February 15, 1954

Conclusions on c-ml; outline of bz-ml; begin c-m2.

I. Review of previous discussion:

1. Considered the origin of mutable c from normal C.

2. Reviewd the evidence suggesting that it arose from transposition of Ds to thelocus of the normal C.

3. Discussed changes in state of c-ml: The ratio of mutations to C to dicentric forming events at Ds.

4. Considered the meaning of this with regard to action of Ds where no phenotypic changes other than dicentric formation could be observed.

5. Conclusion drawn that the number of changes at a mutable locus may not be revealed by study of one type of change -- the mutation, for example. Other changes occur -- "hidden mutations". or "[illegible] changes at locus".

6. The stability of the changed states: Those giving high ratio of mutation to C are much more stable in action in subsequent generations than those giving high ratio of dicentric formations at c-ml.

7. The states of many mutable loci of considerable importance. The presence of hidden mutations suspected or revealed in many cases. These will appear as the discussion continues.

II. Continuation of discussion of behavior of c-ml. Begin pate 7 of outline of February 11.

1. Origin of stable c from c-ml
2. The action of Ac on c-ml and Ds when in same cell
3. The control of Ac on time and place of mutations: Dosages; Stabilized-Ac.
4. The appearance of transposed Ds associated with mutation to C [illegible]

The origin and behavior of bz-ml

I. Kernel having mutations to Bz first noted in cross of:

$$\text{C sh bz wx ds ac female} \times \frac{\text{I}\quad \text{Ds Sh Bz}\quad \text{Wx}}{\text{C}\quad \text{ds sh bz}\quad \text{wx}}\ \frac{\text{Ac}}{\text{ac}}\ \text{male.}$$

a). The appearance of this kernel: C sh bz Wx background with areas of Bz.Wx. Also, areas of wx noted in the non-Bz areas. Like original c-ml kernel, in this respect.

b). The study proved that a mutable bz locus present in a C sh Bz Wx chromosome.

Probably appeard because of a crossover, I to Bz. C sh bz^{m-1}

Variegation - now in plant as well as in kernel. Plant: bz → Bz var. Type = pattern like [illegible]

2. The mutations proved to be Ac controlled. Photo of ear illustrating linkage to sh and ratios in sh class:

Photo (3) Ear

$$\text{C sh bz wx ds ac female} \times \frac{\text{C Sh } \text{Bz}^{s}\text{ Wx}}{\text{C sh bz-ml Wx}}\ \frac{\text{Ac}}{\text{ac}}$$

The gametes: $\frac{Sh \quad bz^{s} \quad Wx}{sh \quad bz\text{-}ml \quad wx}$ $\frac{Ac}{ac}$

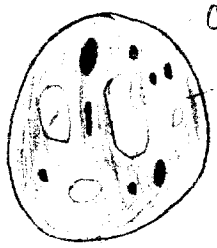
	Constitution of kerneal		Appearance of kernel
Sh-crossovers	C Sh bz^{s} Wx	Ac and ac	C Sh bz Wx, non-var.
on board.	C sh bz-ml wx	Ac	C sh bz wx, var for Bz; var for wx
	C sh bz-ml wx	ac	C sh bz wx
Cross-overs, Region 1	C Sh bz-ml wx	Ac	C Sh bz wx, var for Bz.
	"	ac	C Sh bz wx, non-var.
	C sh bz^{s} Wx,	Ac and ac	C sh bz Wx, non-var.
Re Region 2	C Sh bz^{s} wx,	Ac and ac	C Sh bz wx
	C sh bz-ml Wx	Ac	C sh bz Wx; var for Bz var. for wx
	C sh bz^{m-1} wx	ac	C sh bz Wx

3. If same male crossed to c sh bz wx ds ac, the variegation for mutations and for dicentric breaks readily seen on the ear:

Photo ④ ear

on board

Appearance of kernel:



The dicentric breaks:

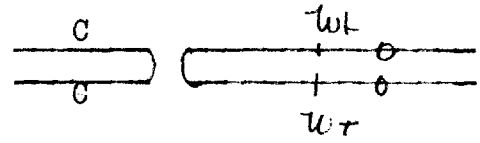


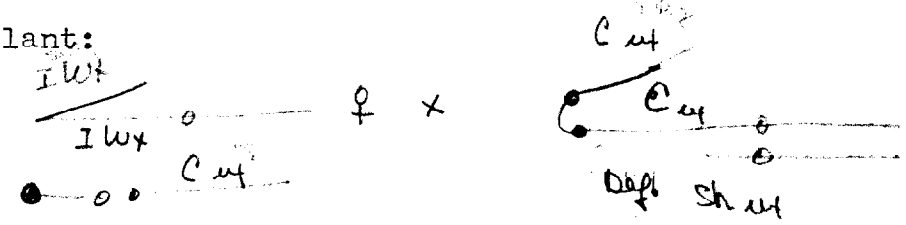
Photo ⑤ kernels.

