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THE GENETICS OF VARIABLE STATES OF THE EN CONTROLLING
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The genetics of variable states of the <u>En</u> controlling

element system in <u>Zea</u> <u>mays</u> L.

by

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DEDICATION

To my mother

I. INTRODUCTION

In maize (<u>Zea</u> <u>mays</u> L.), unstable phenotypes are most often attributed to the activity of controlling elements. Variegation or spotting of kernel aleurone color is one of the unstable phenotypes that is most intensively investigated.

The spotted phenotype results from the activity of two types of controlling elements that operate as a system. One, the receptor element, inserted into a locus or gene governing pigmentation of aleurone (for example, <u>A</u> locus), functions, in cis, to suppress or modify the expression of the gene ($\underline{A} \rightarrow \underline{a}$); second, the regulatory element acts, in cis or trans, to relieve the gene from suppression ($\underline{a} \rightarrow \underline{A}$). The event responsible for the relief is considered as a mutation. In a spotted phenotype, each colored spot represents a single mutation event. The <u>size</u> and the <u>number</u> of spots are related to the <u>time</u> and <u>frequency</u> of occurrence of mutations, respectively, during the development of the endosperm tissue. Thus, the spot size and number constitute a mutability pattern.

The receptor and/or regulatory elements are represented by specific patterns of mutability and referred to as states of a mutable allele (receptor element) or of a regulatory element. The present study deals with various states of the <u>En</u> (regulatory) – <u>I</u> (receptor) controlling element system.

The objectives of this study include:

(1) assessment of the potential of each of the original states

in the origin of new states;

(2) determination of heritability of different patterns of muta-
bility;

(3) derivation of new states of En and I;

(4) confirmation and characterization of the new states in terms
of the mutability patterns elicited.

The different states of controlling elements are comparable to the
wide variety of transposable elements present in bacteria, yeast and
Drosophila. Characterization of these states at the DNA level may enable
us to understand the basis for the origin of diversity among controlling
elements.

II. LITERATURE REVIEW

A. Transposable Controlling Elements in Maize

The presence of controlling elements in maize was made evident because they interrupt the functioning of genes. The change of a non-active gene to an active gene is accompanied by the transposition of an element from the observed gene site to another site in the chromosome complement. These changes are most evident in genes involved in anthocyanin pigment formation and thus have been extensively investigated. Therefore, a brief description of the genetic control of anthocyanin synthesis in maize is appropriate.

1. Genetic control of anthocyanin pathway

Kernel coloration in maize is due to anthocyanin pigment production in the aleurone layer of endosperm. The aleurone is the outermost layer of endosperm cells. For this layer to be colored, at least one dominant allele must be present at each of the following gene loci: A, A2, C, C2 and R. These five genes complement each other in the production of anthocyanin pigment. The C and R loci are considered to be regulatory, while A, A2, and C2 are structural genes (Dooner and Nelson, 1977b) in the control of anthocyanin.

In one of the first attempts to confirm the sequential order of genes acting in the anthocyanin pathway, Reddy and Coe (1962) proposed the following order:

$$CI - C - R - C2 - In - A - A2 \; .$$

In their experimental technique, they pressed together 18-22 day post-pollination aleurone tissues. If the compressed tissue developed color, the genes were considered complementary. Styles and Ceska (1977) proposed an alternate sequence by assaying accumulation of products in aleurone tissues containing different recessive alleles. According to them, C2 acts prior to R in the sequence.

CI, an allele of the C locus, is a dominant color suppressor. It suppresses color irrespective of the residual genotype.

2. **Discovery and definition of a controlling element system**

Transposable controlling elements in maize were discovered following many observations on several unstable mutants that expressed variegation in plants and kernels (McClintock, 1952a). Variegation of the mutants is expressed either as sectors of dominant phenotype in a recessive background or vice versa, depending on the genotypic constitution. The individual cases of variegation that led to the definition of a controlling element system are described here.

McClintock (1946) observed a few variegated kernels on the ear of a self-pollinated plant that had its two chromosomes 9 undergoing chromosome type of breakage cycle. The self-pollinated plant was heterozygous for dominant inhibitor of aleurone color, CI. The variegated kernels were recognized in the heterozygous progeny class that had received both the C and CI-carrying chromosomes.

The kernel variegation was recognized by the presence of colored areas [from changes of CI (no color) to C (color)] or spots in well-

defined sectors. In some kernels, the sectors contained a uniform pattern of small spots. Thus, a pattern has been defined by the number, distribution and size of spots. At this stage of her investigation, McClintock (1946) suggested that the spotted patterns (CI → C) represented the elimination of the I factor from some cells at a particular time and at a particular stage in the development of endosperm tissue.

Cytological examination of the pachytene figures in plants derived from spotted kernels revealed that a break occurred on one of the chromosomes 9 at about one-third the distance from the centromere. This resulted in deletion of the terminal two-thirds of the short arm of chromosome 9 and thus led to the loss of the CI-carrying segment (McClintock, 1946).

McClintock (1947) designated the break point as Ds because a site could be identified that dissociated at the locus. The cytological observations were confirmed by genetic analyses in which dissociation mutations at the Ds locus were traced in all stages of the plant life cycle. In a plant carrying CI, Sh, Bz, Wx and Ds (Sh, Bz and Wx are the dominant alleles of shrunken, bronze and waxy mutants, respectively) in the distal two-thirds of one chromosome 9 and recessive alleles and ds in homologous chromosome 9, a dissociation mutation at Ds results in the loss of the acentric fragment containing all the dominant genes. Consequently, the phenotype of the recessive genes that are present in the homologous chromosome is expressed in the tissue.

The dissociation mutations at the Ds locus were observed only when a specific dominant factor was present in the nucleus. This factor

was designated $\underline{Ac}$ because it activates the $\underline{Ds}$ locus leading to a loss of the distal segment. In the absence of $\underline{Ac}$, the $\underline{Ds}$ locus is indistinguishable from $\underline{ds}$. $\underline{Ac}$ is inherited as a single unit (McClintock, 1947).

$\underline{Ds}$ was observed to change from its original position, a few units to the right of $\underline{Wx}$, to a more distal portion close to the $\underline{Sh}$, $\underline{Bz}$ and $\underline{Wx}$ positions on the short arm of chromosome 9 (McClintock, 1952b). Such transposition of $\underline{Ds}$ was recognized by the appearance of $\underline{Ds}$ associated chromatid breaks at these new positions and concomitantly the disappearance of the breaks at the original position. Also, with the $\underline{Ds}$ at these new positions, some exceptional variegation patterns were derived. McClintock (1948) first observed such a variegation pattern in a single kernel among the progeny of a cross,

$$\underline{c}\ \underline{sh}\ \underline{ds}/\underline{c}\ \underline{sh}\ \underline{ds},\ \underline{ac}/\underline{ac}\ \text{X}\ \underline{C}\ \underline{Sh}\ \underline{Ds}/\underline{C}\ \underline{Sh}\ \underline{Ds},\ \underline{Ac}/\underline{ac}\ .$$

Instead of the expected colored aleurone with colorless sectors, the aberrant kernel was colorless with colored areas. Appropriate genetic analyses indicated that the unexpected variegation was due to a change of $\underline{C}$ in the male parent to $\underline{c}$ that is capable of mutating back to $\underline{C}$ in the presence of $\underline{Ac}$. The mutable $\underline{c}$ was designated $\underline{c-m-1}$.

The similarities in response of $\underline{Ds}$ and $\underline{c-m-1}$ to $\underline{Ac}$, and the known breakage mechanism at the $\underline{Ds}$ locus indicated that $\underline{c-m-1}$ arose when $\underline{Ds}$ was transposed from its standard position to a position within or close to the normal $\underline{C}$ locus (McClintock, 1949). The $\underline{Ds}$ at its new position inhibits the normal $\underline{C}$ activity and as a consequence, no aleurone color

is produced. In the presence of Ac, however, the activity of the

inhibited C locus is restored. The restoration of C activity or

c → C mutation in individual cells of the aleurone is recognized by

colored spots in a colorless background. Since each colored spot

represents a permanent change from c → C, with no further Ds activity,

the mutation event is suggested to involve the removal of the inserted

Ds segment from the C locus followed by the fusion of broken ends that

reestablishes the genic order (McClintock, 1949).

The origin of c-m-1 from transposition of Ds suggested that all

Ac-controlled mutable loci arise from transpositions of Ds at the

respective loci (McClintock, 1949). The mutability depends on the kind

of locus that has been inhibited by the insertion of Ds.

Several Ac-controlled mutable loci were isolated. They included

c-m-2, c-m-4 at the C locus; bz-m-1, bz-m-2 at the Bz locus; wx-m-1,

wx-m-3, wx-m-5, wx-m-6 at the Wx locus; a-m-3, a-m-4 at the A locus,

and a2-m-4 at the A2 locus. The A and A2 loci are located on chromo-

somes 3 and 5, respectively. In bz-m-2 and wx-m-3, no separate activator

was required for the mutability. They were said to be under autonomous

control (McClintock, 1951).

Thus, two classes of controlling systems have been defined

(McClintock, 1952a). They were termed single-unit systems and two-

unit systems. In a single-unit system, the locus is under autonomous

control, i.e. the controlling element is located at the locus, whereas

in a two-unit system, one of the controlling units located at a gene

locus alters the activity of that particular gene. Changes in the unit

at the locus are brought about by a second unit that is independently located in the chromosomal complement. These changes at the locus are reflected in the instability of gene activity.

3. Controlling element systems

Subsequent to the discovery of Ac-Ds two-unit controlling element system, several other two-unit systems have been identified. In general, each of these systems consists of a receptor element located at or near the controlled locus, such as A, and a regulatory element located elsewhere in the genome (Fincham and Sastry, 1974). The receptor element directly controls the activity of the associated gene. The regulatory element controls the functioning of the receptor element by a trans-active signal. The identity of an individual system is based on the specific response of the receptor element to a specific regulatory element.

To date, six two-unit controlling element systems have been identified (Peterson, 1981). They include: Activator-dissociator (Ac-Ds) (McClintock, 1945); Dotted (Dt)-a-dt (Rhoades, 1938; Neuffer, 1955; Doerschug, 1973); Enhancer-Inhibitor (En-I) (Peterson, 1953); Factor cuna (Fcu)-rcu (Gonella and Peterson, 1977); Ubiquitous (Uq)-ruq (Friedemann and Peterson, 1980); Bergamo (Bg)-O2m(r) (Salamini, 1981). Modulator (MP) has been identified with Ac in its effect on Ds (Barclay and Brink, 1954), suppressor-mutator (Spm) with En (Peterson, 1965), and Spf with F-cu (Gonella and Peterson, 1978). Spf induces mutability at the R-r#2 dil allele (Singh et al., 1975) but not at the r-cu

receptor allele. F-cu causes mutability on both the R-r#2 dil and

r-cu alleles (Gonella and Peterson, 1978).

4. Distribution of controlling element systems

The Ac-Ds system was discovered in maize strains which were under-

going a bridge-breakage-fusion cycle within the short arm of chromosome

9 (McClintock, 1946). Ac was found to be functionally similar to Mp

that conditions pericarp variegation (Barclay and Brink, 1954).

Mp has been found in maize populations from U.S., Canada, and South

America (Barclay and Brink, 1954).

The Spm system was recovered in the stocks where Ac-Ds system

originated (McClintock, 1954). The En-I system was originally isolated

from maize exposed to irradiation in Bikini atom bomb test (Peterson,

1953). En has also been found in natural populations of maize from

Colombia, Bolivia, Mexico and Venezuela (Gonella and Peterson, 1975).

The Dt element was originally found segregating in a Black Mexican

sweet corn line (Rhoades, 1936). Later, Dt was also uncovered in two

different strains of maize from Central and South America (Neuffer,

1955). Dt was at different positions in the genome of these strains.

Doerschug (1973) found two other Dt elements from maize plants under-

going bridge-breakage-fusion cycles on chromosome 9. Dt was also found

in Cuna tribal maize from Colombia and South America (Gonella and

Peterson, 1977).

The Fcu-rcu system of controlling elements was uncovered in maize

race called Chococeno from the village Unguia in Colombia (Gonella and

Peterson, 1977). Spf, which is related to Fcu in its ability to cause
mutability at the R-r#2 dil allele (Gonella and Peterson, 1978; Singh
et al., 1975) was found in an unspecified maize line expressing unstable
R alleles (Sastry and Kurmani, 1970).

The Uq-ruq system was initially uncovered in plants of an acces-
sion Ac 538 obtained from G. F. Sprague at the University of Illinois
that included variegations at the a locus (Friedemann and Peterson,
1980). With the a-ruq receptor allele, it has been possible to
canvass a large number of lines for Qu's presence. Uq has been found
in numerous lines that include inbreds B75, color converted W22, a
Longfellow Flint variety and testers a° sh2, a° Sh2, a2 bt, a2-m(r),
En, Ac and Ds.

The Bergamo (Bg)-opaque-2 controlling element system was dis-
covered in the progeny of a cross between two independently and spon-
taneously originated recessive mutants of opaque-2 (O2) (Salamini, 1981).

5. States of controlling elements and the associated
   patterns of variegation

The term "state of a controlling element" has been used to dis-
tinguish the differing potentialities of a mutable allele for express-
ing visible mutations in descendent cells of a tissue (McClintock,
1948). Different states of controlling elements have been described
by different phenotypic patterns of variegation. These patterns of
variegation are dependent on two variables: the frequency and the time
of occurrence of mutations (McClintock, 1947). Since these mutations,
in a two-unit controlling element system, represent specific responses

of the receptor element at the locus to an independently located

regulatory element, a pattern of variegation is specified by the

individual states of these elements.

A change in state of either the receptor or the regulatory element

may occur during the development of a tissue. In the endosperm tissue,

such a change in a cell is reflected in the alteration of variegation

pattern in the descendants of that cell (McClintock, 1947). However, a

change in state during the development of the sporophytic tissue is

observed as altered pattern of variegation in exceptional progeny

kernels.

A change in state of a receptor element occurs only in the

presence of a regulatory element (McClintock, 1955). The new state is

recognized in the presence and also (sometimes) in the absence of the

regulatory element. In the presence of the regulatory element, the new

state is expressed by striking differences in the type of mutation,

and also in the time and frequency of such mutations, whereas in the

absence of the regulatory element, the new state also affects the

degree of gene action ranging from colorless to full colored (Fowler

and Peterson, 1974; Reddy and Peterson, 1976).

A change in state of the regulatory element is recognized by an

altered response of a standard receptive locus. (A standard receptive

locus represents the receptor at a locus without a change in state.)

The altered response includes a change in the time and the frequency of

mutations (McClintock, 1965).

The changes in state of controlling elements and the associated

patterns of variegation have been extensively investigated in En(Spm)-I system. Therefore, this controlling element system will be reviewed with respect to changes in state of both the receptor and the regulatory elements.

a. <u>Changes in state of the receptor element</u>, I   Although the changes in state of I of several mutable alleles have been reported in the literature, only the relevant changes in state of I of the mutable alleles at the A locus are reviewed here.

In the presence of Spm (+ Spm), the original state of I of the mutable allele, a-m-1 exhibits many early-occurring mutations (coarse spots--Figure 2.1) in the aleurone layer of the kernel. The intensity of pigmentation of these mutations ranges from faint to deep. In the absence of Spm (-Spm), restricted gene action (Figure 2.1) occurs and this results in uniformly distributed pale coloration in the aleurone layer (McClintock, 1955).

Derivatives from the original a-m-1 mutable allele representing changes in state of I (Figure 2.1) exhibit varied frequency and time of occurrence of mutations (+Spm) and also degree of gene action at the locus (-Spm) (McClintock, 1968). The patterns of variegation representing the time and the frequency of mutations vary from fine and low (few) to coarse and high (many) spotted phenotypes (upper row). Each of these patterns represents a different state of I of a-m-1 allele in the presence of a standard Spm (in standard Spm, the suppressor - S, and the mutator - M components are stable). In the absence of Spm, the level of gene expression (pigmentation) ranges from null to full color (lower

+SPM

-SPM

A   B   C   D   E   F
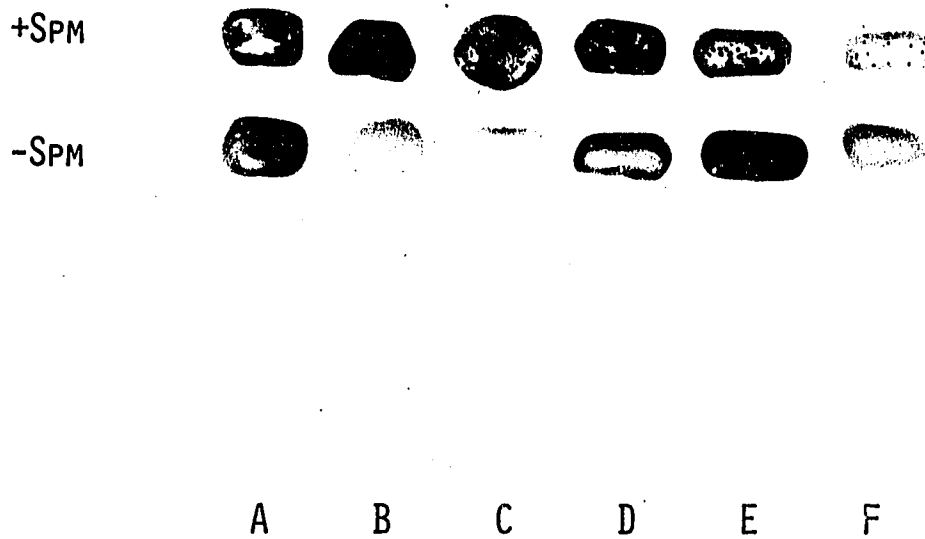
Figure 2.1. Original state of <u>a-m-1</u> (A) and its derivative states
(B-F) with (+) and without (-) Spm (McClintock, 1968)

row).

Similar findings are evident in a study of another mutable allele, a-m-2. The states of I derived from the original a-m-2 allele have been extensively studied with respect to their distinct responses to an Spm that changes in activity. These responses are discussed in a later section along with changes in state of Spm.

Besides these states of I of a-m-2 allele, other modifications of the original state of I differed with respect to their response to the components of Spm (McClintock, 1967a). These states represent the following categories:

(1) respond to S but not to M component;

(2) no response to S but do respond to M component;

(3) no response to either of the components of Spm.

At the A locus, mutability was also observed by Peterson (1961) among pale-green (pg-m) mutable stocks (Peterson, 1960). The original mutable allele, a-m(dense) (a dense pattern of variegation), is an autonomously controlled allele (En at the A locus). From this initial a-m(dense), several exceptional derivative types that were either colorless, pale or spotted were isolated. The colorless derivatives included both En-responsive, a-m(r) and non-responsive, a-m(nr) types. The a-m(r) type derivatives contained no En but exhibited uniform spotting against a colorless background when an En was introduced by appropriate crosses. These tests confirmed that the a-m(r) colorless derivatives represent a changed state of the locus following a transposition of En from the

<u>a-m(dense)</u> locus.

The spotted derivatives from the original <u>a-m(dense)</u> included <u>a-m(flow)</u>, <u>a-m(crown)</u> (Peterson, 1966), <u>a-m(pa-pu)</u> (Peterson, 1970a), <u>a-m(Au)</u> (Peterson, 1978a, 1981) and <u>a-m(pm)</u> (Nowick and Peterson, 1981). These derivatives are shown in Figure 2.2. Except for the <u>a-m(crown)</u>, all other patterns are autonomously controlled.

<u>a-m(crown)</u>: It is basically an <u>a-m(r)</u> type. The crown pattern is

dependent on the <u>En</u> whose activity is limited to the crown region

of the kernel (Peterson, 1966).

<u>a-m(flow)</u>: Fine spotted only at the base of the kernel. The spotting

is changeable to dense type by a wide assortment of <u>En</u> states

(Peterson, 1966).

<u>a-m(pa-pu)</u>: Smaller deeply pigmented spots on large colorless and pale

sectors. The large colorless sectors are interpreted as resulting

from a change of mutating to non-mutating colorless background

(Peterson, 1970a).

<u>a-m(Au)</u>: It is phenotypically indistinguishable from standard <u>A</u> except

for large colorless areas on the kernel (Peterson, 1978a, 1981).

<u>a-m(pm)</u>: Kernels containing this allele exhibit pale coloration with

dark sectors.

b. <u>Changes in state of the regulatory element En (Spm)</u>   A change in state of a regulatory element is discernible by comparing its interaction with a mutable allele before and after the change had occurred. In the <u>En(Spm)</u>-<u>I</u> system, a change in such interaction

A ·   B   C   D   E
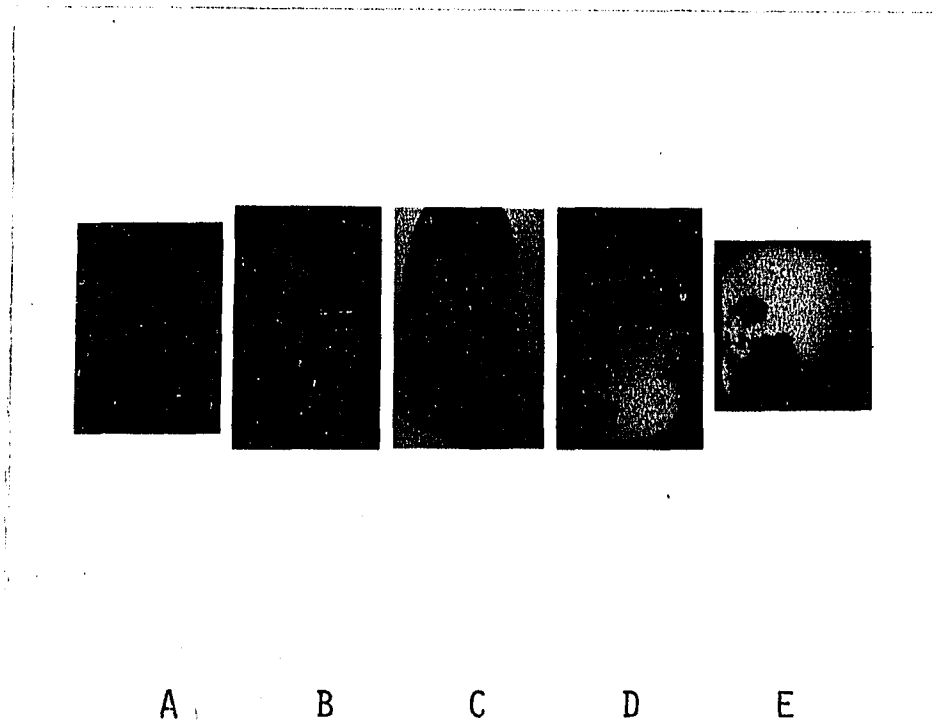
Figure 2.2. Original state of a-m(dense) (A) and its derivative states a-m(crown) (B), a-m(flow) (C), a-m(pa-pu) (D) and a-m(Au) (E) (Peterson, 1966, 1970a, 1978a, 1981; Nowick and Peterson, 1981)

concomitant with a change in state, is observed in terms of time, frequency and place of occurrence of mutations. The changes in time and frequency of mutations in a given plant tissue are reflected in corresponding changes in variegation or spotted patterns (McClintock, 1957, 1963), whereas a change in the place of occurrence of mutations is associated with a spatially restricted phase activity of the En regulatory element (Peterson, 1966).

A change in state of En(Spm) is also recognized by a total inactivity of the element. The inactive element may revert back to the original active state at certain stages of plant development (McClintock, 1961, 1964). Such cyclical change in activity during a period of time can be termed as a temporally restricted phase activity of En (Spm).

McClintock (1957) identified a change in state of the original Spm (Spm^S) by an altered variegation pattern on a kernel with a-m-1 mutable allele. The Spm^S is recognized by many deeply pigmented spots against a colorless background, whereas the new state of Spm (Spm^W) produces only one to several tiny dots of deep pigmentation in a colorless background. Also, the plants with Spm^W are pigmented throughout, in contrast to the Spm^S containing plants with streaks of deep pigmentation in a non-pigmented background. However, the plants with Spm^W become fully colored only at maturity compared to the plants with no Spm which develop pigment at early stages of plant development.

These observations indicated that the M component of Spm^W is very much weakened. The S component, however, is pronounced in kernels (McClintock, 1963).

Several other $\underline{Spm}^w$ states were isolated (McClintock, 1963). They differed in the frequency of reversion to $\underline{Spm}^s$. In some cases, the $\underline{Spm}^w$ located at $\underline{a-m-2}$ locus produced no germinal mutants. However, a reversion from $\underline{Spm}^w$ to $\underline{Spm}^s$ initiated germinal mutations and also transpositions of $\underline{Spm}$ away from the locus.

