2nd lecture. January 11, 1954

The discovery of Ds. Its behavior and its relation to mutable loci.

I. Review of previous talk:

1. Sudden appearance of a large number of newly arisen mutable genes -- expressing variegation-- in progeny derived from self-pollinations.

2. The origin in plants that had undergone the chromosome type of breakage-fusion-bridge cycle in early development. Healed broken ends of chromosome 9 in recovered branch.

3. The various types of plant characters showing variegation. Various types mentioned affecting chlorophyll expression. More types later affecting other characters.

4. About 40 different origins of mutability recognized in early experiment

5. The features shown by the seedling exhibiting variegation:

a). Background - altered phenotype.

b). On background, streaks of normal phenotype

c). The normal streaks present in a decided pattern. Reflect:

(1) Time of change to normal during development of tissue -- determines the size of the streak

(2) The number of streaks: reflects frequency of occurrence of changes to normal phenotype during development.

(3) The relative distribution of streaks: reflect the distribution among a growing tissue in which changes occur in specific cells.

(4): The different types of patterns: The Seedlings:

Many later occurring changes

Few late occurring changes.

many early changes. Leaf a mosaic of many patterns.

d). Within a single progeny, one type of pattern usually predominant.

6. The changes in the pattern of mutations occurring during development.

a). Sectors formed: 1). Increased number of streaks
2). Decreased number of streaks
3). No streaks
4). Large mutant sector

b). The twin-sectors: One sector composed of two parts:

Types: 1). Normal phenotype: altered phenotype - no streaks
2). " " : " " - increase or decrease in number of streaks.
3). No streaks : increased or decreased frequency of streaks.
4). Increased frequency of streaks: decreased frequency of streaks.

c). The interpretation of the twin sectors: Each component of twin derived from one of two sister cells. Both sectors arose from single cell as consequence of a mitotic event which produced two sister cells.

7. Interpretation of the nature of the change that occurred to give rise to single or twin sectors:

a). Particular pattern in seedling leaf reflects a controlled type of expression of time when mutations will occur, and the cells in which they will occur.

b). The factors responsible for this control may be altered as a consequence of a mitotic event.

c). Following alteration, there will be a different pattern of mutation in the descendent cells from that present before the change occurred.

d). The twin sectors suggest that controlling factors segregate at a mitosis: one cell receives something that the sister cell lost.

e). The mutant - altered pattern twin sectors: Suggest that the mutation itself is consequence of a mitotic event altering something.

8. The major questions and propositions:

1. What factor or factors are responsible for producing a particular pattern of mutations during development? i.e., (1) the time during the development of a tissue when mutations will occur. (2) The cells of the tissue in which this will occur; i.e., the frequency and distribution of changes that will occur in particular cells during development.

2. How does the factor, or do the factors segregate in certain cells as the consequence of a mitotic event?

3. Is the process of mutation associated with the same type of event?

4. What component of the mitotic process is involved?

a). Is it a segregation of cytoplasmic components?
b). Is it related to the chromosomes? To changes at specific loci?
c). Is it related to the process of chromosome reduplication?

II. The period of decision and the early investigations.

1. Original prupose, to analyse the genic components within the short arm of chromosome 9.

2. The presentation of a wholly unexpected phenomenon - the variegations.

3. Gradual conviction, following growing of the variegated plants -- that the phenomenon involved in the expression of variegation was far more important than that of the original purposes, stated above.

