Lecture 3. January 14, 1954

The location and cytological behavior of Ds. ← Jan. 18

I. Review of previous discussion

1. Began discussion of variegation produced by loss of segment of chr. 9

2. The pattern of loss during development of tissue followed that shown by mutable genes -- those where mutation occurs from recessive to dominant.

a). In mutable gene, mutation from recessive to dominant controlled by system. Time and cell in which mutation will occur is due to this system.
b). Somatic segregations of factors controlling system
c). Alters time and frequency of mutation in descendent cells.

3. The loss phenomenon -- follows this same pattern but instead of mutatio there is loss of a segment of chromosome 9 short arm.

4. Two apparently different variegation phenomenon have something very much in common.

5. Both seem to be governed by something occurring at a locus in a chromosome during the mitotic cycle. The chromosome loss phenomenon -- effect obviously on chromosome.

II. Need to determine nature of the event involving chromosome loss as it could through light on the nature of the mutation process occurring with mutable loci.

1. First question -- Is all of the chrmosome lost? Is one position in chrom some involved as with mutable loci? Is it specific?

III. The initial test to answer these questions

1. Test started last period:

Female parent	Male parent
Re C wx / Re C wx	I Wx / C wx from variegated kernel.

2. The ear -- I Wx, var. for C wx kernels selected. Plants grown from them. Their constitutions:

3. Normal chromosome 9 I Wx / Inversion " " C wx — For no crossing over in short arm of chr. 9.

4. This plant, as male, crossed to plant with C wx: Kernels on ears (11)

a) 600 I Wx, non variegated
588 I Wx, areas of C wx — Only ½ of I Wx kernels are var.
1550 C wx.

b) The of gamete I Wx : : C wx

5. Same male parent as 3. crossed to plant homozygous for c wx:

Kernels on resulting ear 1 colorless Wx
1 " with wx areas
2 Colored wx, non-variegated.

6. Conclusions: (1) Something carried by the I Wx chromosome responsible for losses.

(2). Something else necessary for losses to occur. Only ½ of the gametes with I Wx produce kernels that show variegation.

(3) Ratio suggests that separate factor present in male parent: Only half of gametes get this factor: Gametes

I Wx with factor

I Wx, no factor.

(4). This proved to be true. Factor given the symbol Ac, for activator. It activates the breakage events in the I Wx chromosome.

(5). This plant: I Wx / C wx 1 Ac (Ac ac) in cross to Cwx

Its gametes:	Kernels on ear
I Wx No Ac	I Wx, non-var
I Wx Ac present	I Wx; C wx areas
C wx Ac	Colored
C wx, no Ac	"

Will consider Ac next period.

III. The tests for the location of factor in I Wx chromosome responsible for loss.

1. Above plant: Normal chromo. 9 I Sh Bz Wx/ Re C Sh Bz wx 1 Ac

2. Crossed to female homozygous for normal chr. 9 with C sh bz wx.

3. Gametes harrying I Sh Bz Wx and Ac produce kernels that are variegated: Sectors present that are C sh bz wx. This due to somatic losses of I Sh Bz Wx during development of kernel.

Appearance of kernels. Photographs of kernels. (1) The Bz rims.

4. These variegated kernels selected from ear. Plants grown from them Their constitutions

I Sh Bz Wx / C sh bz wx, Ac

5. These plants crossed to plants that were C sh bz wx, no Ac.

6. The gametes carrying the I part of chromosome:

4 2 10

I Sh Bz Wx

C sh bz w wx

	Genotype of male gamete	Phenotype of kernel	Non-var.	Var.
Non crossovers:	I Sh Bz Wx	I Sh Wx	59	57
Region 1	I sh bz wx	I sh wx	5	0
" 2and 3	I Sh bz wx and I Sh Bz wx	I Sh wx	52	0

Result: Only chromosome with Wx shows breaks.

The C class of kernels on the ear:

Non-crossover	C sh bz wx	C sh bz wx	128	
Region 1	C Sh Bz Wx	C Sh Bz Wx	2	5
" 2	C sh Bz Wx	C sh Bz Wx	0	1
" 3	C sh bz Wx	C sh bz Wx	31	22

Plus: 2 odd kernels in I class -- to be discussed later. Total, 364

General appearance of the ears showing such ratios: Illustration (2) Ear.