A change in state of an autonomously located $\underline{En}$ regulatory element was also observed when it is transposed to an independent location (Peterson, 1976a). In the autonomously controlled $\underline{a2-m(4\ 1629)}$ mutable allele, $\underline{En}$ triggers very late mutations. It was demonstrated that this $\underline{En}$ does not have sufficiently strong trans-activity as evidenced by the absence of mutability on $\underline{a-m-1}$ and $\underline{a-m(r)}$. However, when a transposition occurred from this $\underline{a2-m(4\ 1629)}$ allele to an independent location, the $\underline{En}$ acquired strong trans-activity and induced regular mutability patterns on $\underline{a-m-1}$ and $\underline{a-m(r)}$ alleles.

Besides these changes in variegation patterns, a change in the state of $\underline{En}$ is also reflected in a change in the place of occurrence of mutations (Peterson, 1966). Such spatially restricted phase activity of $\underline{En}$ was observed with $\underline{a-m(flow)}$ and $\underline{a-m(crown)}$ alleles. In $\underline{a-m(crown)}$, variegation is restricted to the crown region of the kernel, whereas in $\underline{a-m(flow)}$, only the base of kernel is variegated.

This spatially restricted phase activity of $\underline{En}$ was demonstrated by using the genotypes $\underline{a-m-1\ sh2}/\underline{a-m(r)\ Sh2}$, $\underline{En(crown)}$ and $\underline{a-m-1\ sh2}/$ $\underline{a-m(flow)\ Sh2}$. The $\underline{a-m-1}$ allele by itself is pale-purple in the absence of $\underline{En}$ and uniformly spotted in a colorless background in the presence of $\underline{En}$ (McClintock, 1957, 1962). But in $\underline{a-m-1\ sh2}/\underline{a-m(r)\ Sh2}$,

En(crown), the spotting is restricted to the crown area, and in

a-m-1 sh2/a-m(flow) Sh2, the base region is spotted. The non-spotted

areas exhibited pale-purple coloration of a-m-1. This indicated that

the crown and the flow patterns are due to localized phases of En

activity (Peterson, 1966).

A different type of phase activity of En(Spm) involving changes

from an active to an inactive and back to the original active state has

been reported (McClintock, 1958, 1961, 1971; Peterson, 1981). Such

cyclical change in phase of activity of En (Spm) differs from the

changes described earlier in two respects:

(1) The change is from an active to an inactive state. This

is unlike the Spm^S → Spm^W (McClintock, 1963) and the a-m(dense)

(autonomous En) → a-m flow (autonomous En) or a-m crown

(independent En) (Peterson, 1966) changes in which the change

is from an active state to another active state.

(2) The changes are both frequent and infrequent. The frequent

changes occur during the developmental period of a tissue,

whereas the infrequent changes in state are recognized only

in some plant generations.

The cyclical phase activity of Spm was first detected in cultures

of a2-m-1 mutable allele (McClintock, 1958). The a2-m-1 cultures are

distinguished into two classes based on their response to the Spm ele-

ment that undergoes change in phase of activity. Kernels carrying

class I state of a2-m-1 are uniformly pale in the absence of Spm. In

the presence of a single Spm, the kernels have deeply pigmented spots

and pale areas. The spots represent a2 → A2 mutational changes, whereas

the pale areas arise as a result of a change in phase of activity from

an active → inactive state of Spm. The mutant spot size was shown to

depend not only on the state of Spm but also on the time of change of

Spm from an inactive to an active phase of activity. Even when the

Spm present is the one that produces large spots as a result of early-

mutations, a delayed change in phase of activity from an inactive to an

active state would produce small spots.

The pale areas that arise as a result of active → inactive change

often contain smaller colorless areas within them. These colorless

areas within the pale areas represent a cyclical change in phase of

activity of Spm.

The cyclical change in phase of activity was more evident in class

II state of a2-m-1 (McClintock, 1958). The class II state produces

deep pigmentation in both kernel and plant in the absence of Spm. This

pigmentation is similar to that produced by A2. In the presence of an

active Spm, the pigment is produced in the plant but less intense than

that in the absence of Spm. But in kernels, the pigmentation is com-

pletely suppressed by an active Spm. This is in response to the S

component of Spm. The class II state of a2-m-1 does not respond to the

M component of Spm.

A change in state of this active Spm to an inactive Spm in the

kernel is recognized by deeply pigmented areas in a colorless background.

Within these deeply pigmented areas, often colorless areas appeared. In

some cases, the colorless areas in turn contained a deeply pigmented

speck. Such variegation patterns were interpreted to represent fre-
quent changes in phase of activity during the development of endosperm.

The variegation patterns of class II state of a2-m-1 exhibit

dosage effect with an increase in the number of Spm that undergo changes

in phase of activity (McClintock, 1971). An increase in the number of

Spm was shown to decrease the variegation of kernels. The kernels

with one Spm show large pigmented areas in a colorless background; with

two Spm small pigmented spots in a colorless background; with three Spm,

small specks of pigment in a colorless background; and with four Spm,

the kernels are colorless.

Changes in phase of activity of Spm were also studied in a-m-2

cultures that included a-m-2 7977B, a-m-2 7995 and a-m-2 8004 mutable

alleles. In the presence of an active Spm, the a-m-2 7977B and

a-m-2 7995 states exhibit medium size and moderately frequent colored

spots in a lightly pigmented background. However, if the Spm should

undergo a change in phase of activity during kernel development, the

response of either state to the change results in a darkly pigmented

area (McClintock, 1967a).

With a-m-2 8004 state, a change in phase of activity of Spm during

kernel development results in large and small areas outlined by rims

of deep pigment (McClintock, 1967a). The deep pigment of the rims is

interpreted to result from a complementation reaction between the outer-

most cells of the area (with inactive Spm) and a diffusible substance

from the cells surrounding the area in which Spm is fully active.

Another important aspect of change in phase of activity is that the

duration of a phase resides in the event that initiates the change in phase (McClintock, 1961). This was demonstrated in the following experiment.

In plants with an active $\underline{Spm}$, a part of an ear showed reversal in phase of $\underline{Spm}$ activity. Plants grown from the kernels with and without the altered phase were compared with respect to the times and the frequency of occurrence of phase reversals. The patterns of phase reversal were very similar among the plants derived from kernels with the original phase of activity. However, these patterns of change in phase of activity were contrasted with those in plants derived from kernels with the altered phase of activity.

Changes in phase of activity are also observed with individual components of $\underline{En}$ regulatory element (Peterson, 1981). An unstable suppressor $(\underline{S}^u)$ component of $\underline{En}$ was observed to change in phase from $\underline{S}^u \rightarrow \underline{s}$. This change was demonstrated by using the $\underline{a-m-1}$ mutable allele that is dark pale in the absence of an active $\underline{En}$. This allele's response to an active $\underline{En}$ is recognized by deep-colored spots in a colorless background. However, phase changes from $\underline{S}^u \rightarrow \underline{s}$ during the development of kernel result in pale-colored spots or patches. These spots are less intense than the deep-colored spots associated with the mutator $(\underline{M})$ activity. Also, that these spots are not associated with $\underline{En}$ losses has been supported by cyclical change in phase activity $(\underline{S} \rightarrow \underline{s} \rightarrow \underline{S})$.

The unstable $\underline{S}(\underline{S}^u)$ components were detected among newly transposing $\underline{En}$s. These $\underline{S}^u$s varied in the time and frequency of change in phase

of activity. The change in $\underline{S}$ has been shown to be independent of $\underline{M}$ activity.

6. <u>Basis for states of controlling elements</u>:
    <u>position vs compositional hypothesis</u>

The position hypothesis states that the state of a controlling element is determined by its position of insertion within the genome and not by its inherent quality or composition (Peterson, 1976a). The reverse is reasoned in the compositional hypothesis (McClintock, 1958).

To test these hypotheses, Peterson (1976b) conducted an experiment in which the pattern affected by an $\underline{En}$ regulatory element with a standard $\underline{a-m(r)}$ allele was compared with the patterns elicited by the newly transposed $\underline{En}$ in the mutable alleles at $\underline{A2}$ and $\underline{C}$ loci. If the composition of $\underline{En}$ determines the pattern, similar patterns are expected before and after transposition of $\underline{En}$. However, if the pattern is due to the position of $\underline{En}$, no correlation is expected between the pattern of the $\underline{En}$ at its original position and that following its transposition.

Among 18 $\underline{a2-m}$ and 30 $\underline{c-m}$ newly arisen mutable alleles tested, the mutability patterns of these new alleles showed no correlation to the pattern elicited by the original $\underline{En}$ on the $\underline{a-m(r)}$ allele (Peterson, 1976b). In addition, the mutability patterns of $\underline{a2-m}$ and $\underline{c-m}$ are often different although the $\underline{En}$ inserted at these loci originated from the same source. Thus, the diversity of patterns observed in these studies supported the position hypothesis.

Williams and Brink (1972) and Brink and Williams (1973) provided additional support for the position hypothesis. From the isolation of

26 reconstituted mR-nj alleles with common MP, they found a wide variation in mutability. This diversity in expression of the 26 mR-nj alleles is supposedly caused by different sites of MP deposition within R locus.

Recent findings of Dooner (1981) with bz-m-4 and its derivative mutable alleles also support the position hypothesis. The bz-m-4 kernels accumulate traces of anthocyanin pigment, whereas of the seven bz-m-4 derivatives studied, five were fully-purple and two were pale-purple. Two of the five fully-purple derivatives were found to change infrequently to unstable types in response to the regulatory element, Ac, whereas the pale-purple derivatives, in response to Ac, gave a pale-dark-purple variegated phenotype. In addition to these differences in phenotype and response to Ac, the pale-purple derivatives had lower levels of UFGT (UDP glucose:flavonoid 3-0-glucosyltransferase) activity.

In support of compositional hypothesis, both quantitative and qualitative differences were attributed to the differing responses of receptor element to a regulatory element (McClintock, 1958). In Ac-Ds system, changes in Ds were assumed to represent structural differences in Ds element itself or the relation of Ds to the components of locus. Also in Spm system, the strong and weak component differences of Spm were assumed to indicate origin of states by differing in composition of the regulatory element.

7. Gene regulation by controlling elements in maize

Controlling elements in maize are known to reside at a wide variety of gene loci (McClintock, 1964). In residing so, they control gene

action at these various loci. Such control of gene action involves the type and distribution of a gene product in a developing tissue. Also, the gene action is regulated in different tissues at different stages of plant development.

Since the controlling elements regulate the time of expression of a set of genes, they are considered as "genetic clocks"; and the different alleles or states of a gene locus represent different "settings" of the clock (McClintock, 1964). Each state or "setting" of a gene exhibits a characteristic mode of control and it is retained through plant generations unless an alteration occurs in the "setting" by mutation.

In a two-unit controlling element system, the receptor and the regulatory elements represent the component parts of the clock-like regulatory mechanism (McClintock, 1964). Different states of these elements that arise by mutation (change in state or in phase of activity) contribute to the diversity of gene expression. In addition to these changes in state, gene expression is apparently regulated by another gene control mechanism called "presetting" (McClintock, 1964).

a. Presetting and the associated pattern of gene expression
According to McClintock (1964), presetting involves gene regulation that is effected in the presence of Spm early in plant development, and the phenotype resulting from this regulation appears in the progeny only in the absence of Spm. It is called presetting because the gene is preset by the regulator (Spm) to undergo a change that is expressed following the removal of Spm by meiotic segregation.

Figure 2.3. Preset patterns of gene expression (McClintock, 1964)

(a)   Spotted x <u>a</u> sh2/<u>a</u> <u>sh2</u>

            ↓

    Spotted, colorless and presets (mottled)

(b)   Special case where <u>Spm</u> changes in phase of
       activity from active → inactive state in a
       cell that gives rise to part of an ear

          Spotted x <u>a</u> sh2/<u>a</u> <u>sh2</u>

ear sector with	ear sector with
active <u>Spm</u>	inactive <u>Spm</u>
↓	↓

Spotted, colorless, presets (mottled)   Colorless, presets (mottled)

(c)   Special case where <u>Spm</u> in the main plant is
       active and inactive in the tiller

        Tiller   x <u>a</u> sh2/<u>a</u> <u>sh2</u>
      (inactive <u>Spm</u>) |
            ↓

     colorless, presets (mottled)

Figure 2.4.   Tests of spotted kernels of <u>a-m-2 7995</u> or <u>7977B</u> state

(a) colorless x <u>a</u> <u>sh2</u>/<u>a</u> <u>sh2</u>

↓

all colorless

(b) colorless x <u>a</u> <u>sh2</u>/<u>a</u> <u>sh2</u>, <u>Spm</u>

↓

spotted, colorless

(c) Special case where the plant commences develop-
ment with an inactive <u>Spm</u> ('.' colorless kernel)
but a change from inactive → active state re-
sults in a sectored ear

colorless   x <u>a</u> <u>sh2</u>/<u>a</u> <u>sh2</u>
(inactive <u>Spm</u>)

ear sector with   ear sector with
active <u>Spm</u>   inactive <u>Spm</u>

↓   ↓

Spotted, colorless, presets (mottled)   all colorless

Figure 2.5. Tests of colorless kernels of <u>a-m-2 7995</u> or <u>7977B</u> state

(a)

Presets x <u>a</u> <u>sh2</u>/<u>a</u> <u>sh2</u>
(mottled)

1st generation mostly colorless + one or two presets at
the tip of the ear

colorless x <u>a</u> <u>sh2</u>/<u>a</u> <u>sh2</u>

2nd generation   all colorless x <u>a</u> <u>sh2</u>/<u>a</u> <u>sh2</u>

3rd generation   all colorless

(b)

Presets x <u>a</u> <u>sh2</u>/<u>a</u> <u>sh2</u>, <u>Spm</u>
(mottled)

spotted, colorless

(c)

<u>a-m-1</u> <u>sh2</u>/<u>a-m-1</u> <u>sh2</u> x presets
(<u>Spm</u> responsive)

no spotted
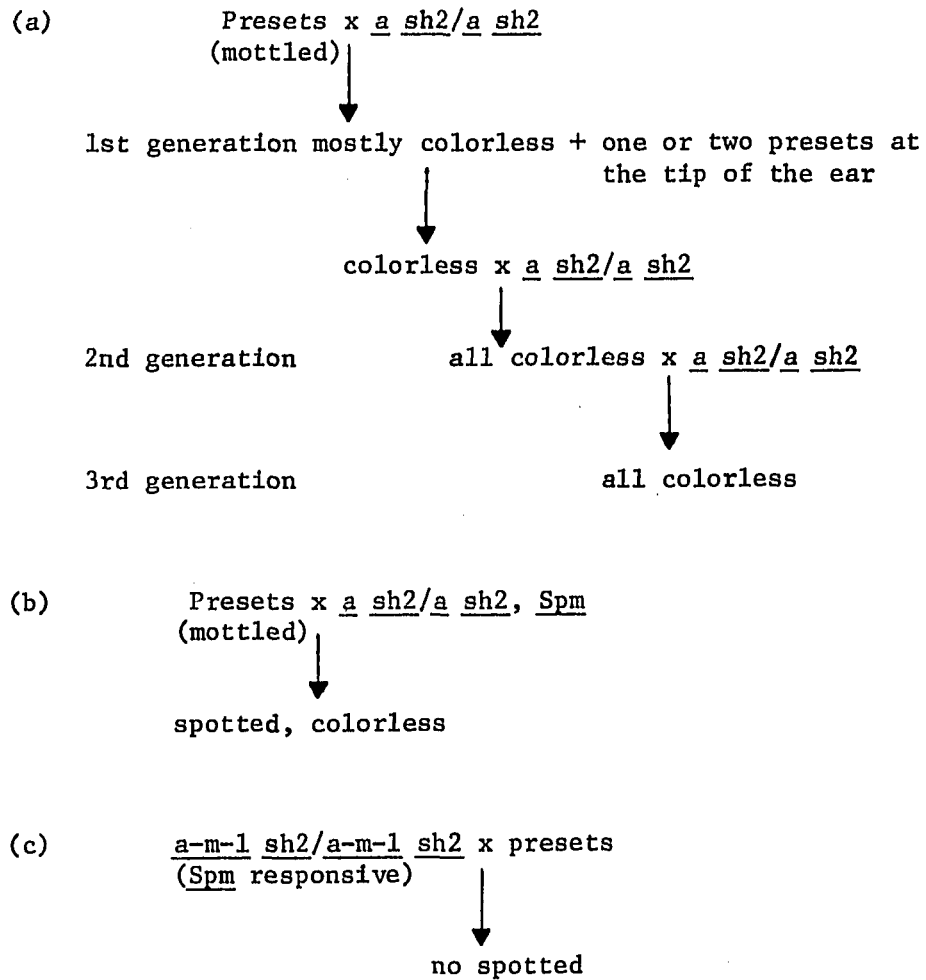
Figure 2.6.   Tests of preset type kernels of <u>a-m-2 7995</u> or <u>7977B</u> state

The preset pattern of gene expression appears as mottled pheno-
type (Figure 2.3). It appeared unexpectedly on an ear of cross
Spm^W a-m-2 (original state) Sh2/a sh2, Spm^S X a sh2/a sh2 (Spm^S is a
standard Spm with strong suppressor and mutator functions; Spm^W has a
weak mutator that induces mutations late and less frequently than Spm^S).
The progeny kernels on this ear are expected to include only spotted
(+Spm) and colorless (-Spm) phenotypes (McClintock, 1963). Similar
results were obtained in crosses

a-m-2 (7977B state) Sh2/a sh2, Spm^S X a sh2/a sh2

and

a-m-2 (7995 state) Sh2/a sh2, Spm^S X a sh2/a sh2

(McClintock, 1964). The states 7977B and 7995 (not autonomous) were
derived independently from the original autonomously controlled a-m-2
state. Both the states have a similar phenotype with (spotted) and
without (colorless) Spm.

The spotted, colorless and preset (mottled) phenotypes of
a-m-2 7995 and a-m-2 7977B progeny kernels were tested to determine
their heritability (Figures 2.4, 2.5 and 2.6) and to formulate the
genetic basis for the appearance of preset kernels (McClintock, 1964).

      1) __Spotted__   The progeny of spotted in test crosses
(X a sh2/a sh2) included spotted, colorless and preset types (Figure
2.4a). In special cases where Spm changed in phase of activity, dif-
ferent heritability patterns resulted. If the Spm changed from an
active to an inactive state in a cell that gave rise to part of an ear,

the kernels in ear sector with inactive Spm included colorless and

presets, whereas those in ear sector with active Spm consisted of

spotted in addition to colorless and preset types (Figure 2.4b). In

another case where the main plant had an active Spm and the tiller

with an inactive Spm, the ear produced on the tiller contained colorless

and preset type kernels (Figure 2.4c).

   2) Colorless   The colorless produced only colorless

among test crosses (Figure 2.5a). In crosses with a sh2/a sh2, Spm,

the progeny included spotted and colorless kernels (Figure 2.5b). In a

special case where the plant commenced development with an inactive Spm

(therefore colorless kernel) but produced a sectored ear as a result of

change in Spm from inactive → active state, the kernels on the ear

sector with active Spm consisted of spotted, colorless and preset types

(Figure 2.5c).

   3) Presets   The presets in most of the test crosses

yielded only colorless kernels (Figure 2.6a). On a few (numbers not

available) test cross ears, however, one or two preset type kernels

appeared. When further advanced through test crosses, the colorless

progeny produced only colorless kernels.

   The presets, when tested on an Spm responsive tester (a-m-1 sh2/

a-m-1 sh2) did not produce spotted progeny, confirming that the presets

lack Spm (Figure 2.6c). In crosses with a sh2/a sh2, Spm, the presets

responded to the Spm yielding spotted and colorless progeny kernels

(Figure 2.6b).

   These analyses showed that the association of an active Spm with the

mutable allele in kernels (compare Figures 2.5a, 2.6b and 2.7a) or in

the plant prior to the development of ear (compare Figures 2.5b, 2.5c

and 2.6c) is required for the preset phenotypes to appear in the

progeny. Since the preset phenotype appeared only in kernels without

Spm (Figure 2.7c), the removal of Spm by meiotic segregation is essen-

tial for the appearance of presets. This set of observations indicated

that presetting of the a-m-2 locus must have occurred early in plant

development.

However, only a few of the progeny kernels without Spm express

preset phenotype. In order to explain this deficiency of the expected

phenotypes, another mechanism called "setting" of the locus was proposed

to occur between the end of meiosis (after the removal of Spm from the

spore nuclei) and the formation of primary endosperm nucleus (McClintock,

1964).

Most often the preset phenotypes are not heritable. But in a few

test cross progenies (presets X a sh2/a sh2), one or two preset type

kernels appeared near the tip of the ear. These observations suggested

that "erasure" of setting occurs at the same stage as the setting of the

locus. The one or two preset type kernels near the tip of the ear are

interpreted as escapes of the erasure mechanism (McClintock, 1967b).

The preset pattern of gene expression has been shown to depend on

suppressor (S) component of Spm (McClintock, 1964). This was indirectly

demonstrated by using plants carrying either Spm^W or Spm^S in individual

crosses with the a-m-2 7977B plants. Although Spm^W has a weak mutator

(M) function, it was as effective as Spm^S in inducing preset patterns

of gene expression. This observation suggested that the $\underline{S}$ component is responsible for the preset pattern. A direct evidence to this effect was obtained in test crosses of a-m-2 7977B plants carrying an Spm whose $\underline{S}$ component is inactive throughout plant development but changed to an active phase during development of the kernels. The preset patterns were not present in the progeny kernels (McClintock, 1964).

The setting phenomenon, similar to the presetting of gene activity in a-m-2 states, has been observed in the F-cu-r-cu controlling element system (Gonella and Peterson, 1978). In this system, the kernel phenotype of r-cu mutable allele ranges from near colorless to almost colored. The variable dilute phenotypes (classified into light, intermediate, and dark dilute) are not heritable; i.e., the test cross progeny of a particular phenotype contain all three different classes of kernels. Such segregation was always observed despite a strong selection for a particular phenotype. These observations suggested that the variability among individual kernels in r-cu pigmentation is an intrinsic property of the r-cu allele.

Since there was no evidence of ear sectoring or the effect of kernel position on the ear with respect to the level of r-cu pigmentation, it has been suggested that the level of r-cu pigmentation capacity is set at some stage between megasporogenesis and fertilization of the polar nuclei. However, this setting does not require the presence of F-cu regulatory element. This is unlike the Spm requirement for presetting in a-m-2 states (Gonella, 1976).

The aspects of gene regulation by controlling elements that are discussed in this section represent only a few of several mechanisms of gene regulation that operate in eukaryotic organisms. Some or all of these mechanisms may operate in any single eukaryote. A knowledge of the molecular aspects of these gene control mechanisms will facilitate a better understanding of gene regulation by controlling elements in maize. Therefore, a general review of the eukaryotic gene regulation is presented in the following seciton.

B. Regulation of Eykaryotic Gene Expression

The genetic mechanisms responsible for eukaryotic gene regulation can be classified into two groups—gene alteration and gene modulation, based on the level of control. Gene alteration includes (a) diminution or loss of genes, (b) gene amplification, (c) gene arrangement, and (d) modification of genes. Gene modulation occurs at (a) transcriptional, (b) posttranscriptional, and (c) translational levels (Brown, 1981).

1. Gene alteration

a. Diminution of genes   The diminution or loss of genes has been observed in some nematodes, crustaceans, insects (Blackler, 1970) and protozoa (Lauth et al., 1976). In these organisms, the gene loss involves elimination of certain chromosomes or DNA during germ cell differentiation. For example, in the protozoan Oxytrichia, a macro-nucleus is formed from micronucleus by a process involving DNA cleavage

elimination of most of the DNA and replication or polytenization of the remaining fragments. The micronucleus retains germ line continuity, whereas the macronucleus is responsible for all of the cell's RNA synthesis (Lauth et al., 1976).

Because the gene loss is detected only in some eukaryotes, it is believed that it is not a general mechanism of gene control. However, a small loss of chromosomal material in other eukaryotes could escape detection (Brown, 1981).

b. Gene amplification Gene amplifications can occur during evolution (Ohno, 1970), as part of a normal developmental process (Brown and Dawid, 1968), and as a compensatory process where deletion of genes is otherwise detrimental to viability (Tartoff, 1975). In evolutionary time, genes occur as repeating structures, such as histone gene clusters in sea urchins (Cohn and Kedes, 1979) and Drosophila (Lifton et al., 1978), rabbit β-like globin genes (Lacy et al., 1979) and chorion multigene family in silk moths (Jones et al., 1979). But most relevant in terms of gene regulation during the life-cycle of a eukaryote is the gene amplification as required by the developmental processes. Examples of gene amplification as part of normal developmental process are the increase in rRNA genes in Xenopus (Brown and Dawid, 1968; Gall, 1968), Tetrahymena (Yao and Gall, 1977) and Drosophila (Endow and Gall, 1975) and in chorion genes in Drosophila (Spradling and Mahowald, 1980). In oocytes of Xenopus, because of amplification of rDNA (rRNA genes), one oocyte synthesizes several thousand times more ribosomes per unit time than a single somatic cell. The amplified

rRNA genes only function during oogenesis (Brown and Dawid, 1968).
In Drosophila, the same chorion gene that is repeated in silk moth
during evolution is present in germ and somatic cells at 1-3 copies
per genome. But in the ovary of flies producing eggs, these genes
are amplified up to 25 copies per ovarian cell genome (Spradling and
Mahowald, 1980).