Photo of ear ; of kernels; pass around ears.

Bogus, Feb. 18 The Origin and Behavior of c-m2.

I. History of the origin must begin with original plant that underwent the chromosome type of breakage-fusion-breige cycle.

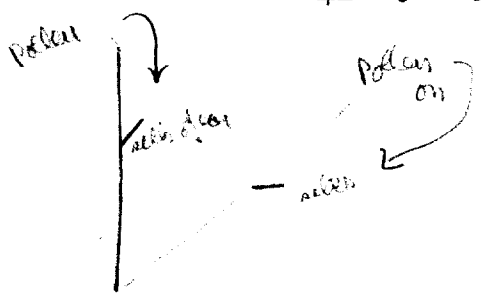
1. Origin of this plant:

a). The cross: 

b). The kernel selected: Broken chromosome from male and female:

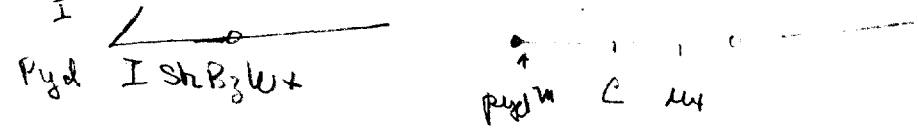
One IWx chr. with broken end + one C m4 chromosome with broken end from ♀ gamete from ♂

c). The plant from this kernel: Number 42-B. Main stalk and tiller self-polli nated:



Bogus Wire Feb. 18

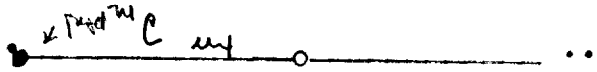
d). Cytology of the tiller: The genetic constitution within each chr:



2. The seedlings from the kernels on selfed ears:

Main stalk: All seedlings green.

The tiller: some kernels derived from C class showed variegat nfor pale-green to green. Proved to be:



3. Plants grown from these seedlings -- both var. and green, in field under culture number 3592.

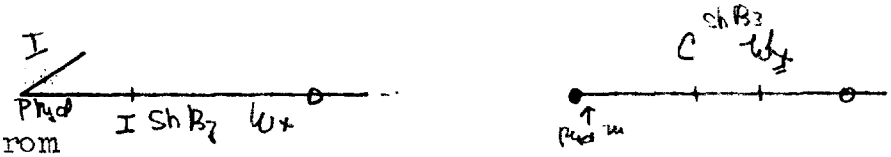
4. The green plants self-pollinated, + crossed. The variegated plants too weak.

5. Appearance of kernels on the ears: New mutables appeared to be segregating.
These were, (1) white to yellow endosperm

(2) altered starch constistency -- like wx to Wx and Wx to wx but staining showed all Wx.
(3) Dicentric breaks -- like Ds-f.l.

6. Kernels on self-pollinated ear of one plant, 3592A, showed var. for starch consistency.

a). The constitution of chromosomes 9 in this plant:



Plants from

7./The I Wx kernels grown in following summer under culture no. 4000 A

The C Wx kernels were in B of this culture.

8. Various tests made with these plants; One was to self-pollinate the ears and to cross to c sh Bz wx plants.

The appearance of the kernels on the ear derived from self-pollination of 4000B-2 one of these plants: Variegated kernels and the non-variegated kernels:

One-fourth of the kernels were colorless and the majority of them showed speckles of color.

In cross to c sh wx plants, C Wx, non-variegated kernels and kernels with areas of color in a colorless background appeared. The type of color produced in the different areas differed markedly from that produced by c-m1. The crosses to plants carrying C, no variegated kernels appeared. Appeared, therefore, that a new mutable c, derived from a previously normal C locus had been produced.

II. The tests of this new mutable c, designated c-m2.

1. Plants grown from the various types of kernels on the self-pollinated ear and on the ear derived from the cross to c sh wx.

2. Tests made. The crosses of the plants derived from the variegated kernels on the self-pollinated ears:

a). Self-pollinated: Again, majority of kernels showed some color. This as small specks of color and not too many of them.

b). In crosses to c sh wx: Nearly all of the kernels were variegated but the patches of color were much larger than on the self-pollinated ear. The types of color patches were not all alike. A wide range in intensity of color and in its distribution within a colored area.

c). The male gametes -- cm2 Sh Wx. On ears, a few areas of wx seen in the majority of the c to C variegated kernels but not many of them.

d). The behavior very much resembled a mutable that was Ac controlled.

