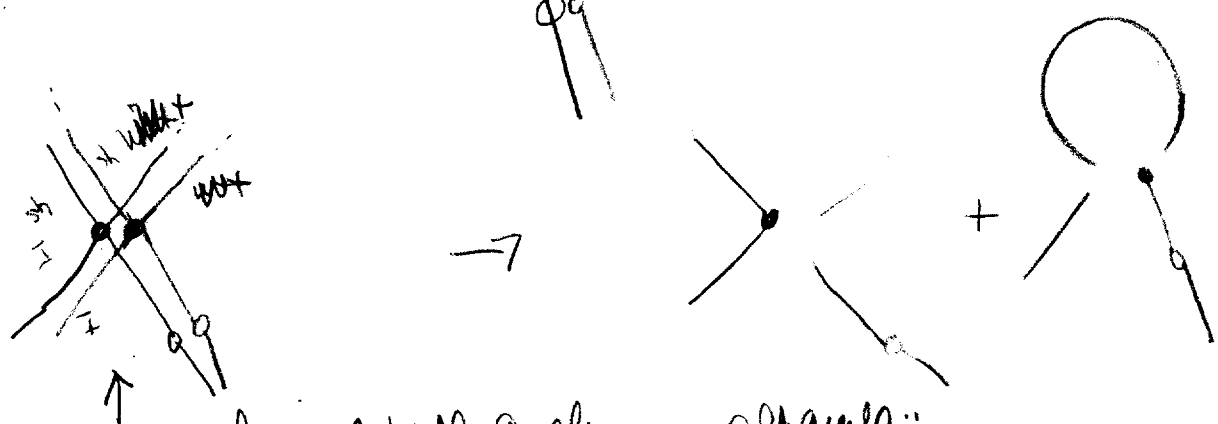
a) Assume single chromosome, before reduplication. native Ds becomes fused with a position in chr 9 between I + Sh:



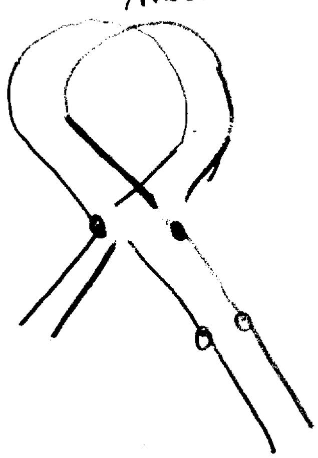
on board

b) Reduplication occurs:

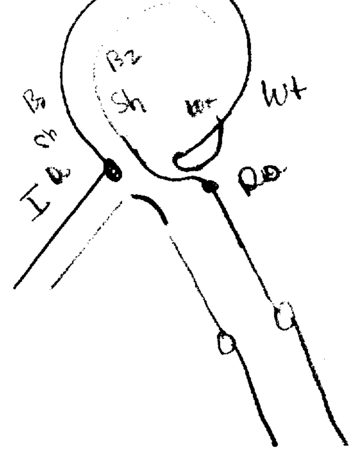




mechanical pull as chromatids separate:



Fusions



I Wx

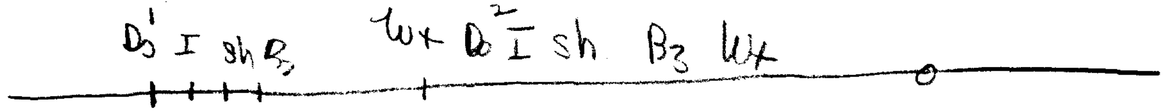
Fusions of broken ends 2+2 = general phenomenon occurring in all examined organisms.

Case II.

1. Origin: C sh bz wx ac ♀ × $\dfrac{\text{I Sh Bz Wx Ds}}{\text{C sh bz wx ds}}$ $\dfrac{\text{Ac}}{\text{ac}}$

occurred the year after origin of Case I.