Gene amplification also is shown to occur in mammalian cells that
are cultured with methotrexate (MTX) (Alt et al., 1978; Schimke et al.,
1979), an inhibitor of dihydrofolate reductase (DHFR). The cells
gradually become resistant to the drug and this resistance is corre-
lated to the increased amounts of DHFR which in turn is related to
increased number of dhfr genes.

The increase in dhfr gene number has been proposed either due to
unequal crossing-over (Bostock et al., 1979) or to saltatory or dis-
proportionate replication (Schimke et al., 1981). In saltatory repli-
cation, replication is initiated at the same origin a number of times
during the S phase of cell cycle (Schimke et al., 1981).

    c. Gene rearrangements   Gene rearrangements have been ob-
served in several eukaryotic organisms (Brown, 1981). Most significant
are the rearrangements in immunoglobulin genes (Tonegawa et al., 1977),
mating-type genes (Hicks and Herskowitz, 1976) and transposable elements
(Ty) in yeast (Cameron et al., 1979), copia-like transposable elements
in Drosophila (Rubin et al., 1976). These systems of gene rearrange-
ments are discussed in a later part of this review. However, a cursory
review of these systems is presented below.

Immunoglobulin genes that code for variable and constant regions of light and heavy chains of the immunoglobulin molecule are widely separated in embryonic cells. Complete immunoglobulin genes are created by somatic recombination that occurs during differentiation of lympho-cyte precursor cells (Tonegawa et al., 1977).

In yeast, mating types (a and α) are controlled by the mating-type locus (MAT) which can harbor one of two genetic blocks, a or α. Silent copies (unexpressed) of a and α genetic blocks reside at two other loci, HMR and HML. Mating-type interconversion occurs by transposition of a copy of mating-type information (a or α) at HMR or HML to the MAT locus (Rine et al., 1981).

Again in yeast, a transposable element (Ty 1) was found to cause heterogeneity in sequence arrangements around a tRNA locus (Cameron et al., 1979). Several transposable elements of Ty 1 type were found distributed in the yeast genome. These elements are characterized as repetitive DNA sequences that move to new locations in the genome by non-homologous recombination events (Fink et al., 1981).

Copia-like transposable elements in Drosophila represent several repetitive sequence families that undergo transposition within the genome. These elements occur at widely scattered locations in the chromosomes. Transposition of copia creates a 5 bp duplication of the target DNA (Rubin et al., 1981).

   d. Modification of genes   DNA modification by methylation of cytosine at the 5-position (5^mC) has been found to be stable and transmitted to progeny cells at each division (Vanyushin et al., 1970).

5^mC is found mainly next to guanine (G) residues (Grippo et al., 1968).
This modification of DNA is the result of post-replicational methyla-
tion of C residues and not of the incorporation of 5^mdCMP during DNA
synthesis (van der Ploeg and Flavell, 1980).

Restriction endonuclease analysis showed that the DNA modification
is site-specific and tissue specific in higher organisms. Bird and
Southern (1978) demonstrated that while most Hha cleavage sites in
Xenopus rDNA were modified at CpG residues, a single Hha site in the
28S rRNA gene was unmodified in about 50% of rDNA repeats at this posi-
tion. Tissue specific DNA modification was found at a CCGG site in
the rabbit β-globin gene. Sperm DNA showed 100% modification at this
site, whereas DNA from other tissues showed lower values (Waalwijk
and Flavell, 1978).

It has been suggested that DNA methylation has a role in differenti-
ation in higher organisms (Holliday and Pugh, 1975). In one of the
studies to correlate the gene expression with DNA methylation, van der
Ploeg and Flavell (1980) have investigated the expression of genes in
the fetal (Gγ, Aγ) and adult (δ, β) phases of human development. These
genes are linked in the order 5' Gγ AγδβЗ' (Flavell et al., 1978). The
data suggest a correlation of the DNA modification around a given gene
and the expression of the gene in the tissue. However, it is not known
whether changes in DNA modification are involved in the cause of gene
expression or as an effect of that expression (van der Ploeg and Flavell,
1980).

2. Gene modulation

a. Transcriptional control   Direct evidence for transcriptional control has been obtained for highly specialized genes of globin, ovalbumin, and fibroin proteins (Brown, 1981). Using clones of DNA complementary to ovalbumin mRNA (cDNA), it has been demonstrated that less than one molecule of RNA homologous to ovalbumin cDNA is present in virgin oviduct tissue before hormone induction, whereas, in fully differentiated oviduct tissue, several hundreds of nuclear RNA and thousands of cytoplasmic RNA copies were homologous to the ovalbumin cDNA (Tsai et al., 1979).

The evidence for transcriptional control has been possible to obtain only for specialized genes which code for a large proportion of cellular mRNA (Brown, 1981). It is because the majority of cellular genes are responsible for only a small number of nuclear RNA and protein molecules (Davidson and Britten, 1979).

There are three other findings that lend support for the transcriptional control in eukaryotes. One, there are three distinct forms of RNA polymerase that transcribe different sets of genes (Roeder, 1976). Another is that in adenovirus (human DNA virus), a single mRNA is produced early in infection, whereas 15 to 20 different mRNAs are produced late in infection, all the transcripts starting from the same starting point in the transcription unit (Nevins, 1982). This differential transcription reflects the fact that early in infection, termination of transcription occurs near the middle of the genome (Nevins and Wilson, 1981), whereas late in infection, transcripts terminate near the end of

the genome (Fraser et al., 1979). The termination of transcript in early infection appeared to be cis-controlled because unreplicated (early form) DNA, when placed into a late infected cell, is still expressed in the early mode (Thomas and Mathews, 1980). This suggests an opposing transcription on the leftward-reading strand could block the rightward-reading transcription (Nevins, 1982).

Similar to the case of adenovirus, different transcription termination sites of a single gene have been shown to be responsible for two forms of μ heavy chain immunoglobulin proteins that differ at their carboxyl termini (Early et al., 1980b).

b. Post-transcriptional control   There are three post-transcriptional modifications that are essential in gene expression. First, an enzymatic complex joins the 5' end of the precursor RNA transcript with an inverted GTP (guanosine triphosphate) residue and then methylates one or more internal adenine residues. Secondly, poly(A) (polyadenylate) residues are added to the 3' end of the RNA molecule (Darnell, 1979). Finally, the precursor RNA is processed by excision of intervening sequences and splicing of the coding pieces of RNA (Tilghman et al., 1978).

Different roles of these processing steps in gene expression have been suggested in recent studies. The GTP addition to the 5' end of RNA transcript (capping) appears to facilitate binding to ribosomes (Kozak, 1978) and enhance stability of mRNA (Furiuchi et al., 1977). Poly(A) addition is suggested to prolong the half-life of mRNA (Heuz et al., 1974). The splicing step has been shown to be essential for

the transport of mRNA from the nucleus into cytoplasm (Gruss et al., 1979).

c. Translation control   One of the most important means for translation control is the stability of mRNA. In silk worm, efficient utilization and stabilization of the mRNA are partly responsible for a high rate of fibroin protein synthesis. Each mRNA serves as a template for the synthesis of about 10^5 protein molecules (Suzuki and Suzuki, 1974). Such prolonged synthesis of protein on stable mRNA is referred to as translational amplification (Brown, 1981).

Another example of stable and inactive mRNA is the so-called "masked" mRNA in unfertilized sea urchin eggs (Gross, 1967). This mRNA is stored for several months.

C. Mutable Loci in Other Eukaryotic Species

Eukaryotic organisms representing several different species contain loci that express mutability similar to that in maize. The organisms include several plant species, mammals, yeast and Drosophila. In the latter two species, the control of mutability is explored at DNA level.

1. Plants (other than maize)

a. Antirrhinum majus   In Antirrhinum majus, magneta spots are observed on ivory colored flowers. The spotting is associated with a mutable allele at the Pal locus, designated pallida-recurrens (pal-rec). The spots are due to mutations of the recessive pal-rec to the dominant Pal allele (Harrison and Fincham, 1964). Since the causal factor for spotting (mutability) is located within or very near

the Pal locus, pal-rec is classified as an autonomous, unstable system. A second mutable allele, designated nivea-recurrens, was discovered at the Nivea locus (Harrison, 1971). This mutable allele also affects flower color similar to the pal-rec allele by inducing colored spots on ivory-colored flowers.

The flowers of pal-rec exhibit high mutability (spotting). From this highly mutable pal-rec strain, several low mutable plants were isolated. This low mutability was suggested due to a semi-dominant modifier, designated stabilizer-St (Harrison and Fincham, 1968). However, when the low mutable plants (pal-rec-low) were crossed by a non-mutable tester (pal-tub), the mutability in the progeny was evoked to varying degrees (Sastry, 1976); the progeny included uniformly low, high and in some cases the individual plants contained both low and high mutability (called shifting) in different parts of a plant. The uniformly low mutable progeny plants (pal-rec-low/pal-tub) were crossed with the highly mutable sib plants (designated pal-rec-low-act/pal-tub) and also with the standard highly mutable pal-rec (pal-rec/pal-rec) plants to determine if the low mutable plants contain a modifier that reduces the high mutability. The results indicated that they do contain a modifier, designated Rp, that reduced the mutability of pal-rec-low-act but not of the standard pal-rec plants (Sastry et al., 1981).

The mutability of the original pal-rec is also found to be suppressed by another modifier factor, designated J (Jeffries, 1977). This factor was isolated from a strain containing a different anthocyanin gene, eos, in a homozygous condition.

Recently, Sastry et al. (1981) discovered a modifier controlling element, Sfm, in a subline of standard pal-rec/pal-rec. The Sfm causes shifting (evoking both high and low mutability) when associated with pal-rec and pal-rec-low. Preliminary observations indicated that Sfm is composed of two components, Sf (suppressor) and m (mutator) similar to those of Spm regulatory element in maize (Sastry et al., 1981).

b. Impatiens balsamina   In Impatiens balsamina, variegation for flower color is caused by an interaction between a mutable allele (designated as p-m) of P-r gene (P-r is one of three genes involved in anthocyanin production) and an independent controlling element, M (Sastry et al., 1981). In the absence of M, p-m/p-m plants produce only white flowers on green stems.

The mutable allele p-m undergoes somatic changes to produce either pale or dark sectors. Also, p-m was observed to mutate either in early or late stages during the development of flowers. The late mutations produce fine spots which in turn are distinguishable into dark and pale.

Differences were also observed between heterozygous (p-m/p, 1 M) and homozygous (p-m/p-m, 1 M) variegated plants in that the former had a large number of whole branches with white flowers in the upper section of the plant. In both cases (heterozygous and homozygous), however, the first two branches (appearing in the axils of cotyledonary leaves) always bore variegated flowers. Vegetative propagation of branches with wholly variegated and wholly white flowers resulted in plants with variegated cotyledonary branches and upper branches with

few or no variegated flowers. These observations indicated two points: One, the flower-variegation is conservative; i.e., mostly cotyledonary branches bear variegated flowers. Secondly, neither a change in p-m (remains responsive) nor loss of M occurs in upper branches with only white flowers (Sastry et al., 1981).

c. Delphinium ajacis   Dawson (1955, 1964) reported an unstable pink flower gene (p*) in Delphinium ajacis. The mutability of p* is dependent on a dominant, independent activator. Several new states differing in mutability were recovered. From the stocks with p* allele, a new mutable gene, g* expressing mutability of the foliage, was isolated. The mutable expression of g* allele was from a yellow-green phenotype to a stable green. The origin of g* allele from p* allele stocks was considered to result from transposition of a controlling element similar to that in maize (Dawson, 1964).

d. Glycine max   Peterson and Weber (1969) reported a variegated leaf character that arose spontaneously in a Glycine max cultivar. The variegation was associated with an unstable dominant allele, designated Y-m. The plants with Y-m have green leaves with yellow sectors, indicating a change from Y-m allele (green) to the y allele (yellow). Reversion of y to Y was never observed, and thus the y allele was considered stable.

e. Nicotiana   In the genus Nicotiana, unstable genes were uncovered among the progeny of interspecific crosses between N. langsdorffii X N. sanderae (Smith and Sand, 1957). A high frequency of the unstable alleles were found at the variegated (V) locus for flower

color (Sand, 1969). The instability was also observed at the $\underline{E}$ locus, which is responsible for pigmentation of the corolla tube and back of the corolla lobes of $\underline{Nicotiana}$ flowers (Sand, 1971).

f. $\underline{Petunia\ hybrida}$ Bianchi et al. (1978) reported on unstable anthocyanin gene ($\underline{An\ 1}$) among the progeny of a red flowered cultivar of $\underline{Petunia\ hybrida}$. The unstable $\underline{An\ 1}$ gene is expressed as red spots on white flowers. The red spots are attributed to back mutations of the $\underline{An\ 1}$ gene. Germinal back mutations were also observed where the progeny included a few plants with red flowers. In addition to the back mutations, changes in spot size and frequency were noticed. Since these changes were very frequent, they are attributed to mutations of a regulatory element associated with the $\underline{An\ 1}$ structural gene.

Factors that influence the spot frequency and the back mutations are also reported. An increase in the spot frequency is observed with an increase in the dosage of an unidentified independent factor located on an extra chromosome in a trisomic plant (Mulder et al., 1981). A decrease in the frequency of back mutations of $\underline{An\ 1}$ gene is identified with a dominant allele, $\underline{In\ 1}$ (Gerats et al., 1982).

2. $\underline{Mammals}$

Whitney and Lamoreux (1982) provided phenotypic evidence for the presence of controlling elements in mammals. Several mutant states of mammalian loci are believed to result from the insertion of transposable elements into or near these loci. In mouse, the mutations at white spotting loci ($\underline{W}$, $\underline{Mi}$), agouti ($\underline{A}$), pearl ($\underline{pe}$) and pink-eye ($\underline{p}$)

are explained in this way. In all these mutations, a high rate of somatic or germinal reversions occur.

a. <u>W-locus</u>   Mutations of the <u>W</u>-locus produce white-spotted fur and slightly reduced (gray) background pigmentation as compared to intense black fur of wild type mice (Whitney and Lamoreux, 1982). In an inbred stock (C57BL/6J) of mice carrying <u>W-42J</u> mutant allele in heterozygous condition (C57BL/6J-<u>W-42J</u>/+), a few mice exhibited islands of wild-type (black fur) in the white areas or in the gray pigmented background. Such somatic reversions to wild type also were noticed in mutant phenotypes of <u>W-37J</u> and <u>W-J2</u> alleles.

b. <u>Mi-locus</u>   Mutations at the <u>Mi</u>-locus also result in white spotting and reduction of intensity of pigmentation. Somatic reversions of <u>Mi-wh</u> mutant to wild-type were observed in 5.3% of mutant mice surveyed (Schaible, 1969). The frequency of these reversions increased when the <u>Mi-wh</u> mutant allele was transferred from C57BL/6J to JU/ctLm strain.

c. <u>Agouti locus</u>   Several of the mutant alleles of agouti locus exhibited somatic and germinal reversions (Schlager and Dickie, 1969). For example, mice heterozygous for viable-yellow mutant allele (<u>A-vy</u>/-) have a mottled phenotype. This mottled phenotype was similar to the phenotype of chimeric mice produced by aggregating two embryos differing in agouti-locus genotype (Mintz, 1971). For this similarity, it is suggested that the mottled phenotype of <u>A-vy</u>/- mice represents clonally expressed reversions to wild-type pigmentation resulting from excision of transposable elements (Whitney and Lamoreux, 1982).

d. <u>Pearl locus</u>   Mice with pearl (<u>pe</u>) mutation exhibit diluted coat-color pigmentation. However, in 6% of the homozygotes (<u>pe</u>/<u>pe</u>) studied, full-colored patches were observed (Russell, 1964). These patches are considered to result from a somatic reversion to wild type (<u>pe</u>$^+$). Germinal reversions also occurred in mice with 5% or more of their coat with full colored patches.

e. <u>Pink-eye locus</u>   An unstable allele, designated pink-eyed dilution (<u>p-un</u>), arose spontaneously in the background of C57BL/6J strain (Melvold, 1971). Mice with the <u>p-un</u> allele had light-coat pigmentation, but 6% of them exhibited dark-pigmented areas in the light-pigmented background. These dark-pigmented are inferred to represent spontaneous somatic reverse mutations and they tend to occur at a certain stage during the development (Russell, 1964).

Similar to these cases in mice, instability of certain phenotypes in dogs and humans are suggested to result from mutations caused by transposable elements. These phenotypes include merle phenotype (fully-intense wild-type pigmented areas on light-pigmented background) in dogs, white-spotting of forelock hair (Waardenburg syndrome) in humans (Whitney and Lamoreux, 1982).

3. <u>Yeast</u>

a. <u>Instability of mating-type locus (mat) in fission yeast</u> (<u>Schizosaccharomyces pombe</u>)   Egel (1976) observed the genetic instability at the <u>mat</u> locus of fission yeast. Two adjacent mating type genes, <u>mat 1</u> ⊖ and <u>mat 2</u> ⊕ are located as a cluster in linkage

group II of the standard map (Kohli et al., 1977). The gene ⊕ at

mat 2 is silent but upon insertion at mat 1, it becomes active (Egel,

1981). In addition to mat 1 and mat 2 at the mating type locus, a

special recognition signal called smt (switching of mating type) maps

between mat 1 ⊕ and mat 2 ⊕. The smt is essential for switching

reaction. The high switching rates observed in homothallic wild type

are reduced to a very low level by the alteration of the switching

signal smt to a restrained state, r (Egel, 1981; Egel and Gutz, 1981).

   b. **Instability of mating-type locus (MAT) in the budding yeast
Saccharomyces cerevisiae**   The mating type locus (MAT) in this yeast

is located on the right arm of chromosome III. It contains either of

the two alleles a or α. The type of the allele present determines the

cell type (Lindegren and Lindegren, 1943). Only the cells of different

mating types conjugate; i.e., a cell type conjugates with α type but

not a with a or α with α.

Genetic instability is observed within a cell by interconversion

of mating type; i.e., MATa to MATα or vice versa. The interconversion

occurs only in the presence of HO and appropriate genes (Hicks et al.,

1977). In addition to HO, interconversion requires the action of

two other loci HML and HMR located on the left and right arm, respec-

tively, of the same chromosome as the MAT locus (Naumov and Tolstorukov,

1973; Harashima et al., 1974). For a → α interconversion HML is

required, whereas α → a interconversion requires HMR. Also, in the

presence of HML and HMR, mutant alleles of MAT were healed by two suc-

cessive mating type switches, e.g. $\alpha^- \rightarrow a^+ \rightarrow \alpha^+$ (Takano et al., 1973);

Hicks and Herskowitz, 1977; Strathern et al., 1979).

On the basis of these genetic analyses, Hicks et al. (1977) proposed the cassette hypothesis according to which MAT locus contains either a or α DNA sequences and the switching of mating type occurs by transposition of a- and α-specific DNA sequences from HML and HMR loci to the MAT locus. The a and α genetic blocks are expressed only upon transposition to the MAT locus.

The physical structure of MATa, MATα, HMRa and HMLα have been analyzed by recombinant DNA procedures (Nasmyth et al., 1981). The a-specific sequence is about 650 bp and α-specific sequence is about 750 bp long. Heteroduplex analysis showed that all loci share two blocks of homologous DNA which are separated by one of two different sequences (Nasmyth and Tatchell, 1980).

The MAT locus has been proposed to code for regulatory proteins that control unlinked a- and α-specific genes (Mackay and Manney, 1974a, b). Two genes, α_1 and α_2 in MATα are proposed to perform this function (Nasmyth et al., 1981). $MAT\alpha_1$-gene product is required for the expression of genes that specify α-mating type. MAT 2 represses a-specific genes that otherwise would be constitutively expressed. This model suggests that MATa plays no role in the specification of mating type (Nasmyth et al., 1981).

4. Drosophila

    a. Mutable alleles affecting wing size   Demerec (1941) was the first to study the mutable alleles at the miniature wing locus (mt) in Drosophila virilis. Mutations of somatic and germinal origin

were observed without any observable chromosomal defects.

Two mutable alleles of miniature wing, mu and dy[73], were identified

in a tandem duplication stock of the white locus (Rasmuson et al.,

1974). These loci are 0.1 map unit distance from each other. Since

the origin of these mutants is from the tandem duplication stock of

the white locus, which is proposed to harbor a controlling element

(Green, 1969), it was suggested that a piece of foreign DNA is

transposed from the white locus to the mu and dy loci (Green, 1975).

b. Mutable alleles affecting the white eye locus   Several

mutable alleles affecting the white eye locus have been investigated.

Mutability was found associated with deficiencies of various sizes,

both on the left and right of the white locus on the X-chromosome

(Green, 1967). A white crimson mutant allele (wc) was observed to

transpose from the white locus to chromosome 3. The transposition and

mutability were explained as due to a controlling element similar to

that in maize (Green, 1969).

A tandem duplication of the white locus resulted in maroon color

phenotype (Rasmuson et al., 1974). The duplication had the right

segment with wl76 (white-eyed) allele, and the left segment with wsp

(white-spotted) allele. Two non-crossover white-eyed phenotypes

designated as wx and wy were obtained from this duplication stock.

The crossovers resulted in white-eyed males, wx⁻ and wy⁻, representing

non-duplicated white eye loci. The wx⁻ and wy⁻ carried only the left

segment of wx and wy, respectively. The white-eyed character in cross-

over types was proposed as resulting from insertions of foreign DNA

blocking the expression of left segment wsp (Rasmuson et al., 1974).

Bingham (1981) isolated a dominant mutant allele of the white

locus, designated w^{DZL}. This mutation arose spontaneously in a single

heterozygous female of a wild-type D. melanogastor stock. w^{DZL}

produces a yellow eye color in females homozygous for the allele and

heterozygous for the allele and a wild type allele. Cytogenetic

. analysis using several mutant markers showed that w^{DZL} resides to the

left of the rst^{CT} mutation and to the right of the w^a mutation at the

white locus. Cytological observations (banding pattern) indicated

that w^{DZL} is associated with an unstable allele that generates dele-

tions, inversions and reciprocal translocations. All these chromosomal

arrangements were shown to share a common break point in or immediately

to the right of the rightmost alleles of the white locus array. It has

been hypothesized that the mutability at w^{DZL} results from the presence

of a transposable DNA segment (Bingham, 1981).

Ising and Block (1981) reported a transposable element (TE) on the

X-chromosome. Cytogenetic analysis showed that the element carries

the genes white (w^a or w^+) and roughest (rst^+). They identified about

150 different transpositions. Each transposition was identified as an

insertion consisting from 1 to 8 distinct bands. Gehring and Paro

(1980) isolated a hybrid E. coli plasmid (61F4) with homologous

sequences to TE. They showed that the segment in the plasmid is

homologous to the copia element, a repeated gene family in Drosophila.

D. Transposable Elements Studied at DNA Level

Transposable elements are sections of DNA that move about the genome of possibly every organism (Starlinger and Saedler, 1976). These elements include the smaller insertion sequences (IS elements), larger transposons (Tn) and the bacteriophage, Mu in bacteria (Starlinger, 1980a), several families of repeated DNA sequences in yeast--e.g. Ty elements (Fink et al., 1981) and in Drosophila--e.g. copia-like elements (Rubin et al., 1981). Recently, retroviruses (eukaryotic viruses) have been regarded as transposable elements (Skalka et al., 1981), although their most common mode of transposition is via reverse transcription and integration of the resultant double stranded DNA copy into cellular DNA (Taylor, 1979). However, studies on the endogenous retroviruses of chickens suggest that an integrated provirus (DNA) can transpose to another location without going through an RNA (viral) intermediate (Tereba et al., 1979).

Some of the general properties of transposable elements include:

(1) contain transcriptional start and stop signals;

(2) transpose within the genome;

(3) induce and regulate the formation of deletions and inversions;

(4) excise themselves from their present site by coding for enzymes that induce excision;

(5) unite unrelated, non-homologous segments of DNA.