4. How could this phenomenon be investigated?

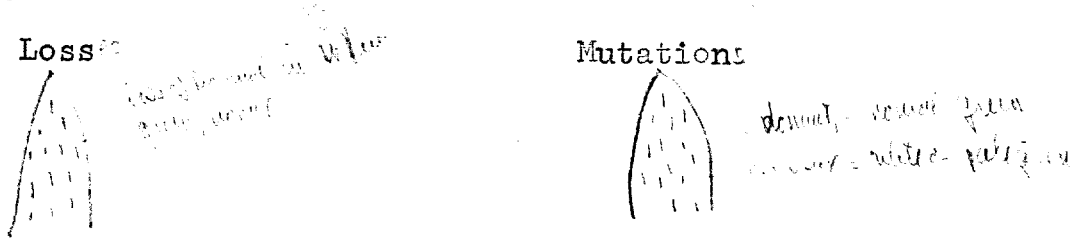
a). At first, did not know.
b). As first step, decided to investigate inheritance behavior of a number of selected cases; and the inheritance aspects of the pattern controlling systems. This in order to get facts with which to think.

5. First year and a half -- Results confirmed earlier observations. Showed that the alterations of phenotype were changes at the locus of a particular gene; a mutable gene. The change resulted in mutation from recessive to dominant. (Mutations).
The control of the mutation process not well distinguished in early studies but suspected a similar type of change as that producing mutation.

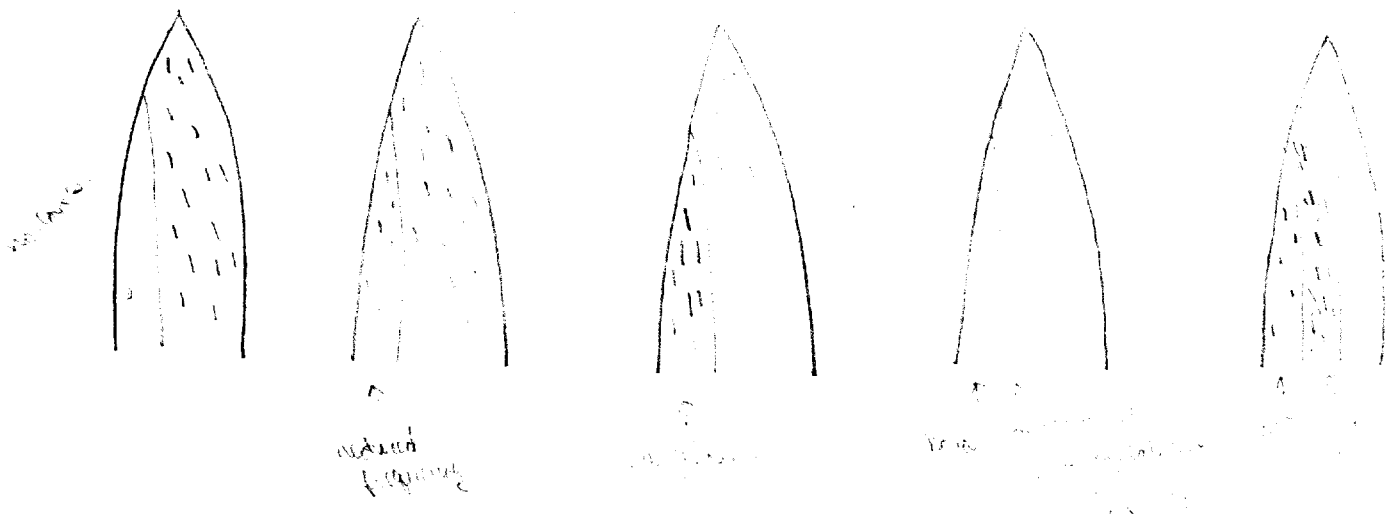
III. The discovery of a variegation system that altered the chromosome composition of nuclei during development. Its major mode of operation:

1. A part of short arm of chromosome 9 eliminated in certain cells during the development of a tissue.

2. The pattern of such losses in the various tissues simulated the patterns produced by the mutable genes, where mutation from recessive to dominant occurs.