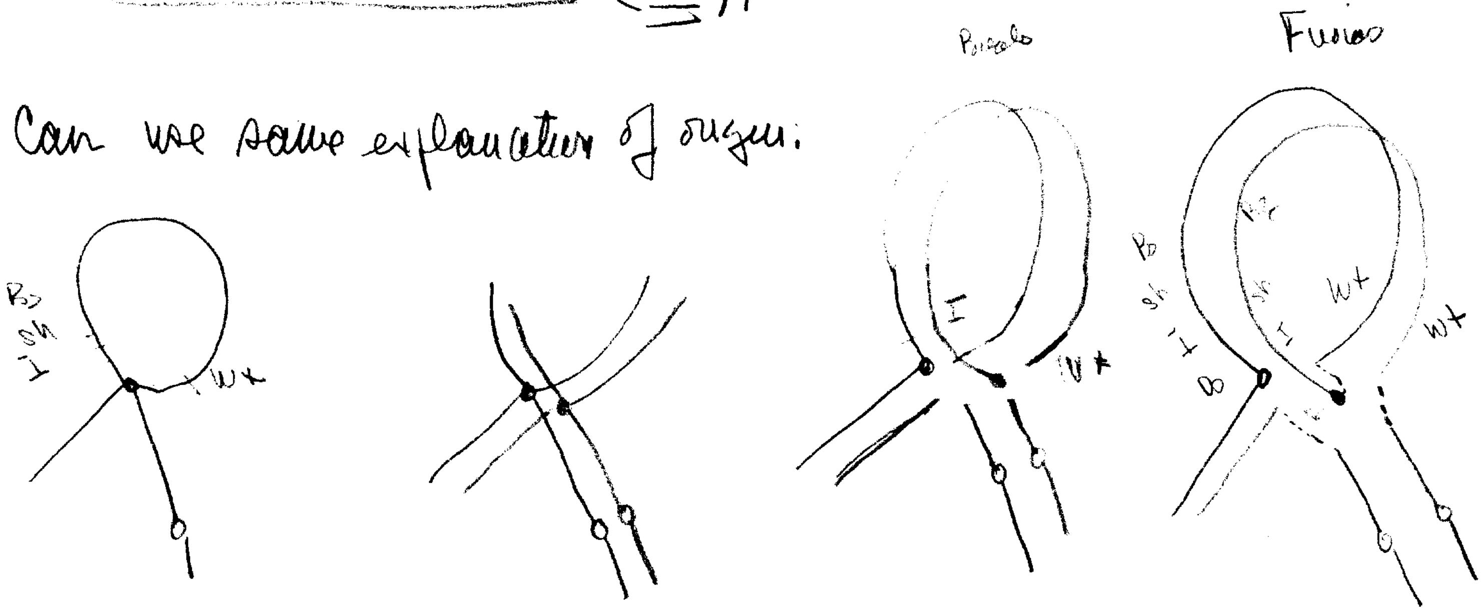
2. The duplication:



3. Must have originated from breaks in 2 sister chromatids of I Sh Bz Wx Ds Chr

4. Again. Positions of breaks of this duplication occurred at Ds standard position + at position of insertion of Ds, and occurred at same position in both sister chromatids (??), as in Case I.

5. Can use same explanation of origin.



Two Ds.

(1). Conclude that mutable c locus present.
(2). The mutable c (designated c-ml) appeared in a single tested gamete of the C Sh wx Ds/C Sh wx Ds Ac/ac grandparent. This shown by the linkage with Sh.

(3). Only ½ of the Sh kernels show variegation -- suggests requiremen of an activator. for mutations to occur.

(4). Ac present from tests of 4204 in Ac/ac constitution. Not linked to factors on c-ml chromosome.

(5). Types of variegated kernels on self-pollinated ear suggested the activator dosage behavior like that of Ac.

(6). Ds present, in the c-ml Sh wx chromosome but not to right of wx, probably; this shown in tests to C sh bz. Variegation in the Sh class and almost no sh kernels were variegated.

<u>Important:</u> None of the 23 kernels that were C showed any C to c variegation. This would be expected if Ds weve present and also Ac. Also, very few of the C areas in the kernels with c to C variegation showed any C to c variegation.

Ds activity certainly present; This clear in the plant variegation and also in the variegation produced in the crosses to bronze.

Question: Where is Ds; what is happening to it when a mutation occurs to C?

VII. The subsequent tests -- a very large number of them. Only an outline will be given of the important tests for our purposes.

1. Tests of plants derived from the c to C Sh Wx kernels in crosses of original plant to females c sh Wx ds ac.

a). Constitution: $\frac{\text{c-ml} \quad \text{Sh} \quad \text{wx}}{\text{c} \quad \text{sh} \quad \text{Wx}}$ $\left[\frac{\text{Ac}}{\text{ac}}\right]$

b). Plants tested for Ac -- in all cases, Ac present.

c). Crosses to c sh wx plants: Ratios of variegation of c to C those expected if c-ml is Ac controlled.

d). The crossovers: c-ml sh Wx and c-ml Sh Wx.

The variegated kernels: Ds present. Sectos of wx present in the colorless areas in kernel. The C areas were Wx (many):

b.f.b. cycles

The non-variegated kernels: c Sh Wx : None shoed any Wx to wx variegation.

The c sh Wx kernels: None showed any Wx to wx var.

e). Ds must be at or close to the c-ml locus as only the c to C var. kernels show presence of Ds type activity.

2. Tests of the colorless Sh Wx class in same cross as above:

a). Majority expected to give plants that are:

$\frac{\text{c-ml Sh wx}}{\text{c sh Wx}}$ probably no Ac.

b). All plants crossed to c sh wx ds ac plants: No variegated kernels appeared on ear, either for c to C or for Wx to wx.

c). Plants crossed to c sh / c sh Ac/Ac (allelic) plants.

