

February 25, 1954

The breakage-fusion-bridge cycle and the origin of instability at gene loci.

I. Review of evidence so far presented:

1. Attempted to present evidence of the origin and behavior of instability at a number of known gene loci in maize.

2. Much of this confined to cases where Ac-controlled the genic action. This done to keep down the need for too much memory of individual cases -- a unified theme for the presentation of cases.

3. Have shown that both Ds and Ac are transposable units and that the transpositions occur in somatic cells at certain times during the development of a tissue, under control of Ac itself.

4. Have shown that mutability arises when Ds activity appears at the locus of the known gene in case of c-ml and bz-ml.

5. Have shown in these cases that mutation at the locus is associated with an event involving Ds -- loss of Ds activity coincident with the mutation.

6. Have shown that changes in state occur at Ds, when at the locus of c-ml or bz-ml or at Ds in other locations.

7. Have indicated the stabilities of the states of c-ml and the succession of states that occur -- one from the other.

8. We know that events at Ds and also at Ac can lead to a physical change in the chromatin at the locus because of : dicentric formation, inversions, duplications, ring chromosome, translocations etc, that have been observed. Evidence points to some change that results in a physical alteration of the chromatin materials at the locus. Evidence suggests that transpositions arise from this process.

9. We know that no alterations occur at the Ds loci, or the loci where mutability is controlled by Ac, if Ac is absent. Mutability will occur, however, when Ac again introduced.

II. The questions: What do Ds and Ac represent? What materials are they composed of? How do such units arise in the nuclear complement? Where do they come from? Are they always there or are they something newly created? What can we find out about them that will help answer these questions?

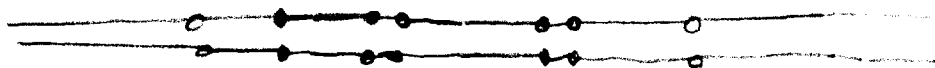
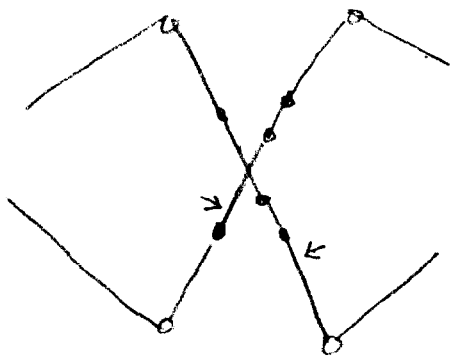
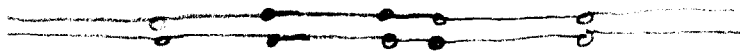
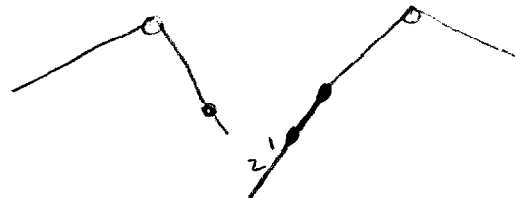
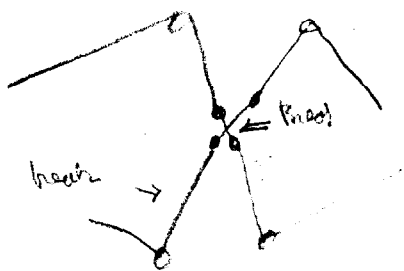
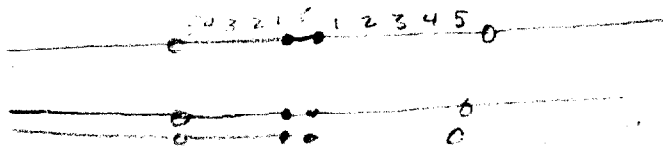
III. The above questions take us back to the very first discussion in this series: the sudden appearance of a great many newly arisen mutable genes in the progeny derived from a set of plants, each of which had undergone the breakage-fusion-bridge cycle. Also, the differences in the mutable conditions exhibited by two branches of the same plant -- one giving a mutable, the other not having this mutable. Some correlations between this cycle and the origin of the mutable loci suspected.

