February 22, 1954

Continuation of discussion of wx-m1; the induction of mutability at selected loci; the states of a_1-m3.

I. Review of previous discussion:
1. The C locus - the origin of c-m2 from C in plants having Ac.
2. The types of mutations occurring at c-m2:

Class I: produces substance 1 in excess and substance 2. Color va ies from very faint to quite dark - a spectrum of mutations in this class. Possibly the spectrum reflects amount of substance 2 produced.

Class II. Produces substance 2 in excess but substance 1 may be limited in those cases where color is light.

Some mutants - plenty of both substance 1 and substance 2. These give rise to cells with exceedingly dark pigmentation -- as great as that produced by the normal C locus in 5 to 6 doses.

The normal C locus -- seems to produce insufficient amounts of substance 1 This may be the explanation of the quantitative expression of dosages.

The hidden mutations -- those cases where change occurs at the c-m2 locus but gives insufficient color to be readily detected in kernel. Detection comes from observations of effects produced on adjacent sector where visible mutation present.

II. The differences between c-m1 and c-m2 quite obvious. c-m1 produces only mutations to the normal C type. Only once has a small sector on a single kernel shown a mutation resembling that of calss I above and many thousands of kernels showing mutations of c-m1 have been examined.
If the units at the C locus -- presumably Ds in the case of c-m1, and something similar in the case of c-m2, -- were the same in the two cases, we should expect to find some c-m2 like mutations arising from c-m1 by some change in Ds that would simulate or replace the former action. This is not observed.
Why do the two mutable C loci - c-m1 and c-m2 differ in their expression? What is controlling the expression of the genic material when a change occurs at the locus, under the influence of Ac?

III. The types of changes that have been examined at the C locus:
(1) - c-m1 from C Ac controlled

(2) - c-m2 from C Ac controlled

(3) c-m4 from C Ac controlled. This case when it first appeared gsve mutations that resembled those produced by c-m1 states that show high frequencies of mutation to C and no breaks.

(4) c-m3. This arose from a normal C locus. It is not Ac controlled The types of mutations: two only a) to the normal C expression; b) to a pale producing mutant -- the same pale color in all cases.

The recessives: Without Ac -- c-m1, c-m2, c-m4 are all stable recessives. The known recessive - c - is also capable of showing color in the presence of a factor -- Blotch -- located in chromosome 6 by Dr. R.A.Emerson. The color produced very clearly differs from that given by mutations of c-m1, c-m2, c-m3 or c-m4.

The common component of all these cases is -- a change at one position in the short arm of chromosome 9 -- corresponds with the C locus. The changes in all cases affect the production of pigment in the aleurone layer of the kernel.

This indicates that there is something at this locus in the chromosome that is associated with the development of pigment. Its mode of action, in this respect, however, can be controlled quite differently. This shown by the differences in the expressions of this pigment in the cases described.

What is the nature of this control? What kinds of changes occur that result in specific types of genic action?

Possibly some answers to these questions can be had in examining the origin of other mutable genes, and the behavior of known recessives to influences on them. The case of wx-ml will illustrate this.

IV. The origin of wx-ml -- previously discussed:

1. wx-ml was present in a single gamete among many thousands observed that were derived from plants carrying c-m2 Sh Wx and Ac.

2. This mutable locus -- proved to be Ac controlled.

3. The time and the cells in which mutations will occur -- like all other Ac controlled mutable genes, -- it responds to doses of Ac, states of Ac, changes in states or doses occurring somatically in the same manner as other Ac-controlled mutables. This proves by combinations of them in the same kernel -- like the cases of combinations of c-m2 and Ds, previously described.

4. The types of mutations occurring at wx-ml: A quantitative series, from a very small amount of amylose to the maximum amount observed in normal material.

Continue with outline, page 4, February 18 discussion.

The induction of Ac-controlled mutability at selected loci.