(b) Nearly all kernels in Sh class showed c to C variegation.

d). Tested for Ac. All plants giving c to C in cross to c sh Ac plants had no Ac.

e). This and other tests established the Ac control of mutability at c-ml.

3. The response of c-ml to Ac doses: exactly like that of Ds.

VIII. The Ds type activity at c-ml.

1. Above tests show that breaks occur at or close to the c-ml locus as well as mutations to C.

2. Cytological evidence obtained for breaks occurring at known position of the c locus in plants carrying c-ml and Ac.

3. c-ml arose in plant homozygous for Ds at standard location:

C Sh wx Ds / C Sh wx Ds; Ac/ac

4. There was no Ds at the standard location in plant arising from the original c-ml carrying kernel. This shown by tests of this c-ml Sh wx chromosome in a number of individuals.

5. c-ml arose in a gamete that had lost Ds from its standard location.

6. Activity appears at locus of c-ml concommitant with origin of mutable c from a previously normal C. Both types of events, mutation to C, and breakage occur at this locus.

7. When mutation to C occurs, all Ds type activity ceases at the locus of the mutated C.

8. Conclusion: the mutable c arose from transposition of Ds to C locus. the mutations arise from removal of Ds from this locus.

Therefore, c-ml, being Ds at C, is Ac controlled.

Ds at the C locus -- CDS -- is c in activity.

The recessive c very stable if Ac absent. Test of over 20,000 kernels obtained from crosses of c-ml/c no Ac to c no Ac.

VIII. Tests for the stability of the mutatědnC from c-ml.

1. Tests of the C kernels arising from germinal mutations:

In crosses of c-ml to c in presence of Ac.

Plants grown from them; Self-pollinated; crossed to c ds ac; crossed to c ds Ac; tested for presence of Ac.

In all cases (over 30 examined), the C is stable;

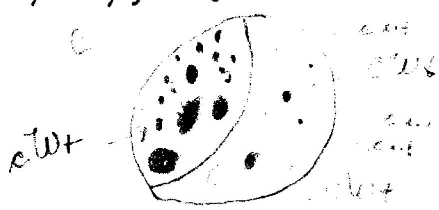
2. Tests of a few cases (rare) in which Ds activity present in the C carrying chromosome.
Some - a new transposition of Ds. In another position in short arm of chromosome 9.
In several cases, Ds still very close to the mutated locus.

IX. Before other tests with c-ml considered, must examine the states of c-ml, and the changes in state.

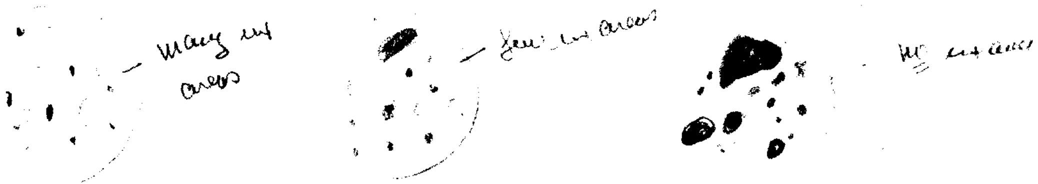
1. The original state of c-ml: Many breaks at c-ml locus; relatively few mutations to C.

2. The appearance of sectors with changed relationships on the kernels:

Constitutions: c-ml / c / c /; Ac. ac ac



3. The appearance of kernels with various altered relationships between frequency of mutation to C and frequency of breaks at c-ml.



4. The tests conducted with the various kernels: The high rate of c to C mutations and low rates of breaks or none will be used as example.

Tests --

Stability in subsequent generations:

5. The relation of the states of c-ml to those of Ds at other positions:

a). Indicates that the Ds-f.l. is like a c-ml with high rates of mutation to C and low breakarates. Therefore, the Ds in the cases of Ds-fl is actually undergoing some change in many cells but these do not show as breaks in the chromosomes. In the case of c-ml, we see the other type of event as a mutation. In the cases where no mutation can be seen, we can observe only the breaks. These a small fraction of the alterations occurring at Ds, however.