1. This history -- no previous investigations made on plants that had undergone this chromosome type of breakage-fusion-bridge cycle.

2. What is the particular significance about this cycle that could produce these new mutable loci? Why might it be responsible for initiating the mutable genes?

on page

Diagrams of i.f.b. cycles - elements.



IV. Examinations made of the chromosome constitutions in a number of the plants that had undergone the chromosome type of b.f.b. cycle -- between 250 and 300 examined. Revealed a striking alteration of particular components of the chromosome complement and this quite unexpected.

1. Alterations involved some kinds of changes in the heterochromatic elements of the chromosome complement, involving chromosomes other than those of chromosome 9 as well as those in chromosome 9.

2. The regions involved: The centromeres, the knobs, the ends of chromosomes, and the nucleolus organizer on chromosome 6.

3. The physical appearance of these components.

4. The positions they occupy in the resting nucleus.

5. The fusions that occur between the knobs and between the centromeres, in normal, resting nuclei.

V. The expected types of changes that should be produced by the chromosome type of b.f.b. cycle: On board.

1. Diagrams on board.

2. If healing of broken ends occurs in the sporogenous cells or in their ancestor cells, the constitution of the chromosomes may be examined in the resulting microsporocytes by analysis of pachytene stages. This could show what is present in the chromosomes 9 that had undergone this cycle. Would expect duplications, deficiencies, reduplications, etc.

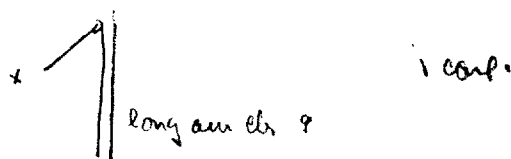
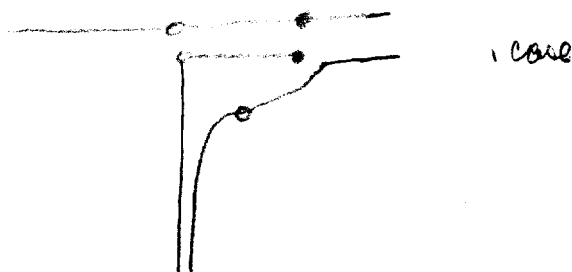
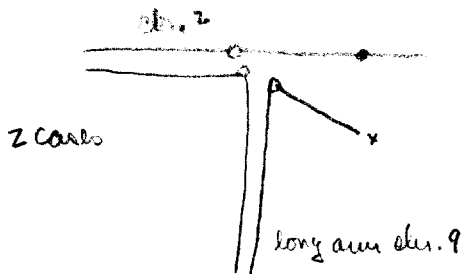
3. In a number of cases, the expected types of alterations, as shown in the diagrams, were found.

4. In about 40 or 50 cases, other types of aberrations observed within the chromosome complement. These not common in plants that have not undergone this cycle.

VI. The classes of abnormal types of chromosome alterations. Involve the broken end or the knob at the broken end of short arm of chromosome 9 with other regions, or the centromeres with other centromeres or knobs.

The types observed: Classes.

1. Centromere to centromere aberrations:

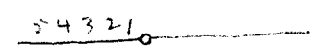


2. Centromere to knob aberrations:

a). Ring chromosome 9 -- centromere to knob



b). Inversion in chromosome 9:

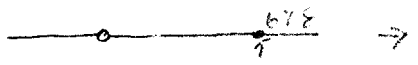


c). Inversions in other chromosomes:



3. Knob to knob or broken end to knob ~~exclusion~~ aberrations:

a). Ring chromosome 9.



b). Duplication:



d). Translocation of end to short arm:

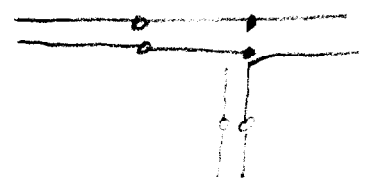


4. Knob to knob and centromere to centromere:



5. Knob or broken end of chr. 9 to knob on another chromosome

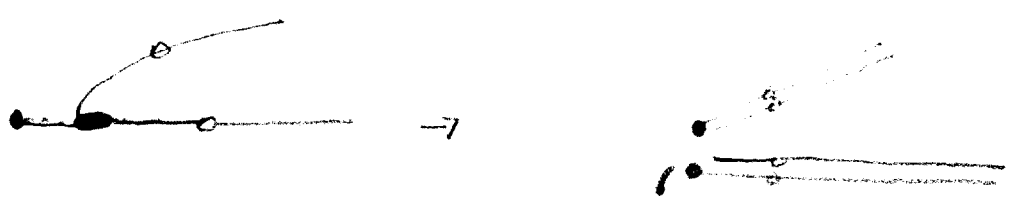
a) Chromosomes 9 and 3, at knob



b) Chromosome 8 long arm, and chromosome 9



c) Chromosome 6 at nucleolus organizer:



6. To end of short arm of chromosome 8 -- many cases: -

VIII. Conclusions:

1. Quite obvious that many other aberrations occurring as consequence of the b.f.b. cycle than that which could be expected.

2. Quite obvious that the majority of these unexpected aberrations occur to particular elements in the chromosome complement -- centromeres, knobs, nucleolus organizer and the end of chromosome 8 short arm.

3. Question: Is there any relationship between the occurrences of these aberrations and the origin of the mutable genes? Can we test this?

4. A single test made, which will be described.

I. History of a_1 - Dt

The a_1 - Dt relationship and the test for induction of Dt.

1. The a_1 , recessive, located in chromosome 3; used for many years in maize genetic studies.

2. This recessive very stable. Reversions to A_1 in the usual genetic stocks not seen; Attempts by Stadler to get reversions by radiation not successful although many thousands of cells irradiated.

3. The origin of a mutable a_1 locus:

a). Randolph stocks: Black Mexican sweet corn. $A_1 A_1$

b). One plant -- on self-pollinated ear, kernels that were colorless and those that were colorless with dots of color were segregating.

c). Rhoades took over the genetic analysis. Thorough analysis over a period of a number of years (1936-1941 and later).

(1) A new mutation to a_1 from A_1

(2) A dominant present, Dt for dotted, which made this a_1 mutate to A_1 in pattern of dots of color in the kernel and fine streaks in the plant. These dots of color were deep -- with the diffusion rims, previously described. Very few early mutations; very few mutations to the pale color.

(3) Occasional anther with mutant tissue, or sector of mutant tissue. Pollinations from them produced the germinal mutations for subsequent study. These proved to have dominants that were not all exactly like the original -- all were higher alleles of the A_1 normal locus.

(4) Tested Dt on the old known recessive. Caused it to mutate in the very same manner as the one which occurred in the Black Mexican Sweet Corn.

(5) The dosage action of Dt: Increased doses, increased frequency of mutation -- more dots, geometric in some cases.

(6) Obtained some a_1 recessives through this process of being with Dt that no longer mutated in the presence of Dt.

(7) Located this dominant, Dt, adjacent to or in the knob terminating the short arm of chromosome 9.

II. The reasoning that lead to experiment to produce Dt by the b.f.b. cycle.

1. a_1 does not mutate in the absence of Dt. It is very stable.
2. If the b.f.b. cycle makes evident the controlling factors, such as Ac or Dt, and does so because it disturbs the heterochromatin or its balance in some manner, then we should be able to get evidence of the origin of such a Dt factor if a_1/a_1 plants have chromosomes undergoing the b.f.b. cycle.
3. This can be done readily, as will be shown.
4. Would expect to find mutations to color -- but in sectors and showing dots of A_1 in these sectors.

This reasoning is based on our knowledge of that state of the normal a_1 locus:

This state gives dots with Dt. Other changes probably occur -- and do occur since stable a_1 in presence of Dt obtained -- and these recognized by the pattern of dots in the kernels having Dt.

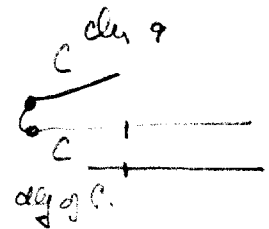
Also, we know have other states of a_1 -- found by Nuffer to have arisen in the Dt stocks. These give types of mutations like those shown early -- early sectorials for both full A_1 and for pales, and various changes in state arise frequently from them.