3. The changes in patterns of loss, as seen in sectors, similar to the changes in mutation time and frequency noted with mutable loci:

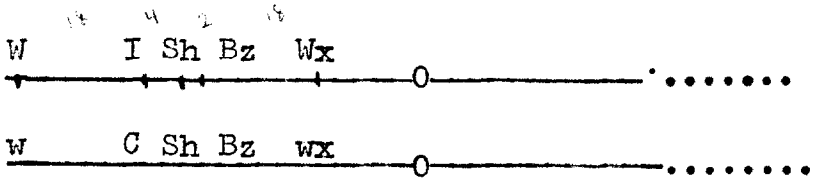


IV. How the system involving chromosome loss was first recognized:

1. First seen in some kernels derived from self-pollination of one plant that had undergone chromosome type of b.f.b. cycle in early development

2. This plant -- carried two chromosomes 9 with newly healed broken ends.

3. The genetic factors carried by each chromosome:



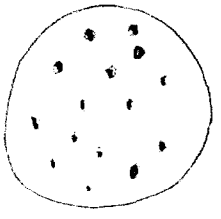
4. The factors carried in the short arm of chromosome 9 -- necessary to know them to continue study:

W, w; Yg, yg. -- At end of short arm.
I, C, c
Sh, sh
Bz, bz
Wx, wx

(a) Crossing over between markers
(b) Physical position in chromosome

ILLUSTRATIONS Ears

5. The pattern of variegation exhibited on some kernels of self-pollinated ear:

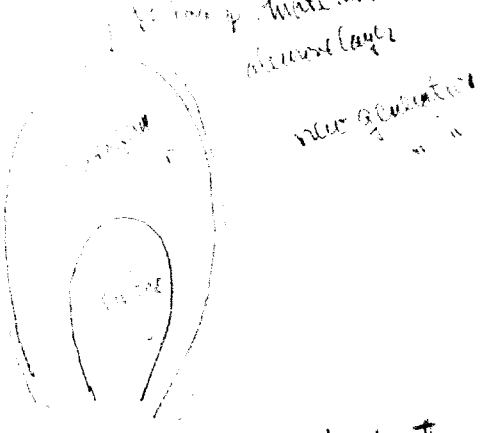


V. The structure of the maize kernel and its development.

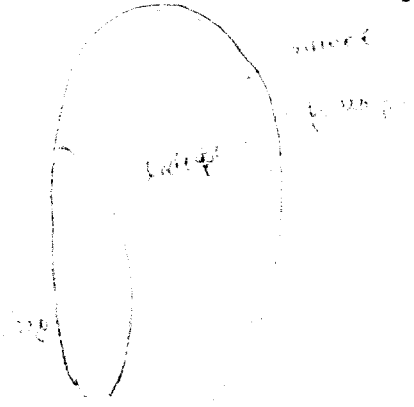
1. Must understand this for any discussion of maize genetics or cytogenetics. Will be given diagramatically for those who are not familiar with the morphology and development of kernel.

2. The morphology of the mature kernel: (ILLUSTRATIONS * Cut kernels).

View from embryo side



Cross section -- longitudinal.

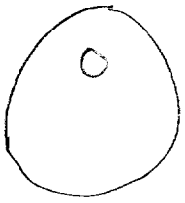


1) Randolph
4) Kuwada
illustrations 3) Cut kernels

3. The origin of the endosperm and embryo in the kernel.

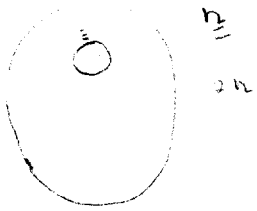
a). The ovule on ear, before fertilization:

Ovule
Megasporocyte

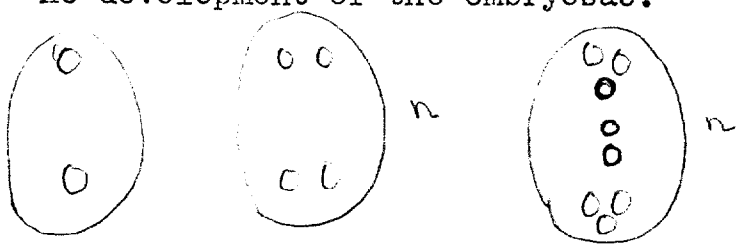


Meiotic mitoses

Megaspore



b). The development of the embryosac: From megaspore n



4. The development of the male gametes:

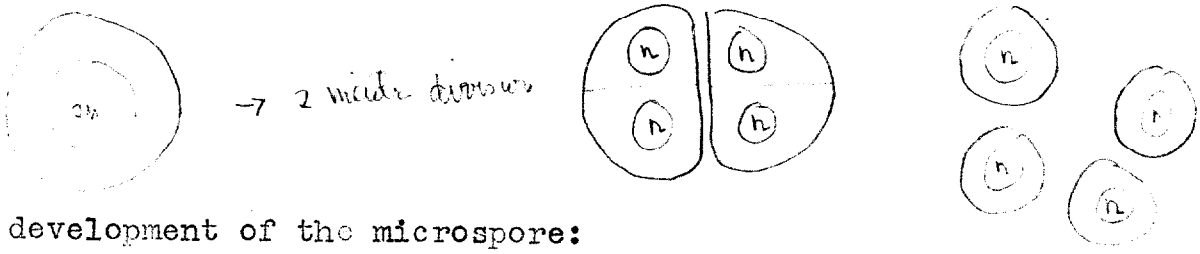
a). The young tassel, inside of leaves: telesppoed effect.

b). Composed of flowe s along branches of tassel.

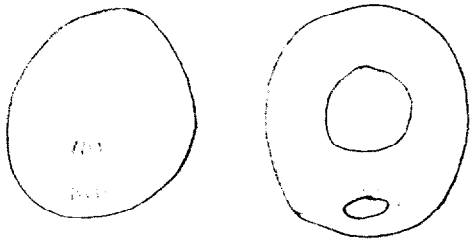
c). Each flower has three anthers.

d). Inside anthers at young stage are enlarging cells - the microsporocytes.

e). The meiotic mitoses and development of microspores:



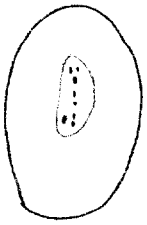
f). The development of the microspore:



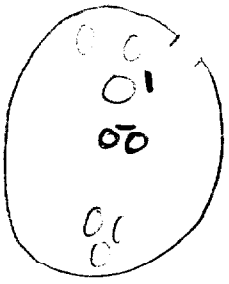
g). The development of the pollen grain and sperm cells:

5. The process of fertilization:

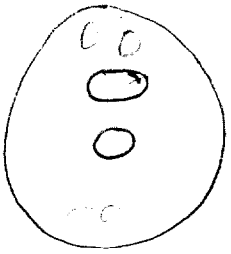
Pollination; pollen tube; break into embryo sac



The nuclear fusions.



The embryo and endosperm develop.



2n
Zygote. Develops embryo

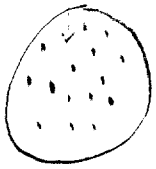
Primary endosperm nucleus 3n

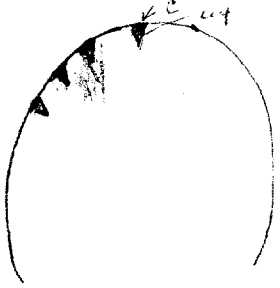
VI. Return to case of chromosome loss variegation. The particular kernels

1. Assume female C wx / C wx x male I Wx

2. Loss of I and Wx during development: Areas of color in aleurone. Underlying areas, exactly corresponding, wx.

3. Cross section of kernel:





4. The pattern of such losses:

a). Exceedingly uniform in some kernels with regard to size and distribution of recessive spots.

b). Not like pattern produced by other known mechanisms that result in losses of chromosomes: The breakage-fusion-bridge cycles
Ring chromosome behavior.

c). Conclusion: A previously undiscovered mechanism at work here that produces the losses of I and Wx simultaneously.