X. The similarity of responses of c-ml and of Ds to changes of Ac.

1. Comparisons between c-ml with few or no breaks and Ds in standard location with many breaks.

2. The response to states of Ac -- example; Stabilized Ac.

a). The pattern produced by stabilized Ac in cross of

C sh bz wx ds ac female x I Sh Bz Wx Ds Ac-stabilized (Photo)

b). The pattern of c to C mutations in crosses of:

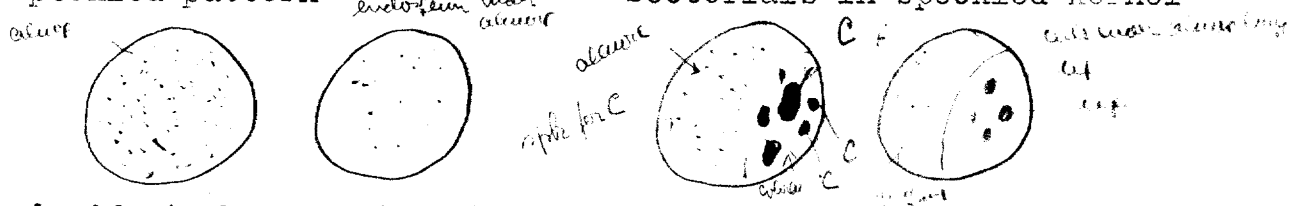
c / c no Ac female x c-ml Stabilized Ac -- the very same.

3. Responses to changes in Ac occuring during development: The type of cross:

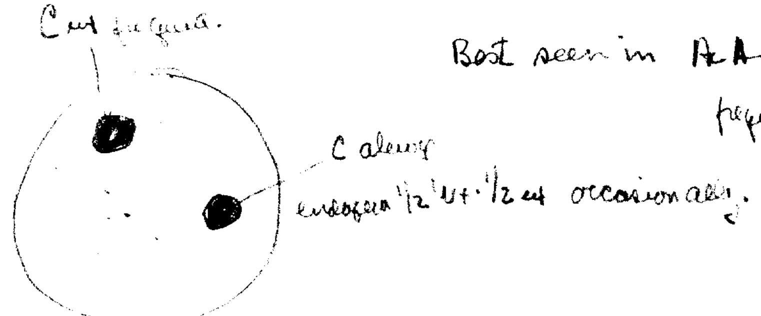
female c-ml wx / c-ml wx Ac x male c^s Wx Ds, no Ac:

The kernels: c^{m1} wx / c^{m1} wx / c^s Wx Ds; Ac Ac ac

Speckled pattern — Sectorials in speckled kernel



4. The coincident changes at c-ml and at Ds-standard in these kernels.



Best seen in Ac Ac ac kernels. The early changes = frequent coincidence.

(Cont remember the ratios of different types as data not here.

I. Origin from Y_g c sh wx ds ca ♀ × Y_g CSh wx Ds / CSh wx Ds $\frac{Ac}{ac}$ ♂

1. The kernels on ear – 3594 – 1/2 CSh wx; – 1/2 CSh wx with c areas.

2. The exceptional kernel – reverse pattern. C areas, no C → c.

II. The plant from exceptional kernel – appearance. Y_g → y_g. Ds type.

1. Tested for Ac: Ac/ac constitution

2. Crossed to c sh wx ds a; C sh y_g wx ds a; y_g Ac tester; ⊗.

3. The kernels on ear from cross to c sh – 1/4 var. in Sh class mainly.

23 C – all Sh

524 c → C var. – 509 Sh; – 15 sh = 3%

2178 colorless – 725 Sh; – 1453 sh

a) The variegation type: The C areas = no C → c or rarely.

4. The tests for Ac control:

a) c → CSh kernels in ears (3) above = all plants have Ac.

b) the c Sh non-var. " " = 96-7% 30 ears:
a) to c sh = all non-var
b) to Ac tester = no Ac (3% C.O.)
c). to c^s/c^s, Ac/Ac = variegation appeared.
d). The dosage action of Ac –
1 Ac = early mutation
2 Ac = spk pattern or sectors
3 Ac = very late spk

c) other tests – to be described later.

Cor project.

5. Tests for position of Ds.

a) c → C sh Wx kernels in crosses of original plant: c m1 Sh wx / c sh wx, to c sh Wx. Plant = $\frac{c\ m1\ Sh\ wx}{c\ sh\ Wx}$

b). Plants from these crossed to c sh wx; C sh of wx.

c). The cross-over classes (colorless var.) = c m1 sh Wx; c m1 Sh Wx.

① Appearance of kernels =

d). The non-var kernels in colorless, non-var. classes = c sh Wx + c Sh Wx = none showed any Wx → wx var.

e). Ds action at C locus. None in position to right of wx. The wx areas = from b.f.b. cycles, no C as consequence.

6. The changes in state of c m-1.

I a) First state - rate of c → C + Wx - wx areas

b) The sectors -

c) The kernels with altered states

d) The tests of plants from these kernels.

e) The stability of states of c m-1. Resemblance to stability of states of Ds.

&

II to c stable = c m1/c m1 Ac/ac x c^s (⊙ 3% colorless kernels

1) Colorless kernels - removed.

2) Tested for Ac +

3) Some = no Ac; some = stable c from c m-1, no breaks.