7. Conclusion: Some factor, located to right of Wx, responsible for the loss phenomenon. These will occur, however, only when Ac also present. Otherwise, no losses occur. Ac will not induce loss in chromosome without the loss factor being present.

8. The second test cross for location of factor: Same male parent to female parent homozygous for c sh Bz wx, no Ac: We will follow only the kernels that are colored.

	Gametes	Kernels Non-var.	Var.
a). The non-crossovers:	C sh wx	99%	1%
Region 1	C Sh Wx	50%	50%
Region 2	C sh Wx	50%	50%

b). Again, factor in I Wx chromosome -- to right of Wx. The few C to c var. kernels in C sh wx class indicate factor is very close to Wx.

c). When this factor present, loss of all markers to left of it occurs to produce the variegation.

d). Question: Does a break occur here in chromosome? What happens because of this factor that produces losses?

IV. Cytological examination of the breakage mechanism.

1. Piece lost is large -- could easily be seen in cells.
2. Losses occur in sporogenous tissues -- therefore sporocytes could be examined for these losses.

3. The reason it was known that losses occurring in some sporogenous cells. Pollen examinations of plants carrying I Wx and factor / C wx no break factor.

Normal pollen from I Wx / C wx, no break factor.	Pollen from above plants. (with Ds and Ac)
1 Wx : 1 wx	Not 1 : 1. More wx grains than Wx. A number of nearly empty grains. Difference between 1 : 1 due to losses of I Wx part of chr.

4. The appearance of the maize chromosomes at prophase of meiosis:

a. The premeiotic growth of chromosomes.

b. The meiotic prophase chromosomes -- long rods. Synapsis.

c. The method of collecting sporocytes and making examinations.
Tassel samples; anthers selected; sporocytes squeezed out on slide in drop of stain; examined microscopically.

d. The 10 bivalents at pachytene Rhoades, figure 3 (4) (1)

Note. 10 rod bivalents - size differences
The centromeres - 7, 9, 10, 2
The knobs, 7, 5, 4
The nucleolus, nucleolus chr. n. or.

e. The contraction period. Rhoades fig. 3 Diplotene (4)

f. Diakinesis - Rhoades figure 3; Metaphase I. Rhoades, fig. 3 (4)

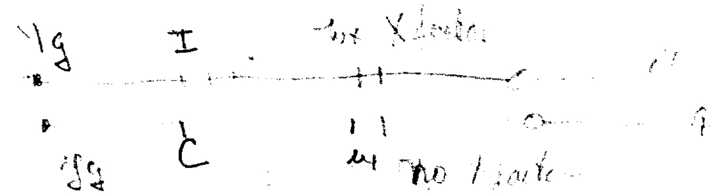
g. AI to A II - Rhoades, figures 4 and 5. (5) (2) + (6) (3)

5. The constitution of the plants first examined: From cross of

Female	Male
yg C wx ds no Ac	Yg I Wx Ds, Ac.

Selection of the variegated kernels I Wx with C wx areas Plants grown from them. Their constitution; <u>appearances</u> Sporocytes examined.

Plant constitution



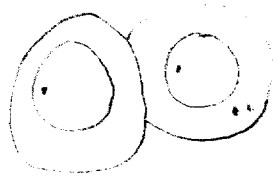
Leaves.

Sporocytes collected from tassel. Examined. Showed:

1. Many cells with normal bivalent chromosome 9:

2. Some individual cells showed break:
Position fits genetic evidence of locus of factor.

3. Some sister cells showed:



4. Cluster of 4 cells in anther:

5. Conclusion: Breaks are occurring at position in chromosome where genetic evidence had located the responsible factor for variegation phenomenon.

4. The question: In cells examined, could not tell which of two chromosomes carries the factor. Do breaks always occur in the Ds carrying chr? Do they also sometimes occur at same position in the homologue?

a). This tested in following way: The two members of the bivalent chromosome 9 must be distinguishable from one another in the short arm. In this way, it can be proved whether or not the breaks occur only in that chromosome 9 carrying the factor.

b). How individuals heteromorphic for short arm of chromosome 9 obtained.

Plant with

Plant with

On ear, I kernels with C areas present. These selected, plants grown from them. The sporocytes collected and chromosomes in them examined.