1. Bacteria: Insertion sequences and transposons

    a. Insertion sequences as start and stop signals

        1) As stop signals   The presence of insertion sequences
(IS 1) was first detected in the galactose operon of E. coli (Jordan
et al., 1968). The insertions act as polar mutations in the operon.
Adhya and Gottesman (1978) hypothesized that the polar effect of the
IS elements is due to rho-dependent transcription termination at a DNA
signal. (The termination factor rho is thought to interact with RNA
polymerase only when a stretch of untranslated RNA is available.)
However, IS 1 does not carry any recognizable signal for termination
by rho (Starlinger, 1980b). The second possibility for the polar effect
is the presence of nonsense codons in IS 1 (Adhya et al., 1974). This
in fact was the case with IS 1 polarity. Nonsense codons are present
in either orientation of IS 1 within the first 100 nucleotides (Ohtsubo
and Ohtsubo, 1978). Although IS 1 in the gal operon did not show rho-
dependent transcription in vitro, such termination is shown to occur
in wild-type strains (Starlinger et al., 1973) and is partially relieved
in strains with mutations of gene coding for rho (Malamy et al., 1972;
Das et al., 1976).

    Saedler et al. (1974) found IS 2 to be polar in orientation I and
carrying a promoter in orientation II. Boyen et al. (1978), however,
observed polar effect of IS 2 in either orientation. The polar effect
of IS 2 in orientation I is in agreement with the finding that IS 2
carries a rho-dependent termination signal (de Crombrugghe et al., 1973).

    A strong polarity was observed when IS 4 is inserted in either

orientation (Besemer and Herpers, 1977). Sequence analysis of IS 4

in either orientation showed a stem and loop structure (Klaer et al.,

1981), which have been described as rho-dependent termination signals

(Adhya and Gottesman, 1978).

2) As start signals   The only well-documented case

of an IS element with promoter signal is IS 2 (Saedler et al., 1972).

In revertants of gal mutants, IS 2 is integrated in orientation II into

the leader sequence and the mutants thus expressed constitutive Gal^+

phenotypes. These revertants are unstable and revert back to Gal^- which

did not have IS 2 in orientation II.

b. Transposition of IS elements and transposons

1) Structural requirements   DNA structural studies

involving restriction and sequence analyses have been useful in determin-

ing the sequence similarities and differences among various transposable

elements. This information provides the basis for possible transposi-

tion mechanisms.

All the bacterial transposable elements analyzed so far terminate

in more or less extended inverted repeats (Starlinger, 1980b). In

transposons Tn 3 and Tn 10, the transposition is blocked if the terminal

inverted repeats are removed. Therefore, the inverted repeats are

supposedly involved in recognition of the transposition enzymes

(Heffron et al., 1977). For a site-specific recombination, Tn 3 was

shown to have a centrally located site (Arthur and Sherratt, 1979).

In addition to the inverted repeats and a cis-located site, two

genes were shown to be involved in the transcription (Heffron et al.,

1977; Dougan et al., 1979). They are located in the unique DNA of

Tn 3. One of them codes for a protein that is involved in transcrip-

tion process and the other codes for a repressor. These two genes

are organized in a self-regulated transcriptional unit (Chou et al.,

1979).

However, in Tn 10 the unique DNA does not seem to be essential

for transposition (Starlinger, 1980b). The inverted repeats, IS 50

and IS 10 that flank Tn 5 (Rothstein et al., 1981) and Tn 10 (Kleckner

et al., 1981), respectively, are essential for transposition. For

Tn 10 transposition, only the right inverted repeat is mostly responsi-

ble (Kleckner et al., 1981). Ross et al. (1979a) showed that the IS-

like inverted repeats in Tn 10 can transpose independently. From this,

a question arises as to whether the E. coli IS elements carry genes for

their own transposition.

Experiments have been executed to explore these questions. Klaer

et al. (1981) have sequenced IS 4 and found a long open reading frame

of 1326 bp (total length of IS 4 is 1426 bp) which can code for a

polypeptide with 442 amino acids. Based on the base sequences in IS 4,

several assumptions have been made for putative sites of ribosome

binding, a start codon and a promoter. These assumptions need further

testing by looking for the products of transcription and translation

in vitro. Another test is to construct mutants in the putative genes

in IS 4 and study the functional consequences (Klaer et al., 1981).

Unlike IS 4, IS 10, and IS 50, IS 1 and IS 2 do not contain

large open reading frames (Ohtsubo and Ohtsubo, 1978; Ghosal et al.,

1979). In addition to the size differences in reading frames, IS 1
and IS 2 are present in several copies in the E. coli chromosome
(Saedler and Heiss, 1973; Deonier et al., 1979), whereas IS 4 is present
in one copy (Klaer et al., 1981) and IS 10 and IS 50 in two copies
(Rothstein et al., 1980). Since IS 1 and IS 2 do not seem to have
genetic information for their transposition, Klaer et al. (1981) raised
a question as to whether these elements depend on a master element.
They have speculated that this situation is similar to the transposi-
tion-active and transposition-passive controlling elements in maize
(McClintock, 1965).

      2) __Involvement of DNA synthesis__   The speculation
that the DNA synthesis might be involved in transposition arose from
the observation of short duplications of target DNA on both sides of
the integrated transposable element. Duplication of 11 or 12 bp for
IS 4 (Habermann et al., 1979); 9 bp for IS 1 (Calos et al., 1978;
Grindley, 1978), Tn 5 (Auerswald and Schaller, 1981), Tn 9 (Johnsrud
et al., 1978), Tn 10 (Kleckner, 1979) and Tn 903 (Oka et al., 1978);
5 bp for IS 2 (Rosenberg et al., 1978; Ghosal et al., 1979), Tn 3
(Ohtsubo et al., 1979; Cohen et al. 1979), bacteriophage Mu (Allet,
1979; Kahmann and Kamp, 1979) and IS 3 (Sommer et al., 1979) have
been reported.

    Several hypotheses have been put forward for possible involvement
of DNA synthesis in transposition. Grindley (1978) and Calos et al.
(1978) have suggested a model in which transposition begins with the
introduction of "staggered nicks" into the recipient DNA. If the

termini of the IS element are linked to the single-stranded protrusions of recipient DNA short gaps are created. These gaps are filled in by repair synthesis. This hypothesis explains how the duplicated segments in target DNA could have arisen at the site of integration.

However, the DNA synthesis is speculated to be involved not only in repair synthesis at the site of integration but also in complete replication of the element at the old site (Ljungquist and Bukhari, 1977; Bennett et al., 1977; Klaer et al., 1980). According to this hypothesis, upon replication of the element, one copy is transposed into a new site while the other remains at the old position. This is based on the observations that the transposable element is present at the old site but at the same time the element is relocated at new sites.

There are several versions of this hypothesis. In one, a covalent complex is formed between two termini of the transposable element in the old position and at the new integration site by single strand cleavages and ligations (Starlinger, 1980b). Shapiro (1979) suggested a cleavage at both 3' termini of the transposable element and ligation to both of the 5' termini of the staggered nicks of the cleaved recipient DNA. He also proposed replication forks at the ligation points which move toward each other.

      c. <u>Chromosomal aberrations associated with transposable elements</u>   A variety of chromosomal aberrations have been observed with transposition. The most common one is an adjacent deletion (Starlinger, 1980b).

1) <u>Adjacent deletions</u>   These deletions extend from one terminus of a transposable element to a variable site in the bacterial DNA. The element itself is not deleted. Reif and Saedler (1975) described the first of its kind with <u>IS 1</u>. Low frequency adjacent deletion is observed near <u>IS 2</u> (Reif, 1975; Ahmed and Scraba, 1978). These deletions in <u>IS 2</u> also terminate at the last nucleotide adjacent to the insertion (Peterson et al., 1979). Similar adjacent deletions are reported at <u>Tn 3</u> (Nisen et al., 1978; Weinstock and Botstein, 1979), <u>Tn 10</u> (Chan and Botstein, 1972; Kleckner et al., 1979), and bacteriophage Mu (Toussiant et al., 1977).

2) <u>Internal deletions and inversions</u>   These aberrations are specifically associated with <u>Tn 10</u>. <u>Tn 10</u> carries a gene for tetracycline resistance (2500 bp) that is flanked by inverted repeats (<u>IS 10</u> = 1400 bp) at both ends (Kleckner et al., 1975). In some mutants, deletions adjacent to the internal termini of <u>IS 10</u> have been reported. In other mutants, a precise deletion of the unique DNA of <u>TN 10</u> and an inversion of one <u>IS 10</u> and some DNA adjacent to it were observed (Kleckner et al., 1979; Ross et al., 1979a).

3) <u>Circular structures</u>   These structures are observed with bacteriophage <u>Mu</u> DNA and an adjacent segment of <u>E. coli</u> DNA (Waggoner et al., 1974; Schröder and van de Putte, 1974). They are formed after infection with bacteriophage <u>Mu</u>.

Recently, Klaer et al. (1981) reported bidirectional deletions around <u>IS 4</u>. In this case, <u>IS 4</u> and the bacterial DNA adjacent to both termini of <u>IS 4</u> are deleted.

d. <u>Excision</u> <u>of</u> <u>transposable</u> <u>elements</u>   It is not clear with

the present transposition models whether excision is related to the

transposition of transposable elements. In some cases, the relation-

ship exists and in others it does not (Starlinger, 1980b).

In <u>Tn 10</u>, Kleckner et al. (1981) described three types of excision

events: (a) precise excision, (b) nearly precise excision, (c) precise

excision of the nearly precise excision remnant.

Precise excision is genetically expressed as reversions of an

insertion mutation. The DNA sequence analysis of two independent $\underline{His}^+$

revertants of Salmonella <u>his G</u>::<u>Tn 10</u> insertions showed that a precise

excision involves a deletion between the short direct repeats of

target DNA that flank an inserted <u>Tn 10</u> element. Such deletion is

shown to restore the wild-type target sequence (Foster et al., 1981).

Nearly precise excisions were identified upon physical mapping

and DNA sequence analysis of <u>Tn 10</u> related rearrangements of phage λ

(Ross et al., 1979a, b). These excisions genetically correspond to

<u>Tn 10</u> polarity-relief revertants which in turn revert to full revertants

at high frequency. Genetic analysis of the polarity-relief revertants

of a <u>his G</u>::<u>Tn 10</u> insertion showed that these revertants are, indeed,

nearly precise excisions in which deletion of the DNA between the

inverted repeats occurs. This resulted in excision of all but 50 bp of

<u>Tn 10</u> (Foster et al., 1981).

The derivatives of nearly precise excision still contain sequences

of both the left and right 9 bp target DNA repeats plus 50 bp inverted

repeats of <u>Tn 10</u>. It is presumed that precise excision of these DNA

repeats should genetically correspond to full reversion of the polarity relief revertants. This has not been confirmed by DNA sequence analysis (Kleckner et al., 1981).

     e. <u>Site specificity of transposable elements</u>   Transposable elements are found in many different positions in <u>E</u>. <u>coli</u> and their plasmids. Some elements seem to have site specificity for integration relations.

    <u>IS 1</u> and <u>IS 2</u> were found to integrate preferentially into the leader sequence of the gal operon (Saedler et al., 1972). Although the leader sequence is less than 1% of the gal operon (Musso et al., 1977), the mutations caused by the integration of <u>IS 1</u> and <u>IS 2</u> into this operon constitute about 20% of all insertions (Starlinger, 1980b)

    <u>IS 4</u> integrates into a single position only within <u>gal T</u> (Shapiro and Adhya, 1969; Pfeifer et al., 1977). The <u>IS 4</u> insertions represent about 40% of all polar insertions in the <u>gal</u> operon (Starlinger, 1980b).

    Transposons <u>Tn 3</u> (Casadaban et al., 1981), <u>Tn 10</u> (Botstein and Kleckner, 1977), <u>Tn 5</u> (Berg et al., 1975) also found to have preferential sites of integration. In a peculiar case, Grinsted et al. (1978) observed that the integration sites of <u>Tn 501</u> into <u>RP 1</u> are determined by the presence or absence of another unrelated transposon <u>Tn 801</u>.

    Starlinger (1980b) identified the sites of preference as "regions of specificity" and "hot-points". On the basis of several comparative studies of the duplicated regions adjacent to <u>IS 1</u> and <u>IS 4</u>, he concludes that the specificity of integration cannot reside within the

duplicated regions. The different preferential integration sites for IS 1 (leader sequence) and IS 4 (at gal T) in the same gal operon were attributed to the differences in IS elements.

    f. Special situations of transposon-like elements in Salmonella and bacteriophages Mu and P1--phase variation   In Salmonella, two genes, H1 and H2, code for the major flagellar structural protein, Flagellin. These genes are located at different regions of the genome (Lederberg and Edwards, 1953). The cells have the ability to switch or alternate the expression of these genes. This alternate expression, termed phase variation, is controlled by a state of a genetic element linked to the H2 gene (Lederberg and Iino, 1956). Another gene, rh1, linked and expressed coordinately with H2, codes for a repressor substance that prevents expression of the H1 gene (Fujita et al., 1973; Silverman et al., 1979). Thus, when rh1 and H2 are not expressed, H1 gene is expressed, resulting in formation of H1 type flagella (phase 1), whereas when H1 gene is suppressed by rh1, H2 product is synthesized, leading to the formation of H2 type flagella (phase 2).

Genetic and physical analysis of the recombinant DNA molecules containing H1 and H2 gene regions showed that the inversion of 900 bp region adjacent to the H2 gene controlled the expression of this gene (Zieg et al., 1977). In one orientation, the H2 operon is "on" and in the opposite orientation H2 operon is "off". This indicated that a promoter of H2 resides in the 900 bp region.

To demonstrate the genetic mechanism of inversion, several mutants defective in switching process have been characterized (Silverman and

Simon, 1980) and the nucleotide sequence of these mutants has been determined (Zieg and Simon, 1980). This analysis, coupled with the identification of a polypeptide (19,000 m.w) in a cell-free transcription-translation system (Silverman et al., 1981), lead to the definition of two functions necessary for recombinational gene switching:

(1) A trans-acting function (hin) specified by a sequence (hin gene) inside the inversion region. The hin gene is presumed to be identical to the independently identified vh2 gene (Iino and Kutsukake, 1981).

(2) A cis-acting function consisting of a pair of 14 bp inverted repeat sequences are located at the boundaries of the inversion region. A homologous recombination between these inverted repeats results in the inversion of the DNA segment between them. Deletion of either of these inverted repeats prevents H2 switching (Silverman et al., 1981).

Similar to this inversion associated phase variation in Salmonella, a specific inversion of a DNA segment in bacteriophage Mu is correlated with the formation of infectious phage particles (Bukhari and Ambrosio, 1978). The G segment of Mu DNA carries out the inversions (Howe and Bode, 1975). A gene designated "gin" (analogous to hin or vh2 in Salmonella) was detected in the β segment of Mu DNA (Chow et al., 1977).

Analogous to the G segment in Mu, an inversion region termed C region has been reported in bacteriophage P1 DNA (Chow and Bukhari, 1976). The P1 DNA was shown capable of inverting the G segment of Mu strain that is defective in trans-acting factor. This indicated a

common inversion system between Mu and P1 (Kamp et al., 1978, 1979).

Kutsukake and Iino (1980a, b) studied the interactions between Salmonella and bacteriophage inversion systems. They demonstrated that prophage genomes P1 and Mu can suppress vh2⁻ (= hin⁻) and markedly enhance the frequency of flagellar variation in phase-stable Salmonella strains. They termed this trans-activity as "din-activity" and the genes responsible as "din". It was inferred that the din genes are located near the C region in P1 and in the β-G segment of Mu. Also, it was further demonstrated that the din mutants that failed to invert the 900 bp region in Salmonella also failed to invert their own C region in P1. Therefore, Iino and Kutsukake (1981) suggested that inversion systems in Salmonella, P1 and Mu operate alike.

2. Yeast (Saccharomyces cerevisiae) transposable elements (Ty)

Cameron et al. (1979) first described a gene family, designated Ty 1, that is represented approximately 30 times in the haploid yeast genome. Most members of this gene family contain about 5300 bp DNA flanked by direct repeats of 338 bp units called delta sequences (Gafner and Philippsen, 1980). The delta sequences are found at numerous sites in the yeast genome not associated with intact Ty 1 elements. They show substantial sequence divergence. The Ty 1 elements are found in different locations in the genomes of different strains. They show considerable sequence divergence (Cameron et al., 1979). The transcript of Ty 1 is about 5500 bases long and it includes delta sequences on at least one end (Elder et al., 1981).

a. $\underline{\text{Ty}}$ $\underline{\text{elements}}$ $\underline{\text{at}}$ $\underline{\text{the}}$ $\underline{\text{his}}$ $\underline{4}$ $\underline{\text{region}}$ Two spontaneous polar

mutations $\underline{\text{his}}$ $\underline{4\text{-}912}$ and $\underline{\text{his}}$ $\underline{4\text{-}917}$ were shown to result from transposi-

tion of $\underline{\text{Ty}}$ elements into the $\underline{\text{his}}$ $\underline{4}$ region (Farabaugh and Fink, 1980;

Roeder and Fink, 1980; Roeder et al., 1980). In each case, a 5 bp

duplication resulted at the insertion site. The two mutants $\underline{\text{his}}$ $\underline{4\text{-}912}$

and $\underline{\text{his}}$ $\underline{4\text{-}917}$ revert to $\underline{\text{His}}^+$ at frequencies of 10^{-5} and 10^{-4}, respec-

tively (Roeder et al., 1980). The $\underline{\text{His}}^+$ revertants were found to result

from a number of different causes such as point mutations, transloca-

tions, transpositions and deletions. The break points in each of these

genetic events were very close to the $\underline{\text{his}}$ $\underline{4}$ region (Chaleff and Fink,

1980; Roeder and Fink, 1980).

Cloning and sequence analysis of the mutant $\underline{\text{his}}$ $\underline{4\text{-}912}$ and

$\underline{\text{his}}$ $\underline{4\text{-}917}$ and the normal $\underline{\text{His}}^+$ genes revealed insertion of $\underline{\text{Ty-912}}$ and

and $\underline{\text{Ty-917}}$ elements at different positions at the 5' non-coding region

of $\underline{\text{his}}$ $\underline{4}$ (Fink et al., 1981). These insertions are 6000 bp long

(Roeder and Fink, 1980) and have 330 bp terminal repeats (Roeder et al.,

1980) similar to the delta units of $\underline{\text{Ty}}$ $\underline{1}$. $\underline{\text{Ty-917}}$ includes a substitu-

tion of 4000 bp DNA with little or no homology to $\underline{\text{Ty-912}}$ sequences.

Two other $\underline{\text{Ty}}$ elements $\underline{\text{Ty}}$ $\underline{1\text{-}B\text{-}10}$ and $\underline{\text{Ty}}$ $\underline{1\text{-}D15}$ with identical delta

regions were sequenced (Gafner and Philippsen, 1980). The delta regions

of these two elements differ by 64 bp from those of $\underline{\text{Ty}}$ $\underline{917}$ (19%) by

54 bp from those of $\underline{\text{Ty}}$ $\underline{912}$ (16%). The homology in delta regions of the

four $\underline{\text{Ty}}$ elements sequences so far suggests a phylogenic relationship

among the transposable elements (Fink et al., 1981).

Mutations in genes unlinked to $\underline{\text{his}}$ $\underline{4}$ were found to alter the

expression of the his 4-912 and his 4-917 mutations. Three regulatory

mutants, spm-1, spm-2 and spm-3, affect both suppression and reversion

of his 4 mutants (Roeder et al., 1980). The spm-1 suppresses the

mutant phenotype caused by Ty 912 and Ty 917 insertions. The spm-2

and spm-3 regulate only the his-4 917 by repressing the mutant pheno-

type. It has been suggested that the wild type alleles Spm-2 and

Spm-3 control the reversion frequency of his 4-917 to His$^+$ (Roeder

et al., 1980). These regulatory elements are assumed to be analogous

to the suppressor-mutator (Spm) element in maize controlling element

system (Fink et al., 1981).

   b. **Ty elements at the ADR-2 region**   The yeast structural

gene ADR-2 codes for the glucose-repressible alcohol dehydrogenase

(ADH II). Mutations in ADR-3, a locus tightly linked to the structural

gene, result in constitutive synthesis of ADH II (Ciriacy, 1975, 1979).

   Nine cis-dominant constitutive mutants of ADR-3 were investigated

by restriction enzyme analysis using the cloned ADR-2 DNA as a hybrid-

ization probe (Williamson and Young, 1981). Seven mutants have

insertions of approximately 5.6 kb near the 5' end of the ADR-2 coding

region. The restriction pattern of four of these insertions is identi-

cal to the Ty 1 as described by Cameron et al. (1979). All the seven

insertions have xhol sites at the boundaries, suggesting that all of

them are bordered by delta sequences. Also, all the insertions are in

the same orientation.

   Two mechanisms have been postulated by which insertion of these

Ty-like sequences might cause constitutive expression of ADR-2

(Williamson and Young, 1981). One is that the insertion of the element alters the regulation of ADR-2 repression by a regulatory site upstream from the insertion. It is known from in vitro translation studies that derepression of ADR-2 is regulated at the level of production of translatable mRNA (Denis et al., 1981). Thus, it is possible that insertion of DNA sequences into the control region of ADH II could alter the regulation of transcription (Williamson and Young, 1981). An alternative suggestion to the alteration of regulation is that the sequences within the inserted Ty elements could be responsible for initiating transcription of ADR-2.

c. Ty elements at the ROAM alleles   ROAM (regulated over-producing alleles responding to mating signals) mutant alleles consti-tute CyC7-H2, $CargA^{+}O^{h}$, $CargB^{+}O^{h}$ and $durO^{h}$ which constitutively over-produce iso-2-cytochrome C, arginase, ornithine transaminase and urea amidolyase, respectively. Overproduction of these gene products is correlated to signals that normally control conjugation in yeast (Errede et al., 1981). For example, CyC7-H2 mutation causes approxi-mately a 20-fold over production of iso-2-cytochrome C (Sherman et al., 1978) in haploid strains but only 1 to 4 fold overproduction in MATa/MATα diploid strains (Rothstein and Sherman, 1980). Similar over-production of enzymes occurs in $CargA^{+}Oh$ (Dubois et al., 1978), $CargB^{+}Oh$ (Deschamps and Wiame, 1979) and $durO^{h}$ (Lemoine et al., 1978) mutants.

Each of the ROAM mutants has normal spore viability and Mendelian segregation. These observations suggested that the mutants have single

site mutations at the structural gene (Errede et al., 1981). However, the normal amino acid sequence of iso-2-cytochrome C in the CyC7-H2 mutant (Sherman et al., 1978) indicated that the mutations are in a regulatory region outside the translated portion of each gene.

Using recombinant DNA procedures, it has been demonstrated that the constitutive over-production of iso-2-cytochrome C in CyC7-H2 mutant is caused by the insertion of Ty 1 element adjacent to the structural gene (Errede et al., 1981). Further control of the overproduction by mating type signals has been suggested to involve Ty 1 elements or portions of Ty 1 elements that may occur adjacent to genes required for conjugation. This may represent a general mechanism of coordinate regulation in eukaryotes (Errede et al., 1981).

3. Drosophila: Copia-like transposable elements

Copia-like transposable elements include three repeated sequence families designated as copia, 412 and 297 (Rubin et al., 1981). Although these elements are non-homologous in nucleotide sequence, they are grouped together as copia-like elements for sharing the following properties:

(a) Each of these elements occurs at about 30 widely scattered locations.

(b) Their sequences are closely conserved and non-permuted at each location.

(c) Copia, 412 and 297 sequences are terminally redundant with direct repeats of 0.3 kb, 0.5 kb and 0.4 kb, respectively.

(d) They undergo transposition within the genome.

(e) They code for abundant poly(A) containing cytoplasmic RNAs.

The number and chromosomal sites for each of these elements dif-
fered among four different strains of D. melanogaster (Rubin et al.,
1981). Also, the individual flies within a strain and homologous
chromosomes within individual flies exhibited differences in the arrange-
ment of copia, 412 and 297 elements.

The stability of copia, 412 and 297 was studied in tissue culture
cells (Potter et al., 1979). Restriction enzyme analysis of the
genome sequences homologous to these elements indicated that most of
the sequences are intact and indistinguishable from those in embryo.
However, the restriction enzyme cleavage sites in DNA flanking these
elements differed among cell cultures and even among cells within a
cell culture population.