III. The test procedure:

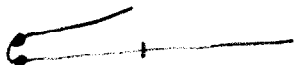
1. The cross:

Female
 a_1/a_1 normal complement
 chr. 3

Male
 a_1/a_1
 normal chr. 3.

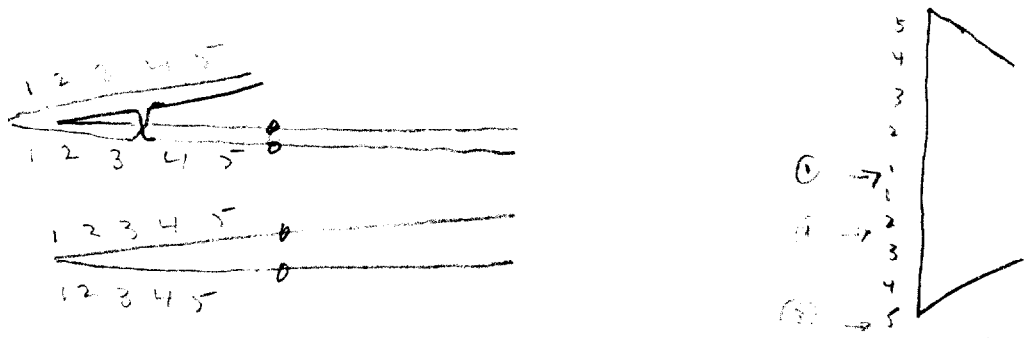


2. The gametes produced by the male:

- (1) Complete Dup. w broken end 
- (2) Def. chr. = non functional pollen
- (3) Chromosomes with newly broken ends

3. The origin of the chromosomes with broken ends:

on base



A I

4. The chromosome constitutions resulting from breaks (1), (2), (3).

5. The functional pollen grains: Majority have a newly broken end. Those grains with near normal constitutions of chromosomes 9, or short duplications function more efficiently in pollination than those with the full duplication of the short arm.

6. The control test of this functioning:

Female
A₁A₁ c/c

Male same = a₁/a₁

The results: See ear.

IV. The results of this test:

315 ears obtained; contained a total of 93078 kernels.

117 kernels with spots of color -- the A₁ type with diffusion rims.
All were dots. No pales, no large A₁ colored areas.

These present on 86 different ears.

The number of dots per kernel:

93 -- 1 A₁ dot only

9 -- 2 A₁ dots

4 -- 3 A₁ dots

6 -- 4 A₁ dots

1 -- 5 A₁ dots

2 -- 7 A₁ dots

1 -- 17 A₁ dots

1 -- 84 A₁ dots distributed over the whole aleurone areas

positions of dots

The appearance of the dots, when more than one present, in sectors.
The resemblance to the dots produced by Dt.

V. The reason for so few cases being found: The Dt factor must be produced early enough in the development of the kernel to have an effect on the cells late in development.

1. The time when these changes could occur: From Anaphase II to early endosperm divisions in order to produce decided sectors:

6 dies

- First division of spore: Both grains with Dt
- Division in pollen grain: " " " "
- First division in endosperm: $1/2$ of kernel
- Second division in endosperm: $1/4$ " "
- Third division in endosperm: $1/8$
- Fourth division in endosperm: $1/16$

VI. Experiment positive. Shows that Dt-like effect can appear in tissues that are undergoing the b.f.b. cycle.

VII. The presence of Dt factors in strains of maize: Those from S.A. examined:

One strain from Peru and one from Brazil found to have Dt. In both cases, the Dt not distinguishable from that found by Rhoades.

In one case, the only one whose test has been reported, the Dt factor is not in chromosome 9. It appears to be in chromosome 6.

These differences in positions -- corresponds to that of Ac.

VIII. The indications so far, suggest that these controlling factors are present or can be made evident in nuclei. Their possible significance will be discussed later.

The case of var. P = Ac

The case of Peterson