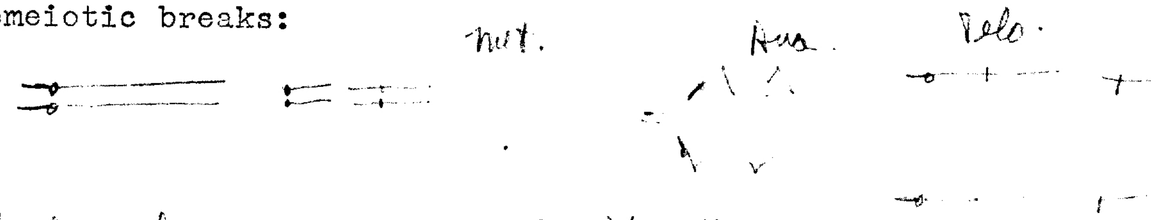
c). The observations:

1. Many cells with bivalent:

2. Some cells, scattered here and there in anther with:

3. The lack of synapsis of the acentric fragment with homologuous part in chromosome.

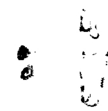
4. The premeiotic breaks:

a): 

b). Meiotic prophase Synapses in each sister cell.
Photo (10) (7)

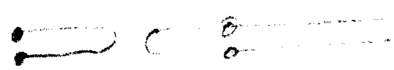
c). The observations.

Photo

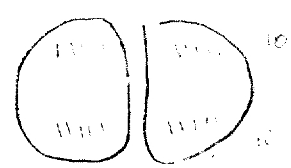
5. The plant homozygous for Ds  + 1 Ac. simultaneous breaks. (11) (9)

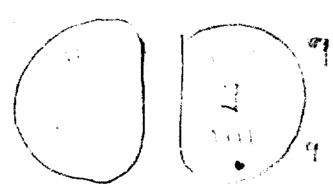
6. What takes place at locus of Ds to produce the dissocaation?

a). Normal chromosome reduplication gives prophase: 

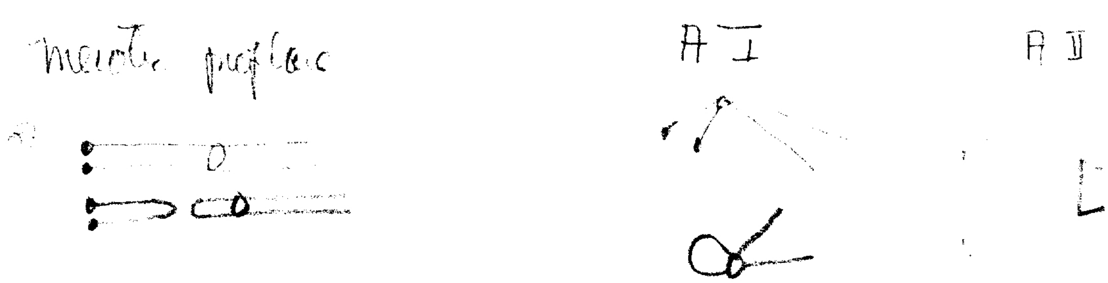
b). When Ds present, and a dissociation event occurs, it results in: 

c). This suggested initially by presence of bridges in the second meiotic mitosis:

Usual appearance: 

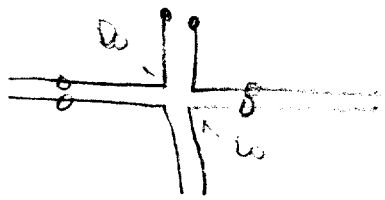
Some cells : 

d). How these latter produced:



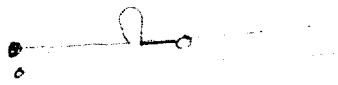
V. The additional types of chromosome alterations produced by Ds. They are a small fraction of the total. Most events at Ds produce the above type of alteration.

1. The translocations: One breakt at Ds in chromosome 9 short arm: Found in few related cells in an anther.

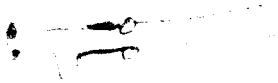


2. Inversions:

3. Duplications:



4. Deficiencies:



5. Ring chrom s omes 9

6. Few with more com lex rearrangements involving short arm of chr. 9.

VI. Emphasize: No breaks of any sort occur at Ds unless Ac is also present.

Ac not in chromosome 9. Shown by earlier ratios.

Ds is completely stable without Ac -- generation after gener.

VII. Ac should not be considered. Where is Ac ? How does it act?

1. Review: In above described crosses, half of gametes carrying Ds chromosome have Ac and other half, no Ac.

Suggests Ac carried by separate chromos e. Segregated at meiosis independently of Ds chromosome.