Sequence analysis of the integration sites showed a repetition of
5 and 4 bp sequence on both sides of copia and 297, respectively
(Rubin et al., 1981). In several cases studied, the base sequence of
copia remained the same but the duplicated 5 bp sequence and the
adjacent regions at the insertion sites had no consistency in base compo-
sition (Strobel et al., 1979). For both copia and 297, identical direct
repeats of 276 bp were observed. However, copia differed from 297
in two nucleotide substitutions. The direct repeat of copia was always
found with the element (Levis et al., 1980). This is unlike Ty 1 in
yeast where independent direct repeats occur (Cameron et al., 1979).

In the Drosophila genome, thus far 15 putative new families of

copia-like elements have been identified (Rubin et al., 1981). In situ hybridization of ^{3}H-labelled DNA of putative copia-like elements to salivary gland polytene chromosomes carrying white, forked and yellow loci showed some homology. But hybridization of more than one copia element to a single mutant locus on a polytene chromosome has been suggested as due to the low resolution of in situ hybridization (often ±100 kb) (Rubin et al., 1981).

E. Similarities Between Controlling Elements in Maize

and Transposable Elements Studied at DNA Level

The controlling elements in maize and the transposable elements in bacteria, yeast and Drosophila are found to be similar in terms of their functions (Peterson, 1970b; Nevers and Saedler, 1977; Nowick and Peterson, 1981; Peterson, 1981). These similarities include:

(1) Insertion of an element into a gene produces a mutant phenotype and its excision leads to restoration of gene activity.

(2) The element excises and transposes by itself and these properties are, in some cases, modified by other factors.

(3) The element inserts into any targeted DNA, but regional preferences for insertion are observed within the targeted DNA.

Structurally, it is not known whether the controlling elements in maize are similar to the transposable elements in any of the organisms studied thus far. However, following a survey of the literature on

Figure 2.7. Model for autonomous control of the B locus by Spm (En)
(Adapted from Nevers and Saedler, 1977)

B   = a hypothetical gene controlled by Spm

P_B = promoter of the B gene

P1 = promoter of Spm that spontaneously starts
    transcription

P2 = promoter of Spm that initiates transcription only
    in the presence of A gene product

A   = an activator gene that induces P2 to start
    transcription

S   = a gene whose product suppresses transcription at
    a site in T sequence

T   = a sequence which terminates transcription in the
    presence of S product

M   = a gene whose product excises at the black boxes
    indicated
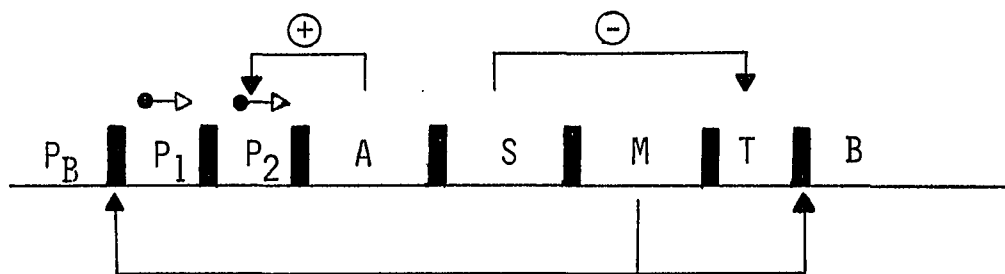
maize elements, Nevers and Saedler (1977) related these elements to
the bacterial transposons and insertion sequences and proposed a
structural model for the Spm (En) regulatory element in maize (Figure
2.7). According to this model, Spm contains two promoters (P1 and P2),
three structural genes (A, S and M) and a termination sequence (T).
Since the S and M components of Spm, when associated with a-m-1 allele,
are recognized to undergo mutations independently (McClintock, 1965),
they are assumed as different genes. The model works as follows.

A spontaneous transcription starts at P1 and leads to the expres-
sion of the A gene. The A gene product thus produced is required for
the activation of P2 leading to the transcription of S and M genes.
In an inactive Spm, the spontaneous transcription at P1 is not initi-
ated, but P2 of the inactive Spm can be activated by the A gene product
from an active Spm. This is conceived from the observations among
a2-m-1 cultures where an inactive Spm is trans-activated by an active
Spm (McClintock, 1968).

In an autonomous system where the entire Spm is integrated into
the locus under control (locus B in Figure 2.7), the S gene product
suppresses the B gene expression by terminating the transcription at
the T sequence. The M gene product excises the whole Spm at the locus
from the black box preceding P1 to the black box after T. Derivation
of a two element system from an autonomous system (Peterson, 1976a) is
explained by the excision of only part of Spm leaving behind the T
sequence bordered by two black box sequences. This residue (a receptor)
can now respond to the S and M gene products of an independently

located Spm.

The black box sequences bordering P1, P2, A, S, M and T components
of Spm are akin to the IS sequences in bacteria. These sequences are
the sites at which the Spm components can undergo inversions, dele-
tions, duplications or transpositions leading to a wide range of
receptor, regulator states that are observed with several mutable
alleles (McClintock, 1951, 1967a, 1968; Peterson, 1966, 1970a, 1976b).

Peterson (1981) proposed a modification to the model of Nevers
and Saedler (1977). This modification includes individual promoters
for S and M components of En instead of one promoter (P1) for both.
It is based on the observation that S and M components change inde-
pendently of each other. A changing S activity was seen with a constant
M activity and vice versa on individual kernels containing the a-m-1
mutable allele.

III. MATERIALS AND METHODS

A. Gene Symbols and Terminology

Allele or element	Description or phenotype
A	An allele of one of the genes (located on chromosome 3) necessary for the synthesis of anthocyanin in the aleurone
a-m(r)	A recessive allele of A, responds to En (a receptor allele for En signals). In the absence of En, the aleurone is colorless; in its presence, colored spots are produced on a colorless background expressing the change from a to A (Peterson, 1961).
a-m-1	A recessive allele of A, responds to En. In the absence of En, the aleurone is pigmented pale; in its presence, colored spots are produced on a colorless background (McClintock, 1958)
et	Etched kernel phenotype, in contrast to the wild type Et-smooth kernel type
Sh2	Round or non-shrunken endosperm. 0.25 map units from A (chromosome 3)
sh2	Shrunken endosperm; a recessive allele of Sh2
I	Inhibitor; a receptor element of the En system that suppresses gene activity when in cis position to the locus (Peterson, 1960), a component of receptor alleles such as a-m(r) and a-m-1
En	Enhancer—a regulatory element; in the presence of

En and a receptor allele such as <u>a-m(r)</u>, colored

spots appear on a colorless background; hypothe-

sized to alter or excise the receptor element, <u>I</u>

so that the locus under control becomes functional

(Peterson, 1960).

<u>Spm</u>   Suppressor-mutator; a regulatory element function-

ally similar to <u>En</u> (Peterson, 1965). Its suppressor

(<u>S</u>) and mutator (<u>M</u>) component functions are evi-

dent with the <u>a-m-1</u> allele (McClintock, 1958);

<u>S</u>--suppresses the dark-pale pigmentation, <u>M</u>--insti-

gates mutation events at receptor allele such as

the <u>a-m-1</u> allele.

Terms	Definition
Controlling element system	A system includes a receptor and a regulatory element that express a specific interaction, example: <u>I</u> and <u>En</u>, <u>Ds</u> and <u>Ac</u>
Receptor elements	Elements such as <u>Ds</u> and <u>I</u> that when in <u>cis</u> position to the locus suppress gene function; also receive signals from a specific regulatory element so that gene activity is restored at the controlled locus (Fincham and Sastry, 1974).
Regulatory elements	Elements such as <u>Ac</u>, <u>En</u> (<u>Spm</u>) that alter or excise the receptor elements <u>Ds</u> and <u>I</u>, respectively, from the controlled allele.
Controlled allele	An allele under the control of a controlling

element system (Peterson, 1976b).

Mutable allele	Same as the controlled allele.
Autonomous control	A mutability control that is inseparable from the locus.
Mutability	Variegated or spotted phenotypic expression in a tissue that is characterized by more than one phenotype such as colored spots or sectors on a colorless background.
Mutable patterns	The phenotypic expressions on a kernel that show differences in the number and size of colored spots that correspond to frequency and timing of mutation events during the development of endo-sperm tissue.
Spotting patterns	Same as mutable patterns; preferably used for the mutable patterns on kernels.
State of receptor element	Characterized in two ways: (a) by the degree of expression of gene activity in the absence of a regulatory element; (b) by the mutable patterns in the presence of a regulatory element.
State of regula-tory element	Described only by a specific mutable pattern elicited in the presence of a mutable allele.
Basic allele phenotype	The phenotype of a mutable or controlled allele in the absence of regulatory element; can range from colorless to fully colored.
Variable pales	Kernels differing in pigmentation but the

individual kernels are uniformly pigmented.

Non-uniform pales   Individual kernels that are non-uniform in pigmentation.

Variable spotted   Kernels differing in spotting pattern (size and frequency of spots)

Speckle spotted   Spotted kernels in which the spots are irregular (not round); speckle spots are often small in size.

Specks   Denote small, round spots intermingled with large spots on individual kernels.

Test cross   Cross between a heterozygous genotype and a tester that tests that genotype. The tester in most cases here described is a sh and is illustrated by this example: a-m-1 Sh2/a sh2 X a sh2/a sh2 or a sh2/a sh2 X a-m-1 Sh2/a sh2.

Straight cross   Test cross in which the a sh tester is used as male.

Reciprocal cross   Test cross in which the a sh tester is used as female.

Sibs   Represent individual kernels or plants derived from a single ear that is obtained in the progeny of a cross.

B. Abbreviations

Genotype/term   Abbreviation

a sh2/a sh2   a sh

a-m(r) Sh2/a-m-1 sh2   a-m(r)/a-m-1

a-m(r) Sh2/a-m(r) Sh2	a-m(r)/a-m(r)
Colored	Cl
Colorless	cl
Shrunken	sh
Round	rd
Spotted	sp
Background	bkg
With	$\overline{c}$

C. Identification of a Plant in a Row

Examples:

$$\text{year} \Rightarrow \begin{matrix} 1981 \\ 1976 \end{matrix} \qquad \text{row number} \begin{matrix} 1 & 4438\text{-}4 \\ & \\ 6 & 1601\text{-}2 \end{matrix} \qquad \text{plant number}$$
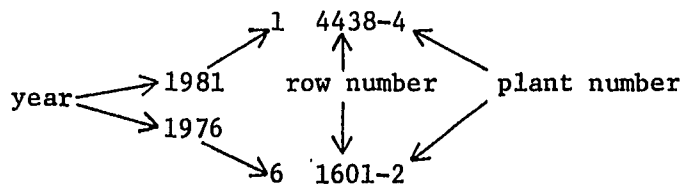
D. Quantification of the Phenotypes of Basic Alleles and Spotted Patterns

Basic alleles and the spotted patterns generated by crossing with different regulatory elements appear in a wide range of phenotypes. In order to be able to properly classify these diverse expressions, each state of a receptor and regulatory elements needs to be identified. There are three criteria that can be applied to describe a spotted pattern: i) Spot _size_ representing the _timing_ of mutations (early mutations produce spots that appear coarse, whereas late mutations result in spots that are fine); ii) Spot _number_ that is associated with the _frequency_ of mutations; iii) The background coloration of the

Figure 3.1.   Standardized diagrammatic array of basic allele pheno-
              types and mutable patterns used to classify the kernel
              types in the genetic analysis

Basic allele phenotypes

Increase in size of spots →

Increase in frequency of spots ↓
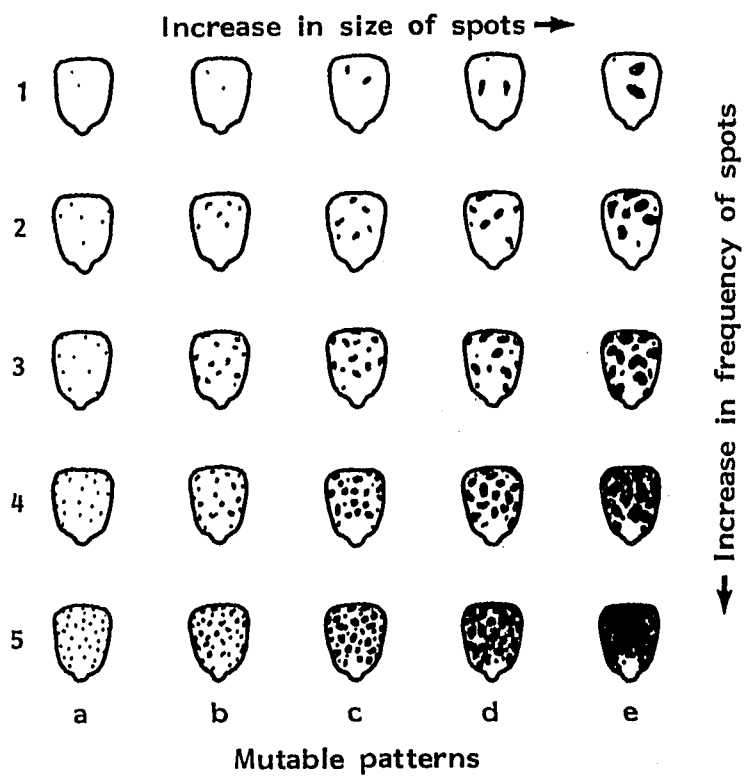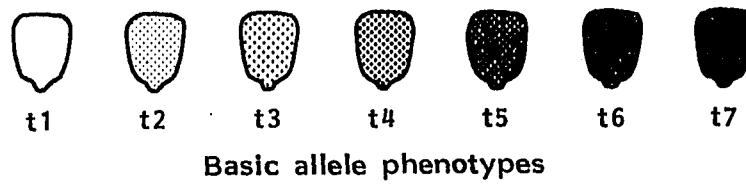
Mutable patterns

mutable patterns which ranges from colorless to full color. These three criteria are considered as parameters by which a particular spotted pattern is described. Since these parameters show variation, each of them is graded in the following manner (Figure 3.1).

i) Size of spots: Size ranges from small (fine) to large (coarse) and is divided into a, b, c, d and e types. Occasionally, very fine spots (1 or 2 aleurone cells) also are observed. They are represented as aa-type.

ii) Frequency of spots: Frequency ranges from a single spot to numerous spots and is graded into 5 divisions--1, 2, 3, 4 and 5. In some cases, the spots are so numerous that the spotted phenotype is almost full-colored. Such type is represented as 5^+.

iii) Background coloration: It ranges from null to full color and is divided into the following 7 phenotypes:

    t1 = colorless

    t2 = very light pale

    t3 = light pale

    t4 = pale

    t5 = slightly dark pale

    t6 = dark pale

    t7 = colored.

Although the spotted kernels on an ear represent the same genotype, the background coloration of these kernels may vary. In such cases, the grade of pigmentation is denoted by more than one type, example t3-4.

Similarly, the basic allele phenotypes (without a regulatory

element) are represented from tl to t7 (Figure 3.1). A specific pheno-
type of a basic allele may show variation and such variation is
expressed by more than one type, example t4-5.

Each of the spotted patterns shown in Figure 3.1 can exhibit a
background coloration from tl to t7. Such a combination of phenotypes
on an individual kernel is denoted as in the following examples:

5a tl = high frequency, fine spotted with colorless background;

3e t4 = medium frequency, coarse spotted with pale background;

1c t6 = low frequency, medium spotted with dark-pale background;

1-2a-b tl = low-medium frequency, fine-medium spotted with colorless
      background;

3-4c-d tl = medium-high frequency, medium-coarse spotted with colorless
      background;

5c tl-2 = high frequency, medium spotted with colorless to very light
      pale background.

                    E. Source of Materials

The material used in this study was obtained from Dr. Barbara
McClintock of Carnegie Institution of Washington, Cold Spring Harbor,
New York. The studies were initiated by Dr. Peter A. Peterson in a
summer nursery in 1976 at the Agronomy farm, near Ames.

                  F. Description of the Materials

The material included in this study consists of five different
mutable alleles. These are three states of a-m-1 (a-m-1 5719A-1,
a-m-1 5996-4, a-m-1 6078) and two of a-m-2 (a-m-2 7977B and a-m-2 8004).

1   <u>A</u>/<u>a</u> <u>A2</u>/<u>A2</u> <u>Ds</u> <u>Pr</u>/<u>+</u> <u>pr</u> <u>Ac</u>/<u>+</u> X <u>a</u> <u>sh2</u>/<u>a</u> <u>sh2</u>   (McClintock, 1951)

2   <u>a-m-1</u>   <u>a-m-2</u>   <u>a-m-3</u>   <u>a-m-4</u>   (McClintock,
       (Spm control) (Spm control) (Ac control) (Ac control)   1952b)

3   <u>5719A-1</u> <u>5996-4</u> <u>6078</u> Several   <u>7977B</u>   <u>8004</u>   Several   (McClintock,
                        others   others   1963, 1968)

-<u>Spm</u>   t7   t1   t2-3   t1   t1
                                   (some $\bar{c}$
                                   faint
                                   pale
                                   areas)

+<u>Spm</u> or   3-4 c-d t1   4a,   t7   3-4c t4, 2b-c t1
<u>En</u> $\bar{c}$ pale   c-d t1 $\bar{c}$ or   3-4c t1 $\bar{c}$
      areas $\bar{c}$ out cl   rimmed
                  areas   areas
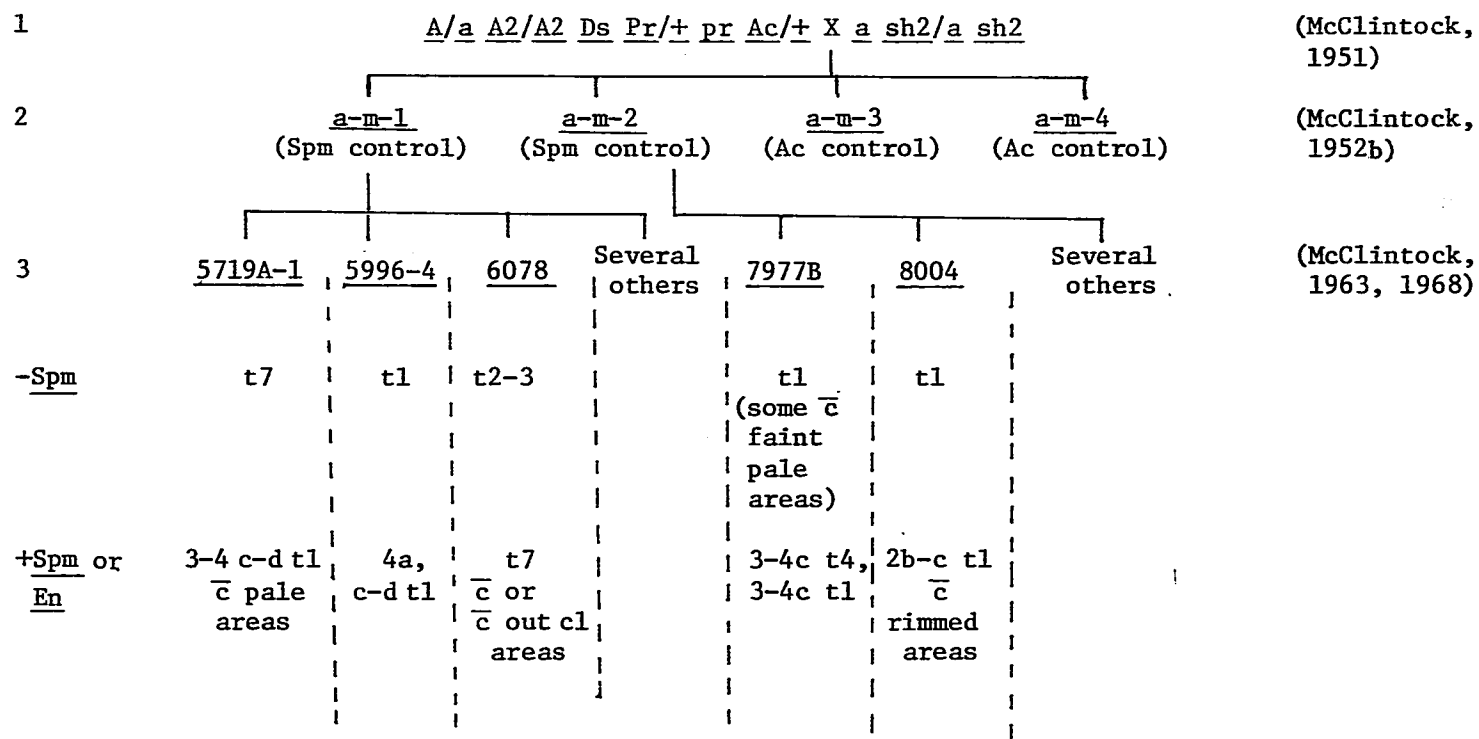
Figure 3.2.   Derivation of different states of <u>a-m-1</u> and <u>a-m-2</u> mutable alleles and their phenotypes with (+) and without (-) <u>Spm</u> or <u>En</u>

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The derivation and the phenotypes (with and without Spm) of these states are given in Figure 3.2. The original derivatives (a-m-1, a-m-2, a-m-3 and a-m-4) were all under autonomous control of Spm or Ac (Figure 3.2, line 2). From these original derivatives, several states with independent control (Spm or Ac not at the locus) were derived (line 3). Five of these states as shown in Figure 3.2 constitute the material for the present study.

In a-m-2 7977B, the colorless phenotype in the absence of Spm often contains faint pale areas. In a-m-2 8004, the rimmed areas in the spotted phenotype represent changes from an active to an inactive state of Spm. No spots are present within these rimmed areas.

1. Determination of changes in state

A change in state is recognized by a change in the spotted pattern (ex. 7b → 3c). Since a specific spotted pattern is produced by a specific interaction between the receptor (I) and the regulatory elements (Spm or En), a change in either or both of these elements is reflected in a change in the spotted pattern. In addition, a change in the basic allele phenotype (phenotype in the absence of En or Spm) is a reflection of a change in I.

Since changes in state occur only in the presence of En or Spm (McClintock, 1955), the exceptional kernels representing changes in state can be isolated among the test cross progeny of plants contain-ing En or Spm. The exceptional kernels may exhibit either a changed spotted pattern or a changed basic allele phenotype. In the case of

a change in the basic allele phenotype, it is evident that a change in

state of the receptor, I (mutable allele), had occurred and the heri-

tability of this new state could be confirmed by appropriate test

crosses. Also, the spotted pattern of the new state will be estab-

lished by crossing with En or Spm containing colorless-shrunken sibs.

If the exceptional kernel is spotted, a determination will be

made to distinguish whether the change involves the receptor I or the

regulatory element En or Spm. These tests are described below:

(1) Heritability tests

    Heritability of confirmed changes can be made by test crosses

    between the spotted exceptional phenotypes and an a sh tester.

    If the exceptional phenotype is heritable, a permanent change in

    state is confirmed. If the test cross progeny contains a basic

    allele phenotype (-En or Spm) that is different from that of the

    original state, a change in state of the receptor element is

    ascertained.

(2) Tests to distinguish a change in state of a regulatory element from

    that of a receptor element

    These tests, in addition to the heritability tests, are required

    to confirm a change in state. The tests involve two types of

    crosses with the a-m(r)/a-m-1 tester that is used as a standard

    for testing all the exceptional spotted phenotypes. The assay

    for the standard is the type of response reflected as an identified

    spotted pattern. Tests for two examples of spotted exceptions

    are illustrated in Figure 3.3. In each example, the following

Example 1.   lc tl ——▶ 4b tl
             (original) (exceptional)

$$\frac{\text{a-m } \underline{\text{Sh2}}}{\underline{\text{a}} \quad \underline{\text{sh2}}}, \ \underline{\text{En}} \ X \ \frac{\underline{\text{a}} \ \underline{\text{sh2}}}{\underline{\text{a}} \ \underline{\text{sh2}}}$$

(lc tl)

lc tl (+En),   tl (-En),   4b tl (+En),   cl, sh
(parental)   (parental) (exceptional) (± parental En)

Test A   Test B
(En in exceptional spotted)   (En in a sh sibs)

$$\frac{\text{a-m(r) } \underline{\text{Sh2}}}{\underline{\text{a-m-l}} \ \underline{\text{sh2}}} \ X \ \frac{\text{a-m } \underline{\text{Sh2}}}{\underline{\text{a}} \quad \underline{\text{sh2}}} \qquad\qquad \frac{\text{a-m(r) } \underline{\text{Sh2}}}{\underline{\text{a-m-l}} \ \underline{\text{sh2}}} \ X \ \frac{\underline{\text{a}} \ \underline{\text{sh2}}}{\underline{\text{a}} \ \underline{\text{sh2}}}$$

(tester)   (4b tl exception)   (tester)   (± parental En)

$$\frac{\text{a-m-l } \underline{\text{sh2}}}{\underline{\text{a-m}} \quad \underline{\text{Sh2}}}, \ \frac{\text{a-m(r) } \underline{\text{Sh2}}}{\underline{\text{a-m}} \quad \underline{\text{Sh2}}}, \ \frac{\text{a-m(r) } \underline{\text{Sh2}}}{\underline{\text{a}} \quad \underline{\text{sh2}}}, \ \frac{\text{a-m-l } \underline{\text{sh2}}}{\underline{\text{a}} \quad \underline{\text{sh2}}} \qquad \frac{\text{a-m(r) } \underline{\text{Sh2}}}{\underline{\text{a}} \quad \underline{\text{sh2}}}, \ \frac{\text{a-m-l } \underline{\text{sh2}}}{\underline{\text{a}} \quad \underline{\text{sh2}}}$$

Spotted
classes

1	2	3	4	1	2
Round, 4b tl + a-m-l- spotting and pale areas (pale areas are unique to a-m-l allele	Round, 4b tl + a-m(r)- spotting	Round, a-m(r)- spotting	Shrunken, a-m-l, spotting and pale areas	Round, a-m(r)- spotting	Shrunken, a-m-l- spotting and pale areas

Distinction between 2 and 3 classes depends on the response of a-m(r) to the En of exceptions. If the response is:
a) low—class 3 is a lower frequency spotted than 2
b) high—classes 2 and 3 may not be distinguishable from each other because of high spotting of both

a) If these spotted patterns are not similar to that of classes 3 and 4 in Test A—a change in En is confirmed.
b) If similar to that of classes 3 and 4 in Test A—a change in I is confirmed.