5. Plants grown from the variegated kernels. Showed:

a). White streaks on normal green background:
more cases.

b). Sectors appeared: no losses, increased or decreased frequency of loss, earlier or later timing of losses.

c). The twin-sectors: No losses -- increased or decreased freq. of loss

Altered pattern of losses in each sector: Often increased in one and decreased in other.

6. Conclusion: The mechanism responsible for loss of chromosome must be basically similar or the same as that producing the gene mutations.

a). The "loss" corresponded to the gene mutation, recessive to dominant.

b). The system controlling the time of loss, and the cells in which it will occur is the same type as that controlling when gene mutations will occur in the cases of the mutable genes.

c). Somatic segregations, occurring at a mitosis, of the factors associated with the controlling system occur during development and this results in altering the time and frequency of losses in descendent cells.

d). Some reciprocal relationship exists in the two sister cells resulting from this particular type of mitosis -- as if one cell gained something that the other cell lost. in comparison with system present in the mother cell.

7. The summary: Comparisons between chr. loss and mutable loci:

a). Mutable loci: Mutation from recessive to dominant; Not chromosome loss.

System present controlling when these mutations will occur.

Controlling system altered as consequence of a mitotic event. Resulting two cells differe from mother cell.

b). The chromosome loss phenomenon: Very same kind of mechanism involved.

Change occurs resulting in chromosome loss

System controlling when this will occur

This system altered as consequence of mitotic event. Resulting cells differ from mother cell with regard to this.

c). Something happens to chromosome to result in loss. What is it?

d). The loss must be gross to include nearly all of short arm of chromosome 9 as seen in the sectors: Losses of W, I and Wx.

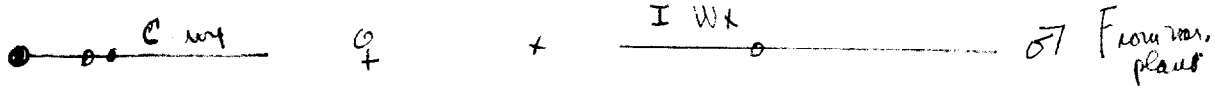
e). Therefore, this mechanism is subject to microscopic examination.
(1) Should be able to find evidence of this loss.
(2)This has been accomplished, and will be described in detail shortly.

8. Genetic tests made to determine if loss confined to particular chromosome 9 derived from variegated plants.

a). Nature of test:

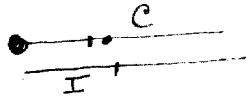
Female plants — Male plants

Reference: Rearranged chromosome 9
Almost eliminated crossing over in short arm of chrom. 9.



b). Selected I Wx kernels with C wx areas - the variegated kernels. from ear

c). Plants grown from them: constitutions:



d). These plants crossed to (1) female plants carrying C

(2) female plants carry c

e). The types of kernels and frequency of type on resulting ears of cross (1):

Table 12 a (page 16) in Ac account.

11 ears gave 600 completely colorless (I) : 588 colorless (i) with spots of C : 1550 colored (C).

Important: Only one-half of the I carrying chromosomeswere variegated.

f). Types of kernels on ear resulting from cross (2): to c

With very few exceptions, ratio of 1 - colorless (I) : 1 colored.
No regular type of C to c variegation.

f). Conclusions:

(1). Losses occur in the chromosome 9 derived from the variegated parent and not in the one derived from the tester plant.

(2). Only half of the kernels receiving the chromosome 9 from the original variegated plant showed these losses.

Reason for this will be discussed later. Due to presence or absence of factor, loacted at position in complement other than chr. 9, required for chromosome loss to occur: Ac for activator. Activator for placement with 9.