3. The tests for Ac -- these conducted. c-m2 proved to be Ac controlled as was c-m1, hz-m1. The same Ac in its action although the derivation differed. Plant 4000B-2 was Ac/Ac in constitution:

$$\frac{\text{c-m2 ShnBz Wx}}{\text{C Sh Bz Wx}} \quad \frac{\text{Ac}}{\text{Ac}}$$

III. The type of variegation produced by c-m2. prpr constitutions -- red shades.

1 General:

1. Areas with very pale pink color to those with fairly deep color.

2. Areas that seem to resemble in all ways that produced by normal C

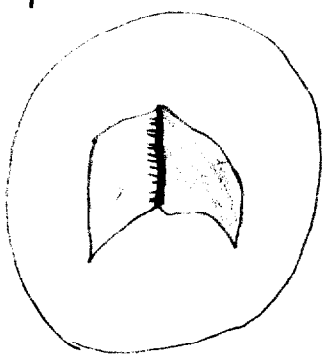
3. In the deeper pink areas, the color distrubution peculiar: Like that produced by a paint-brush:

4. These never present in the normal C areas.

5. The pink-type mutations: form a quantitative series, from almost no color to very deep color but clearly distinquished from those having the normal C phenotype.

2. The details of this variegation:

The borders between a pink and a regular Ctype area:



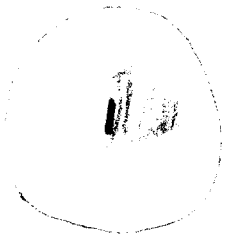
Important: The sharp border between the pink and the colorless areas.
The sharp border between the C and the colorless area.

3. The pink areas: Sometimes the following seen:

In some pink areas, section with deeper pink diffusing into light pink sector, but within the pink sector. Gradiant of color change. Borders of pink area sharp, as if mutation present in a sector bordering the pink area that produces substance that the cells in pink sector can use to increase pigment. (Substance 2). This mutation produces no color in cells having it but the action in the pink sector reflects that such a mutation occurred.

4. The colored areas resembling normal C:

A section on border of such a sector can show deep color grading into color of the sector:



Here, also, looks as if a sector with a mutation is present to left of mutant C area. This mutation does not produce any detectible color, but its presence revealed by the border. The C cells can utilize this substance to increase its pigment. (This si subtance 2.) The "hidden" mutation may be a very light pink, but no color seen.

5. Conclusions so far on mutations at c-m2.

a). Two main classes of visible mutations: The "pink" series, gives sectors with very faint color to those with quite deep color. In the darker colors, the paint-brush like distribution of pigment over aleurone layer is evident.

b). The regular C type mutation, only present in quantitative series also. Here, the color is smooth within the sector in contrast to paint-brush type of mutants in pink series.

c). Both mutant types distinguished by their reactions to one another

Pink - adjacent to "C"
The borders between them: The diffusible substances produced:

Substance 1 produced by pink, can be used by the C mutant cells to increase their color.
Substance 2, produced by "C" areas, can diffuse into pink areas and increase their color.

d). The hidden mutations -- Production of either substance 1 or substance 2 in sector in which no color can be seen.

IV. The tests conducted to substantiate the above conclusions on the production of diffusible substances.

1. Test 1: The diffusible substances produced by these mutations at c-m2 can be used by the normal C carrying cells to increase their pigmentation.

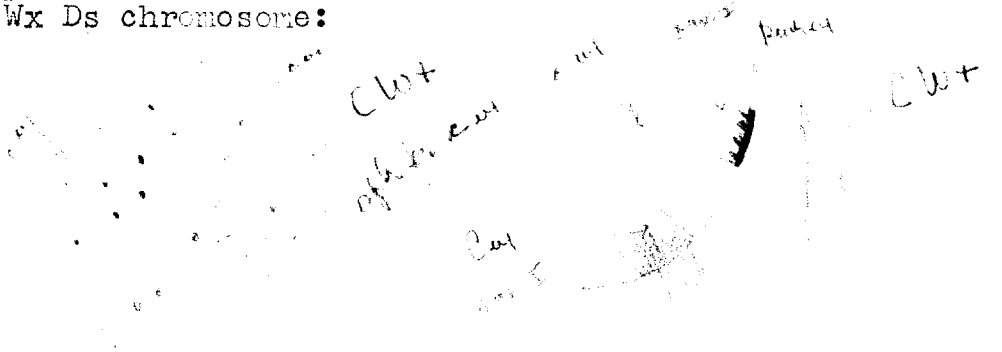
The test cross: c-m2 wx, Ac female x C Wx Ds ac male.