Figure 3.3.   Sample tests to distinguish a change in state of a regulatory element (En) from that of a receptor element (I). The comparisons are between phenotypes as judged from spotted patterns as described in Figure 3.1

Example 2.   5b tl⟶ la tl
              (original) (exceptional)

$$\frac{a\text{-}m \ \underline{Sh2}}{a \ \underline{sh2}}, \ \underline{En} \ X \ \frac{a \ \underline{sh2}}{a \ \underline{sh2}}$$

(5b tl)

↓

5b tl (+$\underline{En}$), tl (−$\underline{En}$), la tl (+$\underline{En}$),   cl, sh
(parental)   (parental) (exceptional)   (± parental $\underline{En}$)

↙   ↓

Test A   Test B
(En in exceptional spotted)   (En in a sh sibs)

$$\frac{a\text{-}m(r) \ \underline{Sh2}}{a\text{-}m\text{-}1 \ \underline{sh2}} \ X \ \frac{a\text{-}m \ \underline{Sh2}}{a \ \underline{sh2}}, \ \underline{En}$$ $$\frac{a\text{-}m(r) \ \underline{Sh2}}{a\text{-}m\text{-}1 \ \underline{sh2}} \ X \ \frac{a \ \underline{sh2}}{a \ \underline{sh2}}$$

(tester)   (la tl exception)   (tester)   (± parental $\underline{En}$)

↓   ↓

$$\frac{a\text{-}m\text{-}1 \ \underline{sh2}}{a\text{-}m \ \underline{Sh2}}, \ \frac{a\text{-}m(r) \ \underline{Sh2}}{a\text{-}m \ \underline{Sh2}}, \ \frac{a\text{-}m(r) \ \underline{Sh2}}{a \ \underline{sh2}}, \ \frac{a\text{-}m\text{-}1 \ \underline{sh2}}{a \ \underline{sh2}} \quad \frac{a\text{-}m(r) \ \underline{Sh2}}{a \ \underline{sh2}}, \ \frac{a\text{-}m\text{-}1 \ \underline{sh2}}{a \ \underline{sh2}}$$

Spotted
classes

1	2	3	4	1	2
Round, la tl + a-m-1- spotting and pale areas (pale areas are unique to a-m-1 allele)	Round, la tl + a-m(r)- spotting	Round, a-m(r)- spotting	Shrunken, a-m-1- spotting	Round, a-m(r)- spotting	Shrunken, a-m-1- spotting and pale areas

Columns 2 and 3 bracketed:

Because the frequency of spots elicited by a-m (exceptional) in class 2 is very low (la), classes 2 and 3 are not distinguish-able from each other.

Columns 1 and 2 (right group) bracketed:

a) if these spotted patterns are not similar to that of classes 3 and 4 in test A—a change in En is confirmed.

b) if similar to that of classes 3 and 4 in test A—a change in I is confirmed.

Figure 3.3.   (continued)

two tests are made with the standard a-m(r)/a-m-1 tester.

Test A: The spotted exceptions are tested on the a-m(r)/a-m-1

tester (Figure 3.3). Among the round-spotted progeny kernels,

those with a-m-1 mutable allele can be distinguished by the

presence of pale areas (class 1). Of the remaining round-spotted

progeny, those of a-m(r) Sh2/a sh2 genotype (class 3) represent

the response of a-m(r) standard allele to the En in exceptional

kernels and those of a-m(r) Sh2/a-m Sh2 genotype (class 2) exhibit

both a-m(r)-spotting and a-m-spotting (exceptional). Spotted

classes 2 and 3 are distinguishable depending on the response of

a-m(r) and the spotted pattern (low or high) of the exceptional

phenotype.

Test B: The a sh sib kernels (collected from the same ear as the

exceptional kernels), one-half of which contain En or Spm, are

crossed on a-m(r)/a-m-1 (Figure 3.3). This test identifies the

original state of En or Spm that is present in the stocks from

which the exceptional spotted phenotype is derived.

In order to distinguish a change in the regulatory element En (Spm)

from that of the receptor element I, the spotted patterns of a-m(r)

Sh2/a sh2 and a-m-1 sh2/a sh2 genotypes in test A, classes 3 and 4 are

compared with those of the same genotypes in test B, classes 1 and 2,

respectively. If these spotted patterns correspond, then it can be

inferred that the En (Spm) in exceptional kernels is the same as the

original and the exceptional phenotype is due to a change in state of I.

But if these spotted patterns in tests A and B do not correspond, then

1   2

Year   Row number   ⟶ 1976   1603   1976   0923
(phenotype of selected kernels) ⟶   (t6-7)   (3b-c t1)

X $\underline{a}$ $\underline{sh}$ or (x)   (x)   (x)

Range or types of progeny phenotypes ⟶   t1-7   3b-c t1, t1

|| Selection || ||

Year   Row number   ⟶ 1977   1025   1977   0501
(phenotype of selected kernels) ⟶   (t1-2)   (3b-c t1)

X $\underline{a}$ $\underline{sh}$ or (x)   X $\underline{a}$ $\underline{sh}$ X $\underline{a}$ $\underline{sh}$

Range or types of progeny phenotypes ⟶   t1-7   3b-c t1, t1, Exceptions

|| Selection || ||

Year   Row number   ⟶ 1978   1751   1978   0123
(phenotype of selected kernels) ⟶   (t6-7)   (t3-4)

X $\underline{a}$ $\underline{sh}$ or (x)   (x)   (x)

Range or types of progeny phenotypes ⟶   t1-7   t3-4

Figure 3.4. A generalized flow diagram illustrating the origin of each of the derivatives from the original source

it can be concluded that a changed En (Spm) is present in the exceptional

spotted kernels.

The tests described above are conducted if the exceptional kernel

was spotted. But if the exceptional phenotype is non-spotted (color-

less, pale or colored), then it is a change in state of the receptor

element I. This change is confirmed by interacting (crossing) the non-

spotted (new basic allele phenotype) with En or Spm in a sh sib kernels.

These tests with spotted and non-spotted exceptions would not only

confirm a change in state of the individual controlling elements, but

also any changes that occur in individual components (suppressor--S,

mutator--M) of the regulatory element, En or Spm. The changes in S

and M components are simultaneously monitored from the response of the

dark-pale a-m-1 mutable allele in a-m(r)/a-m-1 tester.

2. Presentation of results

The results are presented in the same basic format for all the

five states because the strategy is to confirm the heritability of

each of the initial states and to test any derivative exceptional

phenotypes for changes in state. The basic format consists of the

presentation of data in the form of (a) flow diagrams, (b) tables,

and (c) photographs. The flow diagrams are used to assist in tracing

the origin of each of the derivative kernels from an initial state and

then are subsequently discussed. A sample flow diagram is shown in

Figure 3.4. The tables not only contain all the data but also the

field row numbers by which the selected progeny kernels for planting

and crossing in the next generation are identified. The selected
progeny kernels and the row numbers are labelled with a mutually
identifiable mark (asterisk or some other mark). The photographs are
provided to complement the data in the tables and also to facilitate
the description of the phenotypes.

3. Analyses of data

The data in the tables are analyzed, wherever appropriate and
required, by chi-square tests. If the progeny kernel ratios conform to
the expected, then no chi-square tests are used.

IV. RESULTS

A. a-m-1 5719A-1 State

The phenotype of the original a-m-1 5719A-1 state is colored (t7)

in the absence of Spm. In the presence of Spm, this state is 3-4c-d tl

spotted with pale areas in the background (Figure 4.1).

1. Origin of variable pales (t1-6)

From selfed and test cross progenies of the original full-colored

phenotype (t7), variable pales ranging from colorless to dark-pale

(t1-6) were derived (Figure 4.2A, B and C, 1976 progeny). In addition

to the variable pales, there were a few low spotted kernel types among

these progenies, but these phenotypes when tested disappeared among the

progenies after one or two generations of selfing or test crossing

(Figure 4.2A, B and C, 1977, 1979 progenies). Instead, the progeny of

low spotted consisted of kernels ranging from colorless to full-colored

(t1-7). Since these progeny types are similar to those of the original

full-colored (t7) a-m-1 5719A-1 state, the low spotted phenotypes could

either represent a temporary change in state of a-m-1 5719A-1 or an

environmentally influenced temporary physiological change of the kernels.

However, since the original colored phenotype of a-m-1 5719A-1 state

does not contain Spm, a temporary change in state is unprecedented.

The presence of variable pales in the progeny of the original full-

colored a-m-1 5719A-1 state is unexpected and is an expression of

changes at this allele or modifiers of this allele because no other major

genes responsible for anthocyanin pigmentation are known to segregate in

A   B
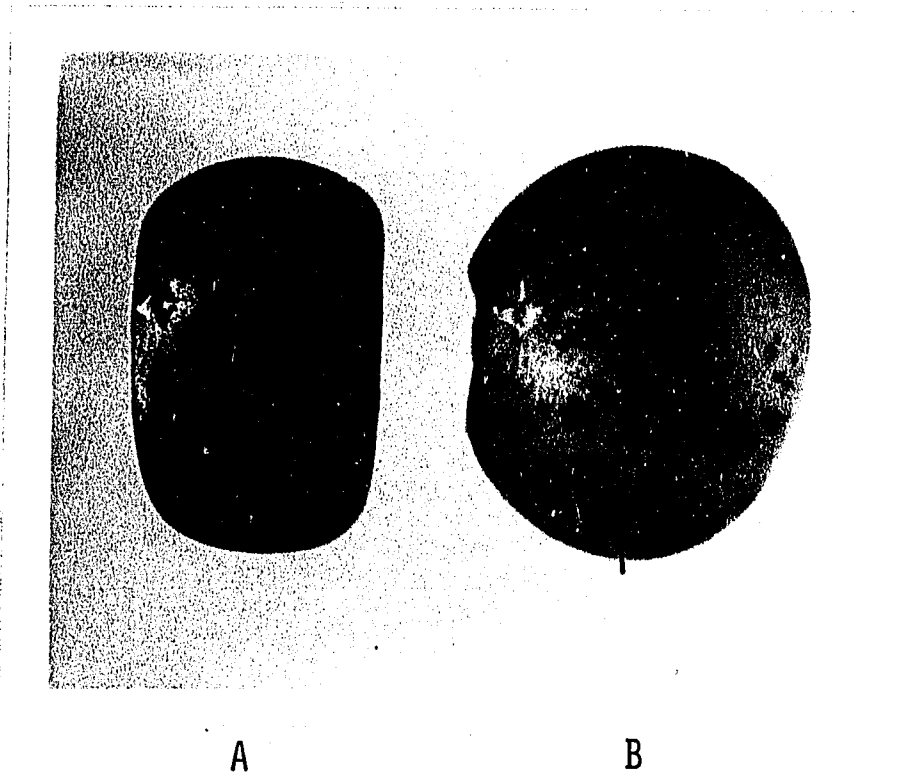
Figure 4.1. Original state of a-m-1 5719A-1

   A. Colored (t7) without Spm

   B. 3-4c-d tl spotted with Spm--note intermixture of pale
    areas (arrow)

Figure 4.2. Flow diagram illustrating different derivatives from the original source of
a-m-1 5719A-1 state

Original   McCl 8615A ⑪/McCl 8615A ⑭   (a-m-1 5719A-1 Sh2/a-m-1 6078 Sh2)
(McClintock,
 1968)

x a sh

t7   (a-m-1 5719A-1)   t2-3 (a-m-1 6078)

1976   1603-1, 1603-1t, 1603-2
    (t7)   (Figure 4.2B,C)

⊗

t7,t1-6 + few low spotted

1977   0931B-1
    (low spotted)

x a sh

t1-5

1979   1636   1635
    (t1)   (t4-5)

⊗   x a sh En

t1-4   3-4c-d t1 c̄ pale areas, t4-7

⊗

t1-7

1980   1356   1350,1353   1351   1352
    (3-4c-d t1 with pale areas)   (t7)   (t4-5)   (t1)

x a sh

x a sh   x a sh En   x a sh   x a sh   x a sh En

3-4c-d t1   t4-7, exceptions   t2-7   3-4c t1,t2-7   t2-5   t2-3   3-4c-d t1,t4-5
c̄ pale areas,   c̄ pale areas,   c̄ pale areas,

1981   1307A   1307B   1307D   1302   1303
    (t1)   (t2-3)   (3c t4)   (t7)   (t3)

A

(see Figure 4.2A)
↓

1976   1603-1t
                (t7)

              ↓ x $\underline{a}$ $\underline{sh}$

          t7, t1-6, few low spotted
                   ‖
1977   0931C-2
            (few low spotted)

      ↓ x $\underline{a}$ $\underline{sh}$ ↓ ⊗

    1-2b t1, t1   1-2b t1, t2-3
        ‖   ‖
1979   1633   1637X
        (t1)   (1-2b t1)

                                                                 ↓ ⊗

      ↓ x $\underline{a}$ $\underline{sh}$ ↓ x $\underline{a}$ $\underline{sh}$ $\underline{En}$ all t1

      t1-7   3-4c t1, t1-3
        ‖   ‖
1980   1348   1354
        (t1)   (3-4c t1)

      ↓ x $\underline{a}$ $\underline{sh}$ ↓ x $\underline{a}$ $\underline{sh}$

      t1-3   3-4c t1, t1, exceptions

                 1308A, 1309A   1308B, 1309B
                   ($5c^+$)   (1b t1)

B

Figure 4.2.   (Continued)

(see Figure 4.2A)

1976   1603-2, 1603-1, 1603-1t
        (t7)   (Figures 4.2A,B)

        ⊗

t7, t1-6, few low spotted

1977   0949-3   0949-5
        (low spotted)   (low spotted)

        ⊗   x a sh

        t1   t1-3
        ‖   ‖
1979   1632   1631
        (t1)   (t2-3)

        ⊗   ⊗   x a sh En (low)

        mostly t4-5   t1-7   2-3b t1, t3-5
        ‖   ‖
1980   1349   1347   1346   1343
        (t4-5)   (t4-5)   (t1)   (2-3b t1)

        x a sh x a sh En   x a sh En   x a sh   x a sh

        t2-3 3-4c t1 c̄   3-4c t1 c̄   t1-5   2-3b t1, t3-7, exceptions
              pale areas, pale areas,
              t4-5   t4-7
1981   1323A   1324
                                                       (1a-b t1)   (1-2a-b t1)

C

Figure 4.2.   (Continued)

97

this material. This change could also be a transient change in state
of a-m-1 5719A-1 allele by some unknown mechanism. To test these
explanations for these changes, colored (t7) and the unexpected variable
pale progeny kernels are selected and tested for their heritability and
for the spotting patterns they produce in response to En.

a. Heritability of variable pales (t1-6) and the full-colored
(t7) phenotype   The variable pales arising from the original
full-colored (t7) a-m-1 5719A-1 alleles are grouped into two classes:
colorless (t1) and pale (t4-5). These two classes and the full-colored
(t7) phenotype of a-m-1 5719A-1 state are test crossed to determine their
heritability.

1) Determination of the heritability of the colorless (t1)
phenotype (Figure 4.2A, 1980 1352; 4.2B, 1980 1348; 4.2C, 1346)   Among
the progeny of the test cross of colorless (t1) (Table 4.1), colorless
(t1), light-pale (t2-3) and pale (t4-5) classes of kernels are found with
the t1 and t2-3 types in the majority. While the t4-5 progeny kernels
occurred infrequently, the colored (t7) class did not appear among seven
test crosses.

It can be concluded that the colorless (t1) phenotype can give rise
to varied pigmented types from colorless to pale (t1-5) among progeny
kernels. The frequency of colorless (t1) kernels differs among the
individual cross progenies. The heritability of the colorless phenotype
averages at a frequency of 0.45 (Table 4.1).

2) Heritability of the pale (t4-5) phenotype (Figure 4.2A,
1980 1351; 4.2C, 1980 1349)   When seven pale (t4-5) kernels are tested

Table 4.1. Heritability of colorless (t1) phenotype derived from the original full-colored (t7) a-m-1 5719A-1 state (Figure 4.2A, 1980 1352; 4.2B, 1980 1348; 4.2C, 1980 1346)

a-m-1 5719A-1 Sh2 ————————————— X a sh2 / a sh2 a-m-1 5719A-1 Sh2 / a sh2	X a sh2 ——— a sh2	Round					
		Variable pales					Frequency
cl,rd (t1)	X cl,sh	t7	t4-5	t2-3	t1	Total	of t1
0 1346-20	X 1431	-	34	274	67	375	0.17
-21	X 1431	-	28	61	--	89	0.0
-22	X 1431	-	--	--	85	85	1.0
0 1348- 3	X 1406	-	--	28	145	173	0.83
- 4	X 1419	-	--	2	22	24	0.91
0 1352-20	X 1432	-	--	92	--	92	0.0
-21	X 0354	-	--	33	1	34	0.3

Average frequency of t1 = 0.45

Table 4.2. Heritability of pale (t4-5) phenotype derived from the original full-colored (t7) <u>a-m-1 5719A-1</u> state (Figure 4.2A, 1980 1351; 4.2C, 1349)

$\dfrac{\text{a-m-1 5719A-1 Sh2}}{\text{a-m-1 5719A-1 Sh2}} \Big/ \text{a sh2} \ \ {}^{\text{X}}\ \dfrac{\text{a sh2}}{\text{a sh2}}$ <u>t4-5</u>	X cl,sh	Round					
		Variable pales					
		<u>t7</u>	<u>t4-5</u>	<u>t2-3</u>	<u>t1</u>	Total	Frequency of t4-5
0 1349-2	X 1530	--	all	--	--	all	1.00
-4	X 1530	--	all	--	--	all	1.00
-5	X 1431	--	--	all	--	all	0.0
-20	X 1431	--	130	110	2	242	0.53
0 1351-20	X 0354	--	93	77	--	170	0.55
-21	X 0354	--	--	225	--	225	0.0˙
-22	X 1433	--	131	130	--	261	0.50

Average frequency of t4-5 = 0.51

Table 4.3. Heritability of colored (t7) phenotype derived from the original full-colored a-m-1 5719A-1 state (Figure 4.2A, 1980, 1350; 1981 1302)

$\dfrac{\text{a-m-1 5719A-1 Sh2}}{\text{a-m-1 5719A-1 Sh2 / a sh2}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$ t7	X cl,sh	Round Variable pales				Total	Frequency of t7
		t7	t4-5	t2-3	t1		
0 1350-2	X 1431	41	--	--	--	all	1.0
-20	X 1431	--	77	--	1	78	0.0
-21	X 0354	173	--	92	--	265	0.65
1 1302-1	X 1351	41	68	--	--	109	0.37
-3	X 1221	28	83	8	--	119	0.23
-7	X 1352	42	116	--	2	160	0.26
-8	X 1221	--	60	130	--	190	0.0

Average frequency of t7 = 0.36

for confirmation, five gave that type in the progeny (Table 4.2). Two

of these five are true-breeding t4-5s. In the other three progenies,

approximately one-half of the round class are t2-3. In two of the

seven progenies, only t2-3 types are present. The t4-5, t2-3 classes

constitute almost all the kernels in the test cross progenies.

When the pale (t4-5) phenotype is selected and tested, the progeny

includes the light-pale (t2-3), in addition to the parental t4-5. Though

the frequency of t4-5 progeny differs among test crosses, the t4-5

phenotype is heritable at an average frequency of 0.51 (Table 4.2).

3) __Heritability__ __of__ __the__ __colored__ (__t7__) __phenotype__ (__Figure__ __4.2A__,

__1980__ __1350__, __1981__ __1302__)   When seven selected t7 type kernels are

tested, the t7 type appears in five of the progenies (Table 4.3). In

one of these five, it is the only class. In the other four, it varies

in frequency and is distributed along with t4-5 and/or t2-3 classes.

The t4-5 type kernels are present in five and the t2-3 in three of the

seven test cross progenies.

The colored (t7) selection bred true in one of the seven progenies

indicating that it is heritable. But it is not heritable in two and

only partially heritable in the rest of the progenies. The t7 pheno-

type occurs at an average frequency of 0.36 (Table 4.3).

In summary, tests of the heritability of colorless (t1), pale

(t4-5) and colored (t7) classes demonstrate the following:

    (1) The phenotype of each of these classes is not fully heri-

           table because the progeny kernels vary in pigmentation within

           and among test crosses.

(2) Although the progeny kernels vary in the pigmentation, there

is a tendency for the progeny kernels to fall closer to the

parental class. For example, the progeny kernels of colored

(t7) phenotype contain a few or none of the colorless (t1)

class (Table 4.3). Similarly, kernels of colorless (t1)

phenotype do not contain any of the colored (t7) class

(Table 4.1).

It can be concluded that the phenotypes are heritable or have a

unidirectional heritability. It is not known, however, how each of the

variable pale types (t1, t2-3 and t4-5) arose from the original full-

colored (t7) a-m-1 5719A-1 state. The original allele that gave rise

to selfed or test cross progeny was not in the presence of Spm or En

(Figure 4.2A, B and C, 1976 progenies). These changes of t7 to t1,

t2-3 and t4-5 could be the property of the allele itself that generates

the variability.

b. Response of variable pales to En   The same three classes--

colorless (t1), pale (t4-5) and colored (t7)--were tested for their

response to En. A comparison could then be made to test if it is dif-

ferent from that of the original a-m-1 5719A-1, which would be expected

if a change in state had occurred.

1) Response of colorless (t1) to En (Figure 4.2A, 1979

1636, 1980 1352; 4.2B, 1980 1348)   The colorless (t1) phenotype is

responsive to En of the a sh + En line yielding 3-4c-d t1 spotted

progeny kernels (Table 4.4). However, these spotted are of two types--

with and without pale areas. The spotted with pale areas are present

Table 4.4. Response of colorless (tl) phenotype to En (Figure 4.2A, 1979 1636, 1980 1352; 4.2B, 1980 1348)

| $\frac{a\text{-}m\text{-}1\ 5719A\text{-}1}{a}\ \frac{Sh2}{sh2}$ X $\frac{a\ sh2,}{a\ sh2}$ En | | Round | | | | | | Shrunken |
| | | Spotted | | Variable pales | | | | |
tl	X cl,sh	(3-4c-d tl $\bar{c}$ pale areas)	(3-4c-d tl $\bar{c}$ out pale areas)	t7	t4-5	t2-3	tl	Color-less
9 1636-20	X 3311-5	24	--	--	20	--	--	45
-21	X 3311-6	93	--	9	18	--	--	101
0 1348-6	X 1426-6	--	83	--	--	27	75	191
1352-6	X 1529-1	76	--	--	78	--	--	1/2

Table 4.5. Response of pale (t4-5) phenotype to _En_ (Figure 4.2C, 1980 1347, 1349)

| a-m-1 5719A-1 Sh2 / a-m-1 5719A-1 Sh2/a sh2 — t4-5 | X a sh2 / a sh2, En — X cl,sh | Round | | | | | | Shrunken |
| | | Spotted | | Variable pales | | | | Color-less |
		(3-4c-d t1 c̄ pale areas)	(3-4c-d t1 c̄ out pale areas)	t7	t4-5	t2-3	t1	
O 1347-20	X 1427-7	25	--	18	10	--	--	53
-21	X 1529-1	114	--	41	98	6	--	--
-22	X 1529-5	80	--	51	--	--	--	135
1349-1	X 1531-8	123	--	--	72	--	--	--
-5	X 1530-2	33	--	--	35	--	--	--
-8	X 1427-7	75	--	--	74	--	--	--

Table 4.6. Response of colored (t7) phenotype to En (Figure 4.2A, 1980 1350, 1353)

| a-m-1 5719A-1 Sh2 / a-m-1 5719A-1 Sh2/a sh2 | X a sh2, En / a sh2 | Round | | | | | | Shrunken |
| | | Spotted[a] | | Variable pales | | | | |
t7	X cl,sh	(3-4c-d tl c pale areas)	(3-4c-d tl c out pale areas)	t7[b]	t4-5	t2-3	t1	Color-less
0 1350-12	X 1529-11	54	--	97	--	--	--	151
-13	X 1529-11	56	--	63	--	--	--	126
-14	X 1530-5	186	--	180	--	--	--	--
-15	X 1531-6	96	--	263	--	--	--	--
1353-20	X 1528-9	48	--	--	31	18	--	--

[a]Include a few low-spotted types.