I. From above discussions of origins and similarites of mutable loci derived from particular plants carrying known mutable genes, one should be able to predict the origin of new mutable genes and their behavior.

a). If we assume that c-ml, c-m2, bz-ml, wx-m-1, 5,6,7,8, c-m4; arose from insertion of some controlling material at the locus of thegene. If we know that this controlling material can be transposed from one location to another, then:

(1) In plants carrying both Ds and Ac, transposition of Ds could occur to various loci, among them, the locus of a known gene.

(2) By setting up the proper test conditions, such insertions, after they occur, should be detectible.

II. The nature of the tests conducted: The original test made as a "pilot" experiment as the frequency of occurrence of transposition of Ds to a particular locus not known. The original test was small and no controls were done.

1. The nature of the pilot experiment:

a). Number of plants carrying A_1 Sh_2 / A_1 Sh_2 in chromosome 3 and A_2 Bm_1 / A_2 Bm_1 in chromosome 5 short arm grown. Each plant had Ds in one chromosome 5 long arm and Ac at an unknown location in the chromosome complement:

b). Pollen from two sourses used on these plants:

(1) a_1 sh_2 No Ac; neither recessive mutates in presence of Ac

(2) a_2 bm_1 " " " " " " " " "

2. The results:

a) 72 ears from cross (1). All kernels with normal appearing aleurone color except 1. This kernel showed colorless background with colored spots.

b). 120 ears from cross (2). All kernels normal in color with exception of three. These three found on three different ears. Each showed a colorless background with colored spots or areas.

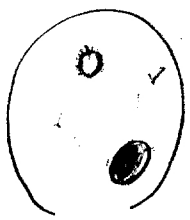
3. The tests of the single kernel from cross (1). It proved to be a mutable gene -- designated a_1-m4. It was Ac controlled. Manner of control exactly like that of all other Ac-controlled mutables examined:

a). Without Ac -- completely coloress, stable.

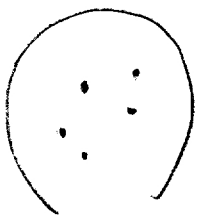
b). With Ac: mutations of two main types: pale color, and full A_1 color.

The differences in expression of these two classes of mutants:

Pale
No diffusion rims



Full A_1
Diffusion rims



c). Mutations in the plants -- similar; will be considered shortly.

d). The germinal mutations -- like the mutations in kernels to give sectors. Pale; full A_1.

e). The changes in state: Few changes in state noted. One type appeared on several occasions: colorless with only a few small spots of the full Ac type -- diffusion rims.

The meaning of changes in state at the mutable a_1 locus will be presented shortly in the examination of a_1-m3, another Ac-controlled mutable.

4. The tests of the three variegated kernels from cross (2).

a) The female parent:

$$\frac{A_2 \quad Bm_1 \quad O}{} \qquad \frac{Ds \; Pr}{}$$

$$\frac{A_2 \quad Bm_1 \quad O}{} \qquad \frac{ds \; pr}{}$$

b). The three kernels removed, plants grown from them. Tests conducted to determine the nature of the instability expressed in the kernels.

(1) One plant: In the tests of this plant, the chromosome 5 contributed by the female parent was not transmitted to the following generations. Something wrong with it. No cytological examination made. This mutable condition lost, therefore.

(2) Plant 2: Proved to have a new mutable a_2 locus. This arose in the A_2 Bm_1 carrying chromosome. In this plant, something wrong at the Pr locus or close to it -- not male transmissible. The crossovers recovered -- carried a_2-m4 Bm_1 ds pr.

(a). a_2-m4: Proved to be Ac controlled, and again this expression the same as that shown for all other Ac-controlled mutable loci with respect to time of occurrence of mutations as an expression of Ac dose and state. The nature of the mutations -- many very pale mutations, few like full A_2 with diffusion rims:

Low rate of visible mutations in equal state.

The range of pales -- very light to quite dark; no diffusion rims. From frequencies observed in this initial state, many hidden mutations suspected. They do not give any perceptible amounts of color.

(3) Plant 3: A new origin of mutability at A_2 in the A_2 Bm_1 chromosome. This mutable is <u>not</u> Ac-controlled. Designated a_2-m3.