The constitution of the gametes in above crosses:

I → Ac = Variegated kernels produced in resulting kernel
I → ac = Non-variegated kernels

C → Ac and no ac, -- all non-variegated. in resulting kernels

9. Tests conducted to determine whether or not a whole chromosome or only part of chromosome 9 being lost.

a). Example given only. Many different types of tests conducted:

Variegated plant with: $\frac{\text{I Sh Bz Wx}}{\text{C sh Bz wx}}$ 1 Ac

crossed as male to tester plant that was C sh bz wx.

Diagram normal crossing over between markers: See below

Resulting plant: $\frac{\text{I C Sh Bz Wx}}{\text{C sh bz wx}}$

The types of kernels obtained:

non-recombs
I Sh Bz Wx = 1 colorless Sh Bz Wx : 1 variegated for C sh bz wx see photograph
C sh bz wx = all C sh bz wx

Reg. 1
I Sh Bz wx = all Colorless sh bz wx . non-var.
C Sh Bz Wx = 1 C Sh Bz Wx non-recomb. : 1 C Sh Bz Wx with recomb. sh bz

Reg. 2
I sh bz wx = all colorless sh bz wx non-var
C sh Bz Wx = 1 C sh Bz Wx non-var: 1 C sh Bz Wx with wx bz

Reg. 3
I Sh Bz wx = all Colorless Sh Bz wx non-var
C sh bz Wx = 1 C sh bz Wx ; no var for wx : 1 C sh bz with wx, var.

b). Conclusion: Some factor located to right of Wx, associated with loss phenomenon. This is because Wx locus required for losses to appear. All crossovers up to this locus, bringing in wx, do not show the loss phenomena.

c). Cross of same plant as male to plant that is c sh Bz wx:

c sh Bz wx x $\frac{\text{I C Sh Bz Wx X}}{\text{C sh bz wx x}}$

Types of kernels expected from non-crossing over and crossing over:

I Sh Wx X → 1 colorless Sh Wx : 1 Colorless Sh Wx with wx spot
C sh wx → nearly all C sh wx non-var. About 1% of C class and in C sh wx kernels showed wx area. pattern see photo
I Sh wx
C Sh Wx = 1 C Sh Wx : 1 C Sh Wx with sh wx area
C sh Wx = 1 C sh Wx : 1 C sh Wx with wx area.

Results: Same type of result obtained as in previous cross except that a few of the C sh wx kernels showed losses of C.

The exceptional kernels -- expected type of variegation pattern as if X were present.

g). Conclusions: Some factor present, close to and to right of Wx that is responsible for somatic losses of the short arm of chromosome 9.

Diagram:

10. Question: Does the position of X indicate the position of break in chromosome 9 producing the losses? If so, then would get:

W I Sh Bz Wx ——————o——————.....

Acentric fragment Centric segment.

11. Cytological examinations proved the above to be true. These will be described next time.

12. This X factor given the symbol Ds for dissociation.

The mutation-like event occurring at Ds results in the dissociation of the chromosome at this position.

13. The genetic consequences of Ds events --The meaning of the sectors in the kernel and the plant:

Female

C sh bz wx ds I Sh Bz Wx Ds

Losses of I Sh Bz Wx during development. Due to loss of acentric fragment.

The time of loss - shows as the size of the sector of C sh bz wx

The number of spots -- shows the frequency of loss.

14. General conclusions:

a). Genetic factor, Ds, to right of Wx in short arm of chromosome 9.
b). When Ac, another factor present, breaks occur at Ds.
c). Breaks result in loss of all factors in short arm of chromosome 9 from Ds to the end.
d). When Ac absent, no breaks occur. Would not know Ds present.
e). The break phenomenon at Ds compafable to mutation from recessive to dominant in general behavior pattern.
f). Two components in system: Ds, the locus showing change, and Ac, the factor responsible for this change occurring -- controls the breaks.

15. Next period: will discuss the cytological evidence for breaks at the locus of Ds.