[b]Include some non-uniform pales.

in 3 out of 4 progenies which also contain colored (t7) or pale (t4-5)
sib kernels of the basic allele phenotype (-En type). Whereas, the
spotted without pale areas are prevalent in only one of the progenies
(Table 4.4, 0 1348-6 X 1426-6) that contains light-pale (t2-3) and
colorless (t1) sib kernels of -En type. The distinction between the
pale area types and the non-pale area types is clearly due to the
presence of a particular basic allele and is not a consequence of the
behavior of En. With the t2-3 or t1 type, any changes in En would not
be evident whereas the pale areas in spotted kernels correspond to the
colored (t7) or pale (t4-5) basic allele phenotype. The pale areas
thus correspond to changes in the activity of the suppressor component
of En, indicating that it has become inactive.

2) Response of pale (t4-5) phenotype to En (Figure 4.2C,
1980 1349, 1347)   The same spotting pattern (3-4c-d t1) occurs with
the t4-5 in response to En as that with the colorless (t1) kernels (Table
4.5). Here, however, the pale areas are present on spotted kernels of
all the progenies. This is consistent with the presence of colored
(t7) or pale (t4-5) sib kernels (-En type) in each of the progenies
allowing for changes in En activity to be expressed.

3) Response of colored (t7) to En (Figure 4.2A, 1980 1350,
1353)   The response of colored phenotype to En (Table 4.6) is similar
to that of the tests with the colorless and pale selections. In all
the progenies, the spotted (3-4c-d t1) kernels contain pale areas.
The presence of these pale areas corresponds to the occurrence of
colored (t7) or pale (t4-5) kernels (-En type) in all the progenies.

The segregation patterns are not consistent. In two of the
progenies (Table 4.6, 0 1350-12 X 1529-11 and 0 1350-15 X 1531-6), the
spotted (+En) are much fewer than the colored (-En) (1:1 ratio is
expected on the basis of segregation of an independent En). Such a
disproportionate ratio might result from two kinds of events. Either
a loss or a change in phase of activity of En (from an active to an
inactive state) during microsporogenesis in the a sh En line could
explain these discrepant ratios.

In summarizing the response of the t1, t4-5 and t7 types to En,
it is evident that the spotted patterns in the progenies are con-
sistently the same (3-4c-d t1) in spite of differences in the parental
kernel phenotype. The pale areas in the spotted kernels are an expres-
sion of the state of En.

There is a significant difference in the pigmentation expression
of the basic allele between the heritability (-En) study and that of
the En-responsive (+En) study. In heritability study, the -En type
kernels in the progeny of a particular phenotypic class are lighter
and more varied in pigmentation than those in En-response study. For
example, the progeny kernels of colored (t7) phenotype vary from t2
to t7 in the heritability study (Table 4.3), whereas those of the same
colored type are colored in most of the progenies in En-response study
(Table 4.6). Because the basic alleles used are the same in each study,
either the En or some other factors in a sh En plants used in En-response
st dy are responsible for the darker pigmentation of progeny kernels.

c. Derivation of exceptional phenotypes in test cross progenies of spotted kernels   When exceptional phenotypes are derived in test cross progenies of spotted kernels, either there is a change in state of the receptor element (I) (mutable allele) or of the regulatory element (En). A change in the state of En is recognized by a changed spotting pattern. For a change in the state of a mutable allele, there are two ways to define such a change: the spotting pattern as well as the basic allele phenotype (-En state) will provide a definitive change.

Exceptional phenotypes are derived in the test cross progenies of three different spotted kernels as shown in Figures 4.2A (1980 1356), 4.2B (1980 1354) and 4.2C (1980 1343). These three spotted types and the exceptional phenotypes derived from them include:

Parental spotted pattern	Exceptional progeny kernels
1. 3-4c tl	$5c^+$ (1 1308A, 1 1309A), 1b tl (1 1308B, 1 1309B) (from Table 4.7)
2. 3-4c-d tl with pale areas	3c t4 (1 1307D), t2-3 pale (1 1307B) tl (1 1307A) from Table 4.10)
3. 2-3b tl	1a-b tl (1 1323A), 1-2a-b tl (1 1324) (from Table 4.12)

The exceptional kernels are selected and tests are made to determine whether the change in state occurred in En or I of the a-m-1 5719A-1 allele. These test procedures are outlined in Materials and Methods.

1) Tests of exceptional phenotypes obtained in test cross progenies of 3-4c tl spotted kernels (Figure 4.2B, 1980 1354 and Table 4.7)

(a) $5c^+$ spotted (1 1308A, 1 1309A)

The test cross progenies of $5c^+$ spotted exceptions confirm the

Table 4.7. Derivation of exceptional phenotypes in test cross progenies of 3-4c t1 spotted (Figure 4.2B, 1980 1354)

| $\dfrac{a\text{-}m\text{-}1\ 5719A\text{-}1}{a}\ \dfrac{Sh2}{sh2}$, En X $\dfrac{a\ sh2}{a\ sh2}$ | | Round | | | | | Shrunken |
| | | Spotted | Exceptions | | Pale | Color-less | Color-less |
3-4c t1	X cl,sh	3-4c t1	$5c^{+}$	1b t1	t3-4	t1	
0 1354-1	X 1419	96	4*	1*		97	197*
-8	X 1419	50	3**	3**	6	62	115
-19	X 1431	64	2	1	5	118	182
-20	X 2158	69	--	1	--	95	146
-21	X 2158	39	5	1	--	110	148
1981 rows containing selected kernels[a]			(1309A*) (1308A**)	(1309B*) (1308B**)			(1309C*)

[a]Selected kernels and their assigned row numbers in a column are identified by the same superscript.

Table 4.8. Tests of 5c$^+$ spotted exceptional phenotypes obtained in the test cross progeny of 3-4c tl spotted kernels (from Table 4.7)

$\dfrac{\text{a-m-1 5719A-1 Sh2, En}}{\text{a} \qquad \text{sh2}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$		Round			Shrunken
$5c^+$ X cl,sh		Spotted 3-4c-d c̄ 3-4a specks (5c$^+$)	Pale t2-3	Color-less tl	Color-less
a) Progeny test					
1 1308A-1	X 1352	69	--	59	1/2
A-2	X 1353	157	40	48	1/2
A-3	X 1230	92	1	47	1/2
1 1309A-1	X 1352	85	7	75	1/2
A-5	X 0549	121	5	112	1/2

$\dfrac{\text{a-m(r) Sh2}}{\text{a-m-1 sh2}}$ X $\dfrac{\text{a-m-1 5719A-1 Sh2, En}}{\text{a} \qquad \text{sh2}}$
Cl,rd   X   5c$^+$ Round, spotted types

b) Test for type of En in 5c$^+$ spotted kernels

1253	X 1308A-1	4-5c tl	4-5c tl c̄ pale areas	4-5c tl fine pale spots
1254	X 1308A-2	"	"	"
1253	X 1309A-1	"	"	"

$\dfrac{\text{a-m(r) Sh2}}{\text{a-m-1 sh2}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$ En sibs
cl,rd   X   cl,sh   Round, spotted   Shrunken, spotted

c) Test for type of En in colorless-shrunken sibs

1255	X 1309C-1	4-5c tl	4-5c tl c̄ pale areas
1254	X 1309C-2	"	"
1259	X 1309C-4	"	"
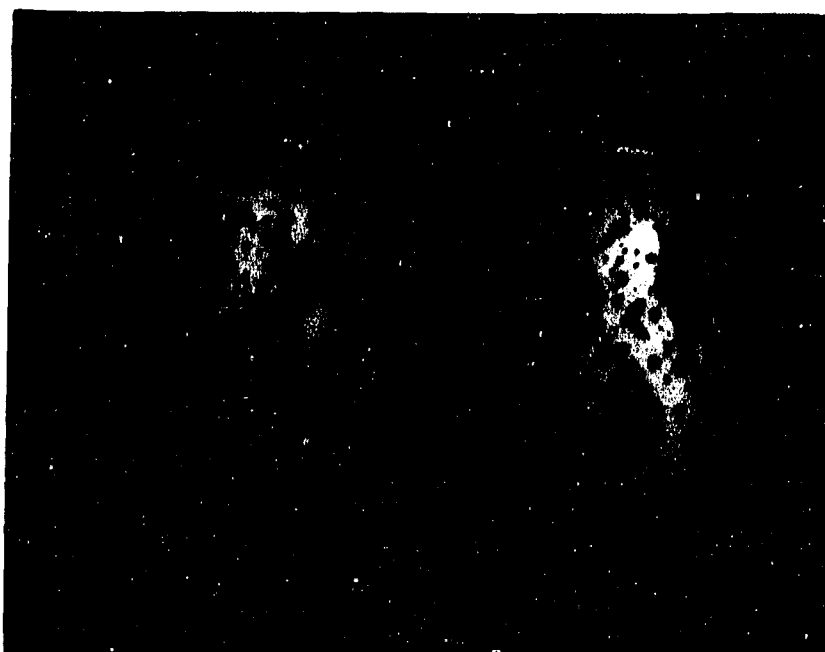
Figure 4.3. $5c^+$ spotted exceptional kernels arising from the original a-m-1 5719A-1 (Figure 4.1). Note the distribution of the two distinct spot types--3-4c-d and 3-4a

selected type as a truly derived exception from the parental 3-4c tl

type (Table 4.8a). Spotted ($5c^+$), light-pale (t2-3) and colorless

(tl) kernels are included in the progeny. In a comparison of the

figures of the parental (Figure 4.1B) and the derivative (Figure 4.3),

the difference is clearly evident. The $5c^+$ pattern of the derivative

is formed from medium size spots (3-4c-d) plus fine pale spots (3-4a)

that are only clearly discernible when examined with a dissecting

microscope. It is the fine pale spots that give an appearnace of $5c^+$

spotted pattern to the naked eye.

In order to determine whether there is a change in En, tests are

made (Table 4.8b, c). In (b), the $5c^+$ spotted (a-m-1 5719A-1 Sh2/

a sh2 + En) are crossed on a-m(r) Sh2/a-m-1 sh2 (abbreviation -

a-m(r)/a-m-1), a standard En-responsive tester. Among the round

progeny kernels, the spotted patterns include 4-5c tl (a-m(r) Sh2/

a sh2 + En), 4-5c tl with pale areas (a-m-1 sh2/a-m-1 5719A-1 Sh2 + En)

and 4-5c tl with fine pale spots (a-m(r) Sh2/a-m-1 5719A-1 Sh2 + En).

The assignment of genotypes to these spotted types can be made because

it is known that the pale areas in spotted kernels appear only in con-

junction with the a-m-1 allele of the tester.

In order to test the En of the exceptional kernels (Table 4.8b)

against the parental En (Table 4.8c), the spotted patterns are compared

from the two sets of progenies. The spotted progeny in test (c) include

4-5c tl (round) and 4-5c tl with pale areas. On the basis of the

parental genotypes, these spotted progenies represent a-m(r) Sh2/

a sh2 + En and a-m-1 sh2/a sh2 + En, respectively. When the same

Table 4.9. Tests of exceptional 1b tl spotted obtained in the test-
cross progeny of 4c tl spotted kernels (from Table 4.7)

$\frac{\text{a-m-1 5719A-1 Sh2, En}}{\text{a} \qquad \text{sh2}}$ X $\frac{\text{a sh2}}{\text{a sh2}}$		Round			Shrunken
		Spotted	Pale	Color-less	Color-less
1b tl	X cl,sh	1b tl	t2-3	tl	

a) Progeny test

1 1308B-1	X 1352	---	few	1/2	1/2
B-2	X 1213	---	few	1/2	1/2
B-3	X 1214	---	few	1/2	1/2
1 1309B	X 1351	---	few	1/2	1/2
Reciprocal crosses					
1230	X 1309B	---	29	131	1/2
1221	X 1309B	---	27	133	1/2

$\frac{\text{a-m(r) Sh2}}{\text{a-m-1 sh2}}$ X $\frac{\text{a-m-1 5719A-1 Sh2, En}}{\text{a} \qquad \text{sh2}}$	Spotted

b) Test for type of En in 1b tl spotted kernels

1255	X 1308B-1	None
1253	X 1308B-2	None
1254	X 1308B-3	None

genotype (a-m(r) Sh2/a sh2 + En) in test (b) and (c) is compared, the
results show that the spotted pattern is identical (4-5c tl) indicating
that these two En are similar in expression. It can be concluded that
the exceptional 5c$^+$ spotted pattern represents a change in the receptor
component of the a-m-1 5719A-1 allele and not the En.

(b) 1b tl spotted (1 1308B, 1 1309B)

The test cross progenies of the 1b tl spotted do not contain any
spotted kernels (Table 4.9a). Mostly colorless (tl) and a few light-
pale (t2-3) kernels constitute the progeny. When tested on the
standard a-m(r)/a-m-1 tester (Table 4.9b), the 1b tl spotted did not
produce any spotted progeny kernels.

These results suggest two possible explanations for the non-
heritable nature of 1b tl spotted phenotype. One, in spotted kernels,
the 1b tl expression is confined only to the endosperm because only the
endosperm nucleus received an En whereby the embryo is devoid of an En.
Secondly, a cyclical change in phase of activity of En in alternate
plant generations would result in the appearance of spots on kernels
only when En is in an active state. In the present case, the active
state of En is reflected in low and fine spots in a colorless background
(1b tl).

Since the 1b tl spotted exceptions were derived in the test cross
progenies of 3-4c tl spotted (Table 4.7), the basis of this change
may reside in a change in state of either the En or a-m-1 5719A-1
allele. It can be concluded that the change is in En, because a
change in the a-m-1 5719A-1 allele would have produced 4-5c tl spotted

progeny in tests on the a-m(r)/a-m-1 tester (as in test (c) of Table 4.8). However, this new state of En is neither heritable nor does it trigger mutations of a-m(r) or a-m-1 alleles (Table 4.9b). The question is whether the non-heritability and the lack of activity are the properties of this new state of En. If so, the colorless (t1) and a few light-pale (t2-3) progeny kernels (Table 4.9a) when test crossed should yield a reactivated state of En.

It is possible that the origin of 1b t1 spotted exceptions is dependent on a pre-existing inactive regulatory element (such as Spm) before the introduction of a standard En into the a-m-1 5719A-1 cultures (Figure 4.2B, 1979 1633-colorless). The standard En of a sh En line could trigger the inactive Spm, but the expression of the activated Spm is not discernible in that generation (1980 1354) because it is masked by the highly active En expression (3-4c t1). In the following generation, however, the activated Spm is expressed (1b t1) in a few progeny kernels (1981 1308B, 1309B exceptions) in the absence of En. The presence of such an inactive Spm is supported by the previously unexplained occurrence of spotted (or 1b t1) kernels in the progenies derived from the original a-m-1 5719A-1 cultures (Figures 4.2A, B and C 1977 cultures) that could not be recovered in subsequent test crosses.

2) Tests of exceptional phenotypes obtained in test cross progenies of 3-4c-d t1 spotted with pale areas (Figure 4.2A, 1980 1356) The four exceptional kernels include one colorless (1 1307A), two t2-3 pale (1 1307B) and one 3c t4 spotted (1 1307D) (Table 4.10). Appropriate tests (as outlined in Materials and Methods) are conducted

Table 4.10. Derivation of exceptional phenotypes in test cross progenies of 4c-d tl spotted with pale areas (Figure 4.2A, 1980 1356)

| $\dfrac{\text{a-m-1 5719A-1 Sh2, En}}{a \quad \text{sh2}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$ | | Round | | | | | | Shrunken |
| | | Spotted[a] | | Pale | | | Color-less | Color-less |
4c-d tl c̄ pale areas	X cl,sh	3-4c-d tl c̄ pale areas	Exceptions	t7	t4-5	t2-3	t1	
0 1356-19	X 1432	119	—	84	50	—	—	253
-20	X 1419	118	—	25	17	—	1*	156
-21	X 1419	108	1*(3c t4)	12	22	2*	—	141*
-22	X 1420	104	—	20	10	—	—	128
1981 rows containing selected kernels[b] ⟹		(1307D*)				(1307B*)	(1307A*)	(1307E*)

[a] Spots are slightly irregular in shape.

[b] Selected kernels and their assigned row numbers in a column are identified by the same superscript.

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to distinguish whether the changes are in _En_ or in the _a-m-1 5719A-1_

allele.

(a) Colorless-tl (1 1307A)

When tested with colorless-shrunken sibs (1 1307E), half of which

contain _En_, the colorless phenotype does not respond (Table 4.11A). The

progeny included only colorless kernels. The absence of spotted progeny

indicates that this exceptional colorless phenotype is non-responsive to

_En_, thus confirming a change in the _a-m-1 5719A-1_ allele.

(b) t2-3 pales (1 1307B)

When the t2-3 pale exceptions also are crossed with the sib _a_ _sh_ _En_,

the progeny include 4b-c tl spotted with pale areas, colored (t7) and

pale (t4-5) kernels (Table 4.11B). These progeny types are similar to

the + and -_En_ types of the parental source (Table 4.10). Thus, the

t2-3 pales do not represent a change, but are representative of the vari-

able pale types that often appeared in the progenies of original full-

colored (t7) _a-m-1 5719A-1_ allele (Figure 4.2A, 1976 progeny).

(c) 3c t4 spotted (1 1307D)

Only colored kernels appeared among the test cross progeny of the

3c t4 spotted (Table 4.11C), indicating that the spotted phenotype is

not heritable. The progeny represent the original -_En_ state of the

parent allele. The selected exception is an ephemeral expression of

events taking place in the endosperm that can be due to changes in _En_

or _I_.

Table 4.11. Tests of exceptional phenotypes obtained in the test cross progenies of 4c-d spotted with pale areas (from Table 4.10)

A. Test of colorless (t1) (1 1307A)

$\frac{a\text{-}m\text{-}1\ 5719A\text{-}1\ Sh2}{a\qquad sh2}$ X $\frac{a\ sh2 \pm En}{a\ sh2}$ sibs		Round					Shrunken
t1	X cl,sh	Spotted[a] 4b-c t1 c̄ pale areas	t7	t4-5	t2-3	Colorless t1	Colorless
1 1307A X 1307E-3 +[b]		--	--	--	--	1/2	1/2
A-t X 1307E-2 +		--	--	--	--	1/2	1/2
Reciprocal cross							
1 1307E-3 X 1307A-t		--	--	--	--	1/2	1/2

B. Test of t2-3 pale (1 1307B)

$\frac{a\text{-}m\text{-}1\ 5719A\text{-}1\ Sh2}{a\qquad sh2}$ X $\frac{a\ sh2 \pm En}{a\ sh2}$ sibs

1 1307B-1 X 1307E-3 +		61	41	16	--	1	1/2
B-2 X 1307E-4 +		72	50	15	--	--	1/2

C. Test of spotted (3c t-4) phenotype

$\frac{a\text{-}m\text{-}1\ 5719A\text{-}1\ Sh2,\ En}{a\qquad sh2}$ X $\frac{a\ sh2}{a\ sh2}$

1 1307D X 1206		--	1/2	--	--	--	1/2
Reciprocal cross							
1 1226 X 1307D		--	1/2	--	--	--	1/2

[a] Spots are slightly irregular in shape.

[b] Presence of En confirmed on a-m(r)/a-m-1.

3) Tests of exceptional phenotypes obtained in test cross progenies of 2-3b tl spotted kernels (Figure 4.2C, 1980 1343) The exceptional phenotypes include 1-2a-b tl and 1a-b tl spotted (Table 4.12, Figure 4.4). These two spotted exceptions differ only slightly in their spot frequency.

(a) 1-2a-b tl (1 1324)

The test cross progenies included two +En types (1a tl and 1a t6 spotted) and three -En types (t7, t4-5 and tl) (Table 4.13a). The five 1a t6 spotted represent exceptions to the parental type.

The tests of 1-2a-b tl spotted on a-m(r)/a-m-1 tester yielded 1-2a-b tl spotted progeny (Table 4.13b). When the colorless-shrunken sibs were tested on a-m(r)/a-m-1, the progeny included 2-3b-c tl (Table 4.13c). Because the En expressions differ in comparative tests, it can be concluded that the 1-2a-b tl spotted phenotype represents a change in state of En.

The 1a t6 spotted exceptions differ in the background coloration (tl vs t6). This could represent a change in the S component of En from an active to an inactive state while there is no change in M component. Further tests will be necessary to confirm the heritability of this En.

In addition to the -En colored (t7) types, there are colorless (tl) kernels in the test cross progenies. The ratios of progeny types suggest that these represent cases where En did not trigger mutations since it was a low acting En.
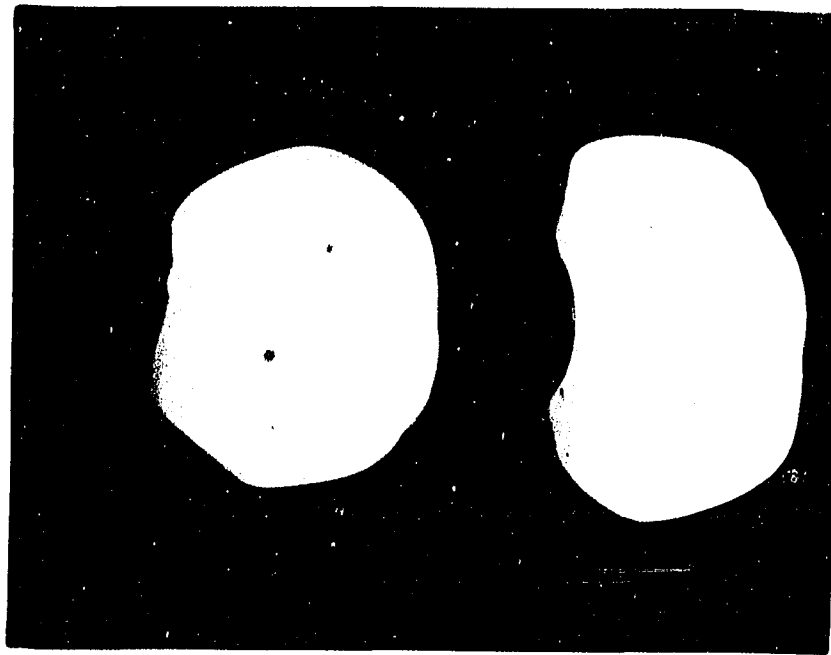
A B

Figure 4.4. Exceptional spotted types obtained in test cross progenies
of 2-3b tl (Table 4.12)

A. 1a-b tl

B. 1-2a-b tl

Table 4.12. Derivation of exceptional phenotypes in the test cross progeny of 2-3b tl spotted kernels (Figure 4.2C, 1980 1343)

$\dfrac{\text{a-m-1 5719A-1}}{\text{a}}$ $\dfrac{\text{Sh2,}}{\text{sh2}}$ $\underline{\text{En}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$ 2-3b tl X cl,sh	Round						Shrunken
	Spotted		Pales			Color-less
	2-3b tl	Exceptions	t7	t4-5	t2-3	
0 1343-17 X 1419	41	1(1a-b t1)	23	17	--	90
-18 X 2159	91	5*(1a-b t1)	--	28	54	182
-19 X 2151	74	--	--	48	23	130
-20 X 1419	93	--	38	33	26	179
-22 X 1419	33	4**(1-2a-b t1)	43	--	--	59
0 1344-1 X 1419	74	1(1a-b t1)	38	26	3	129*
1981 rows containing selected kernels[a] ⟹	(1323A*) (1324**)					(1322C*)

[a]Selected kernels and their assigned row numbers in a column are identified by the same superscript.