The constitution of the kernels examined:

wxg wx

c-m2/ c-m2 / C Wx Ds Ac Ac ac

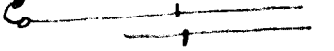
Appearance of the sectorial kernels -- those with an early break at Ds in the C Wx Ds chromosome:



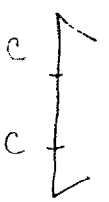
The control: c wx / c wx / C Wx Ds, Ac Ac Ac:

The observatio s show that two types of mutations occur at c-m2: To "pink" which produces substance 1, allowing normal C carrying cells to increase their color, and to full C type, that has no contribution of a diffusible substance to the cells carrying the normal C.

Test 2 The germinal pink mutations: (Relatively stable in presence of Ac)

The cross: pink-carrying mutant plant x 

The kernels examined, those which have received a newly broken end from the male parent. How this broken chromosome will behave in the developing kernel:



(6) Photo ear = 6 (6 ears)

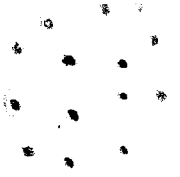
The control: c/c female x broken chromosome from male: Appearance of variegation:

See photo ear

Comparisons of the color patterns produced by two crosses described:

Conclusion: The germinal pink mutant is produce some substance - substance 1 that the normal C carrying cells can use to increase color but, these normal C carrying cells also produce substance that the germinal pink mutants can use. There are no sharp borders in any of the sectors produced through the breakag-fusion-bridge cycles.

Test 3 Germinal pink mutations selected. Used as females in crosses with plants carrying c-ml. The Ac Ac kernels:



Mutations to full C at c-ml occur late, to produce speckles with this dose of Ac.

The specks of color all have exceedingly deep centers, surrounded by a diffusion rim, the color gradually fading into that of the pink background. Tests for substances 1 and 2.

V. The color produced by various doses of C.

Quantitative -- higher the dose, the deeper the color:

color intensity

C c c

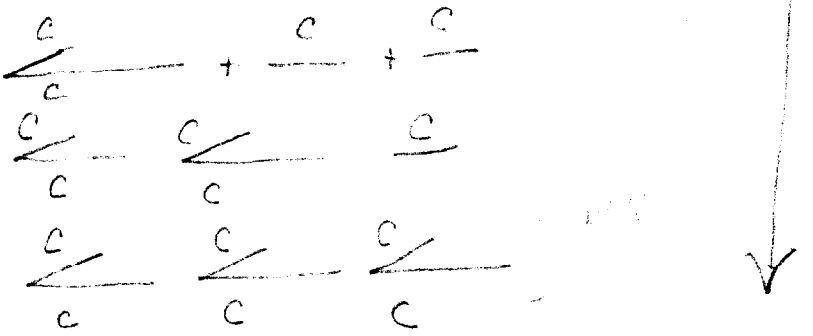
C C c

C C C

C C C C

C C C C C

C C C C C C



2. The quantitative action of C, and the tests of increase in color produced by substance 1 when only 1 C present, suggests that the normal C color produced by different doses of C reflect the amount of substance 1 that is present and produced by this C gene.

a). Pink mutant can greatly intensify the color produced in cells with 1 C. It often resembles the highest intensity produced by the dosage studies.

Conclusions:

Both substances 1 and substances 2 can be produced without color appearing -- see kernel types.

In "pink mutants", substance 1 present in excess. Some other factor limiting, this may be substance 2.

In the "C types," possibly the quantitative grades reflect the amount of substance 1 produced, as the light colors and the dark colors are present.

Differences also shown by the pattern of color distribution within each type of mutant sector or in the germinal mutations -- the "paint brush" pattern vers the smooth pattern.

Quantitative actions within the "pink" series:
The breakage-fusion-bridge cycles. c / broken pink

One, two and three doses of pink -- increased color intensity.

VI. The stability of the mutants produced by c-m2.

1. A number of apparently stable c types recovered. Stable in presence of Ac.

2. The pink mutants -- show considerable stability with Ac, but new alterations or mutations arise, as sectors, or as germinals.

3. The mutants of pink, in absence of Ac, are stable.

4. The full C mutants -- probably stable, but tests insufficient.

5.
VII. The States of c-m2.

1. Original state -- many hidden mutations; many mutations to pink series.

2. Changed state -- the mutations to dark pink and to full C very frequent. The hidden class much reduced. Photo of ear.