It mutable behavior very different from that of the Ac-controlled types. Many of the mutations to full A_2 expression and some to pales of varying intensity. Many early occuring mutations in the plant; many changes to stable a_2. Many changes in state occur, and these take place early in development of plant. Plants may be highly sectorial for many changed states. Can be extracted from these plants and maintained as altered states.

5. <u>Conclusions:</u> Although test is small, and no control, it is clear that new Ac-controlled mutable loci may be obtained and at loci previously selected for this. The mechanism of control can be anticipated <u>but</u> the types of mutations that will occur <u>can not be anticipated.</u> This will be shown in the examination of a_1-m3, an Ac-controlled mutable locus derived from a normal A_1 in plant carrying Ds and Ac.

THE STATES OF a_1-m3.

I. The origin of A_1-m3.

Constitution of plant:

Tests being conducted to investigate the behavior of Ds in the Pr chr. Ear; self-pollination of this plant: Some variegated kernels appeared; type of variegation suggested change at the A_1 or A_2 locus.

Crossed to a_1/a_1 plant: Showed that the locus involved was A_1 No mutables present at this locus in sister plants.

II. The subsequent tests: This new mutable proved to be Ac-controlled. The expression of mutations differed markedly from those of a_1-m4 just described.

1. Must be emphasized: The time of occurrence of mutations completely controlled by Acand its dose and state. The type of mutations controlled by conditions present at the a_1-m3 locus.

2. The original state: Rather dark pale background with very deep A_1 spots or areas, each showing diffusion rim.

3. Changes in state occurred frequently in comparison to the infrequence of such occurrences in a_1-m4.

4. The types of change in state are important. Effects seen in both the kernel and the plant.

III. The types of states: See chart.

IV. Summary:

1. Origin of a_1-m3 in A_1/A_1 plant. One of the A_1 loci affected. This plant carried Ds in chromosome 5 and Ac in unknown location.

2. Original state -- dark pale with no Ac; Mutations with Ac to full A_1, to lighter pales, to colorless.

3. In course of study, a number of different states isolated. Each characterized by the types of mutations that occur in the presence of Ac.

4. The relation of one state to that of the known a_1-Dt condition. The changes in state observed in this a_1-Dt association -- by Nuffer.

5. The state of the normal recessive, a_1. What type of mutation phenomenon would be expected if a new Dt were made:

Dots of A_1 mainly. Diffusion rims.

6. The state of the mutable loci - very important aspect of the control system. What differences exist among these states that serve to produce these effects?

Hard - Types II in pale group =
Time of mutation to A_1 - dots.
... = ... [illegible]

1 Ac = when types better than with 2 Ac, are ... late with 2 Ac.

A_1 A_1 Ds Ac plant.

One A_1 allele - mutable: Dark pale background: mutations to full A_1 in presence of Ac. Throws many different states of a_1^{m-3}. These distinguished by background color, with no Ac, and by types of mutations when 1 Ac present.

Appearance when no Ac present: Kernel	Plant	When 1 Ac present: Kernel	Plant
Dark pale Type I	dark color (regions)	mutations to full A_1 different times " to lighter pales, various. " colorless	mutations to A_1 in leaf tissue
med. pale "	med dark color (with ^)	mutations to full A_1 - different times " " lighter pale + colorless " darker	Dark streaks on med dark background sometimes seen
lighter pale "	light anthocyanin color	" to full A_1 " darker pale " colorless.	Dark streaks on light colored background
colorless Type I	no anthocyanin	" to full A_1 - early, different times " " pales - " no " " " colorless "	green with dark or pale streaks.
colorless Type II	" "	" " full A_1 - all late only dots of A_1 " " pales - early + late mutation. (large areas, pale areas)	"
colorless Type III	" "	" " full A_1 only - all late = dots (large dots) and occur in sectors of kernel. " " 1 Ac = ...	Hidden mutation prob. Some lowered Sh activity.
" Type IV	" " Green	mutations to pales only	mutations to color - various classes.