Table 4.13. Tests of 1-2a-b tl spotted exceptional phenotypes obtained in the test cross progeny of 2-3b tl spotted kernels (from Table 4.12)

$\dfrac{\text{a-m-1 5719A-1 Sh2, En}}{\text{a} \qquad\qquad \text{sh2}}$ x $\dfrac{\text{a sh2}}{\text{a sh2}}$		Round					Shrunken
		Spotted		Colored	Pale	Color-less	Color-less
1-2a-b tl	X cl,sh	1a tl	1a t6	t7	t4-5	tl	
a) Progeny test							
1 1324-1	X 0529	57	5	40	45	53	215
-2	X 1218	96	1	104	--	4	229
-3	X 0529	76	-	78	--	--	1/2
-4	X 1216	63	1	83	--	7	1/2

$\dfrac{\text{a-m(r) Sh2}}{\text{a-m-1 sh2}}$ x $\dfrac{\text{a-m-1 5719A-1 Sh2, En}}{\text{a} \qquad\qquad \text{sh2}}$	Spotted—round	Spotted—shrunken

b) Test for type of En in 1-2a-b tl spotted kernels

1258	X 1324-1	1-2a-b tl	very few (mostly colorless)
1259	X 1324-3	1-2a-b tl	very few (mostly colorless)

$\dfrac{\text{a-m(r) Sh2}}{\text{a-m-1 sh2}}$ x $\dfrac{\text{a sh2}}{\text{a sh2}}$, En sibs	Spotted—round	Spotted—shrunken

c) Test for type of En in colorless-shrunken sibs (Table 4.12, 1 1322C)

1258	X 1322C-6	2-3b-c tl	2-3b-c t-1
1258	X 1322C-9	2-3b-c tl	2-3b-c t-1

Table 4.14. Tests of la-b tl spotted exceptional phenotypes obtained in the test cross progenies of 2-3b tl spotted (from Table 4.12)

$\frac{a-m-1\ 5719A-1\ \underline{Sh2}}{\underline{a}\qquad \underline{sh2}}$, $\underline{En}$ X $\frac{a\ \underline{sh2}}{a\ \underline{sh2}}$		Round				Shrunken
		Spotted	Pales		Color-less	Color-less
la-b tl	X cl,sh	1-2a-b tl	t7	t4-5	tl	

a) Progeny test

1 1323A-2	X 1354	90	75	35	6	1/2
-4	X 1353	91	63	37	4	1/2
-20	X 1353	80	53	23	2	1/2
-21	X 1354	75	47	28	1	1/2

$\frac{a-m(r)\ \underline{Sh2}}{a-m-1\ \underline{sh2}}$ X 1323

Spotted—round   Spotted—shrunken

b) Test for type of En in la-b tl spotted kernels

1259	X 1323A-2	1-2a-b tl	very few
1258	X 1323A-4	1-2a-b tl	very few

(b)   1a-b t1 (1 1323A)

The progeny tests (Table 4.14a) and the tests on <u>a-m(r)/a-m-1</u>

tester (Table 4.14b) indicate that the 1a-b t1 represents a change in

the state of <u>En</u> because the basic allele phenotype (variable pales)

is unchanged from the parental source (Table 4.12). In the progeny

tests, however, the spot frequency of progeny kernels is slightly

higher (1-2a-b t1) than that (1a-b t1) of the parental kernels.

Similar discrepancy between the parental and the progeny spotted pat-

terns is noticed with 1-2a-b t1 exceptional phenotype in the preceding

section (Table 4.13a) indicating that these discrepancies are due to

environment and therefore the 1-2a-b t1 (1 1324) and 1a-b t1 (1 1323A)

exceptions represent one and the same change in <u>En</u>.

B.   <u>a-m-1 5996-4</u> State

The original <u>a-m-1 5996-4</u> state is colorless (t1) in the absence

of <u>Spm</u>. In the presence of <u>Spm</u>, this state is 4a, c-d t1 spotted

(Figure 4.5, note two distinct types of spots—a, c-d).

1.   Derivation of Variable Pale Types from the
Colorless (t1) Basic Allele Phenotype

The colorless (t1) basic allele was crossed by <u>a sh En</u>. Progeny

consisted of 4a, c-d t1 spotted, colorless (t1), and a few exceptions

(Figure 4.6, 1979 3215 progeny). In test crosses, the 4a, c-d t1

spotted, in turn, yielded varied progeny (Table 4.15). In all the four

progenies, the -<u>En</u> type is not the expected t1 but it is t2-3 pale.

Some of the progenies also contained a few t1, t4-5 and t6-7. The +<u>En</u>
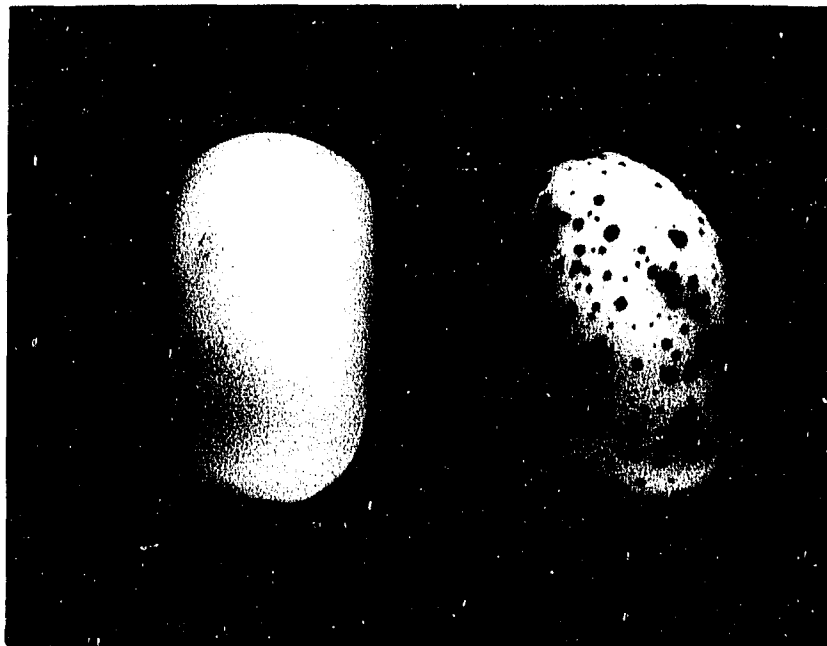
A   B

Figure 4.5. Original state of <u>a-m-1 5996-4</u>

A. Colorless (t1) without <u>Spm</u>

B. 4a,c-d t1 spotted with <u>Spm</u> (note two distinct types
    of spots—a,c-d)

Original   McCl 8618A⑤ I/8680 ⑪ (<u>a-m-1</u> 5996-4 <u>Sh2/a</u> <u>sh2</u>)   (McClintock, 1968)

x <u>a</u> <u>sh</u>

1976   1604
        (4a,c-d t1)

x <u>a</u> <u>sh</u>

<u>t1</u>, 4a,c-d t1, exceptions

1979   3215
        (t1)

x <u>a</u> <u>sh</u> <u>En</u>

t1, 4a,c-d t1, exceptions

1980   1401
        (4a,c-d t1)

x <u>a</u> <u>sh</u>

4a,c-d t1,   t1,   t2-7 (mostly t2-3),   exceptions

1981   fewer spotted   more spotted   1339E   1338D   1338B   1336C   1336D   1338A
        than expected   than expected   (t2-3)   (t4-5)   (t6-7)   (1c t2) (3b t2) (1-2a t1-2)
        1338C   1337F
        (4a,c-d t1)   (4a,c-d t1)
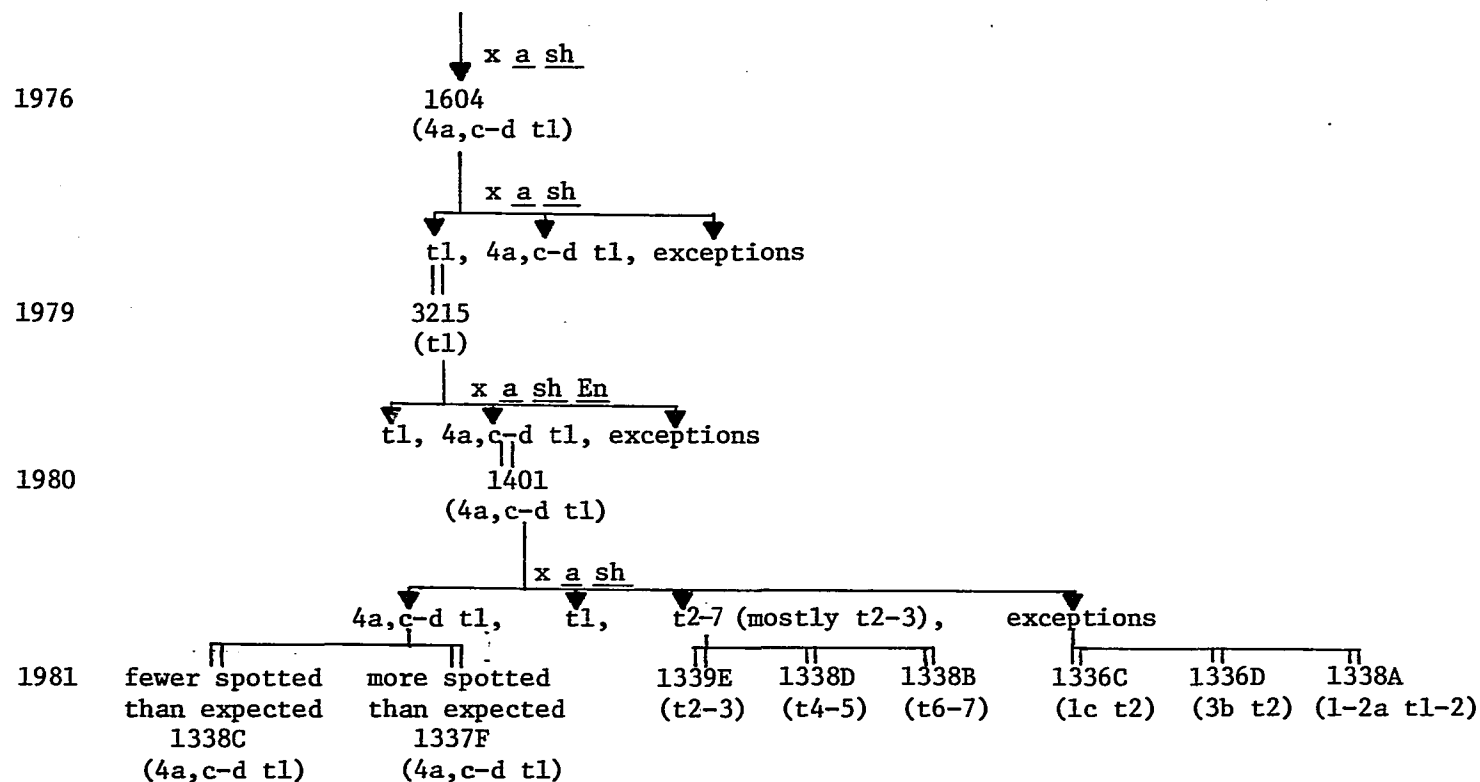
Figure 4.6.   Flow diagram illustrating different derivatives from the original source of
              <u>a-m-1</u> 5996-4 state

Table 4.15. Test cross progeny derivatives of 4a,c-d tl spotted kernels (Figure 4.6, 1980 1401)

$\dfrac{\text{a-m-1 } 5996\text{-}4}{\text{a}}$ $\dfrac{\text{Sh2,}}{\text{sh2}}$ En X $\dfrac{\text{a sh2}}{\text{a sh2}}$ 4a,c-d tl	X cl,sh	Round Spotted 4a,c-d tl	Exceptions	Pales t6-7	t4-5	t2-3	Color-less tl	% spotted	$\chi^2_{1:1}$ a	Shrunken Color-less
0 1401-2	X 1420	5*	2*(1-2a tl-2)	2*	5*	149*	8	4.1	S	168
-20	X 1420	38	1(1-2d t2)	--	--	150	17	18.9	S	195
-21	X 1420	26	1(2d t2)	--	10	136	4	15.2	S	171
-22	X 1431	138**	2**(3b t2 (mottled bkg)	--	10 (mottled bkg)	88 (mottled bkg)	--	59.5	S	225*

1***(1c t2 (mottled bkg)

3(4-5c-d t3)·

| 1981 rows containing selected kernels[b] ⟹ | (1338C*) (1337F**) | (1338A*) (1336D**) (1336C***) | (1338B*) | (1338D*) | (1339E*) | | | | | (1341G*) |

[a]$\chi^2_{1:1}$ – test for 1:1 spotted to non-spotted; S is significant at 0.05 probability level.

[b]Selected kernels and their assigned row numbers in a column are identified by the same superscript.

type, however, is represented by the parental type spotted (4a,c-d t1).
But three of the four progenies have fewer and the remaining one (Table
4.15 0 1401-22 X 1431) has more than the expected (based on independent
En) number of spotted kernels. There are, in addition, some exceptional
spotted derivatives.

It is important to note that the variable pale types (t2-3, t4-5
and t6-7) were not present in the progeny of original colorless basic
allele (t1) X a sh En cross (Figure 4.6, 1979 3215). But their appear-
ance in the test cross progenies of spotted kernels (Figure 4.6, 1980
1401) indicates that the presence of En is necessary to elevate the pig-
ment level (mostly to t2-3 pale type).

a. Response of pale types to En   The different pale types
(t2-3, t4-5 and t6-7) obtained in the test cross progeny of 4a,c-d t1
spotted (Table 4.15, 0 1401-2 X 1420) are tested with the En in a sh
sibs. If differences in response to En are observed, then it could
be deduced that changes in the state of the a-m-1 5996-4 allele
occurred.

1) Response of t2-3 pales to En   When the t2-3 pales
are crossed with the parental En (in a sh sibs), spotted (4-5b-c t1-3)
and variable pale types (t6-7, t4-5 in addition to the parental t2-3)
appear in the progeny (Table 4.16a). These phenotypes are distinctly
different from that of the original state (4a,c-d t1 and t1), indicat-
ing that the t2-3 pales represent a change in state of the a-m-1 5996-4
allele.

Table 4.16. Response of light-pales (t2-3) to $\underline{En}$; light pale kernels selected among test cross progenies of 4a,c-d tl spotted kernels (from Table 4.15)

$\dfrac{\text{a-m-1 5996-4 Sh2}}{\text{a} \quad \text{sh2}}$ X $\dfrac{\text{a sh2, En}}{\text{a sh2}}$		Round						
		Spotted	Variable pales			Color-less	% spotted	$\chi^2_{1:1}$ a
t2-3	X cl,sh	4-5b-c tl-3	t6-7	t4-5	t2-3	tl		
a) Response of light-pales (t2-3) to $\underline{En}$								
1 1339E-1	X 1341G-1 +[b]	79	22	64	23	--	42.0	S
-3	X 1341G-1 +	76	--	55	27	--	48.1	NS
-5	X 1341G-4 +	65	25	59	40	--	34.4	S
-7t	X 1341G-5 +	35	--	2	42	--	44.3	NS

$\dfrac{\text{a-m(r) Sh2}}{\text{a-m(r) Sh2}}$ X $\dfrac{\text{a-m-1 5996-4 Sh2}}{\text{a} \qquad \text{sh2}}$

b) Test for the presence of $\underline{En}$ in light-pales (t2-3)								
1 1247 X 1339E-1	--	--	--	--	all tl-2	--
1247 X -3	--	--	--	--	all tl-2	--
1247 X -7	--	--	--	--	all tl-2	--

$^a\chi^2_{1:1}$ – test for 1:1 spotted to non-spotted; S is significant at 0.05 probability level and NS is not significant.

^{b}Presence of $\underline{En}$ confirmed in crosses with $\underline{\text{a-m(r)}}/\underline{\text{a-m-1}}$.

The t2-3 pales do not give spotted when tested on a-m(r)/a-m(r)

confirming that they do not contain En (Table 4.16b). In this test,

the progeny (without En) include only colorless to very light pale

(t1-2) in contrast to different -En pale types (t2-3, t4-5, t6-7)

in crosses with a sh En sibs (Table 4.16a). These differences in

pigmentation of -En progeny can be ascribed to the presence of some

unknown factors in a-m-1 5996-4 cultures. Also, the same factors

could be responsible for a different response (4-5b-c t1-3) of t2-3

pales to En (Table 4.16a) from that (4a, c-d t1) of the original

colorless (t1) basic allele of a-m-1 5996-4. Alternatively, the vari-

able pigmentation of the progeny and a change in response to En could

be attributed to changes in state of the a-m-1 5996-4 allele itself.

Judging from the ratios (Table 4.16a) of spotted to variable pale

(t2-3, t4-5 and t6-7) progenies (fewer spotted than expected), some of

the pales may represent changes to non-responsive type.

2) Response of pales (t4-5) to En   The t4-5 pales repre-

sent another phenotypic class of variable progeny obtained in test crosses

of 4a, c-d t1 spotted kernels (Figure 4.6, 1980 and Table 4.15).

There are only a few kernels of this phenotype in the progeny. In

crosses with a sh sibs with En, these pales yielded 3-4c t1 spotted

and t1-4 variable progeny (Table 4.17a and Figure 4.7). In contrast to

the parental phenotype (t4-5), the t1-4 variable progeny in the crosses

are lighter pigmented. This is also in contrast to the tests of the

t2-3 pale sib derivatives (Table 4.16a) where many of the variable

progeny kernels are darker (t4-5 and t6-7) than the parental phenotype

Figure 4.7. Progeny ear of a cross a-m-1 5996-4 Sh2/a sh2 (t4-5 pale)
X a sh2/a sh2, En sibs showing 3-4c t1 spotted and t1-4
· variable progeny (Table 4.17, 1 1338D-1 X 1341G-5)

Table 4.17. Response of and tests for En among pales (t4-5); pale kernels selected among test cross progenies of 4a,c-d t1 spotted kernels (from Table 4.15)

$\frac{\text{a-m-1 5996-4 Sh2}}{\text{a}}$ X $\frac{\text{a sh2, En}}{\text{a sh2}}$		Round			% spotted	$\chi^2_{1:1}$ [a]
		Spotted	Variable	pales		
t4-5	X cl,sh	3-4c t-1	t6-7	t1-4		
a) Response of pales (t4-5) to En						
1 1338D-1	X 1341G-5 +[b]	56	--	108	34.1	S
-2	X 1341G-1 +	42	--	57	42.4	S
-20	X 1341G-4 +	17	--	69	19.7	S
Reciprocal crosses						
1 1341G-6 ?[c]	X 1338D-1	6	2	156	3.6	S
-3 -[d]	X 1338D-2t	--	--	159	--	S

$\frac{\text{a-m(r) Sh2}}{\text{a-m(r) Sh2}}$ X $\frac{\text{a-m-1 5996-4 Sh2}}{\text{a}}$ $\frac{}{\text{sh2}}$

b) Test for the presence of En in pales (t4-5)

1 1247 X 1338D-1	--	--	all t1-2	--
1246 X -2	--	--	all t1-2	--

[a] $\chi^2_{1:1}$ – test for 1:1 spotted to non-spotted; S is significant at 0.05 probability level.

[b] Presence of En confirmed in crosses with a-m(r)/a-m-1.

[c] Presence of En is not confirmed.

[d] Absence of En; confirmed as the + cases.

(t2-3).

In crosses with <u>a-m(r)/a-m(r)</u>, the t4-5 pales produced no spotted

progeny confirming the absence of <u>En</u> (Table 4.17b). The progeny kernels

are colorless to very light pale (t1-2) as compared to t4-5 parental

phenotype and also in contrast to the variable progeny (t1-4) of

t4-5 X <u>a</u> <u>sh</u> <u>En</u> sibs (Table 4.17a). These differences again can be

attributed to some unknown factors, or to changes in the allele itself.

     3) <u>Response of dark pales (t6-7) to En</u>   One of the two

dark-pale (t6-7) kernels (Table 4.15, 0 1401-2 X 1420) was tested for

response to <u>En</u> in <u>a</u> <u>sh</u> sibs (Table 4.18a). Several ears were obtained

from one straight cross and several reciprocal crosses. The progeny

do not include the original spotted type (4a, c-d t1) and in addition,

there was considerable variation among the spotted patterns in both

spot size and frequency (3-4c, 3-4a-b, 4-5b-c) (Table 4.18a, Figure

4.8). Also, the spots on some of the progeny kernels are not uniformly

distributed (Figure 4.8 arrow). The basic allele phenotypes (-<u>En</u> types)

in the progeny are lighter pigmented than the parental type (t6-7).

These include colorless (t1), t2-3 and t4-5 pales. This same range of

variable pigmentation occurred among the progeny of t4-5 pales (Table

4.17).

The crosses of the selected dark-pales (t6-7) with the <u>a-m(r)/a-m(r)</u>

tester yielded only colorless to very light pale (t1-2) progeny (Table

4.18b) again illustrating the non-heritability of the dark selections.

The absence of spotted kernels in these tests confirms the absence of

<u>En</u>. The presence of only colorless to very light pale (t1-2) progeny

135

Figure 4.8. Progeny ear of a cross a-m-1 5996-4 Sh2/a sh2 (t6-7) X
a sh2/a sh2, En sibs containing variable spotted kernels.
Note some kernels with non-uniformly distributed spots
(arrow)

Table 4.18. Response of dark-pales (t6-7) to $\underline{En}$; dark-pales selected among test cross progenies of 4a,c-d tl spotted kernels (from Table 4.15)

$\dfrac{\text{a-m-1 } 5996\text{-}4}{a} \dfrac{Sh2}{sh2}$ X $\dfrac{a \; sh2}{a \; sh2}$, $\underline{En}$		Round			% spotted	$\chi^2_{1:1}{}^{a}$
	Spotted	Pales		Colorless		
t6-7 X cl,sh		$\underline{t4\text{-}5}$	$\underline{t2\text{-}3}$	$\underline{tl}$		
a) Response of dark-pales (t6-7) to $\underline{En}$						
1 1338B-t X 1341G-9 +[b]	81(3-4c t1)	23	90	30	36.1	S
Reciprocal crosses						
1 1341G-2 + X 1338B	15(3-4a-b tl)	6	87	30	10.8	S
-5 + X 1338B	26(3-4a-b tl)	18	120	29	13.5	S
-15 + X 1338B	11(3-4a-b tl)	6	73	14	10.6	S
-1 + X 1338B-t	110(4-5b-c tl)	--	45	44	55.3	NS

$$\dfrac{\text{a-m(r) } Sh2}{\text{a-m(r) } Sh2} \; X \; \dfrac{\text{a-m-1 } 5996\text{-}4}{a} \dfrac{Sh2}{sh2}$$

b) Test for the presence of $\underline{En}$ in dark-pales (t6-7)						
1 1247 X 1338B	--	--	--	all tl-2		
1238 X 1338B-t	--	--	--	all tl-2		

$^{a}\chi^2_{1:1}$ - test for 1:1 spotted to non-spotted; S - significant at 0.05 probability level and NS - not significant.

[b]Presence of $\underline{En}$ confirmed on $\underline{\text{a-m(r)}}/\underline{\text{a-m-1}}$.

kernels is in contrast to the variable pale progeny in crosses with a sh sibs with En (Table 4.18a).

In summarizing the results, it is clear that there is a similarity of the En-induced spotting patterns of the t4-5 and t6-7 pales (3-4c t1) as well as the range of pigmentation among the -En kernels (t1 to t4-5). The t2-3 pales (Table 4.16) on the other hand show a heavier spotting pattern (4-5b-c t1-3) and darker pigmented basic allele types (t2-3, t4-5 and t6-7). These confirmed differences from the parental type authenticate these selections as true changes in state of the original a-m-1 5996-4 allele.

In addition to the confirmation of changes in state, it is important to note that the ratios of spotted (+En) to non-spotted (-En types--all the variable pales together) progenies in t2-3, t4-5 and t6-7 X a sh En sib crosses (Tables 4.16, 4.17, 4.18) fit 1:1 (expected on the basis of independent En) only in a few or none of the progenies. The spotted are fewer than the non-spotted in most of the progenies. The fewer spotted also occurred in parental progeny (Table 4.15, 0 1401-2 X 1420) from which the t2-3, t4-5 and t6-7 pale types were selected for En-response study indicating that the property of fewer spotted is inherited through the pale types. The fewer spotted property is heritable despite that the a sh En sibs used in crosses with the pale types were derived in the progeny (Table 4.15, 0 1401-22 X 1431) with more than the expected (with an independent En) number of spotted kernels.

2. Heritability of fewer and more than the
    expected number of spotted progeny

The heritability of fewer and more than the expected spotted is

further investigated by selecting the spotted kernels from two test

cross progenies (Table 4.15)--one with fewer than the expected

(O 1401-2 X 1420) and the other with more than the expected

(O 1401-22 X 1431) number of spotted kernels.

When the spotted progeny kernels representing fewer than the ex-

pected (Table 4.15, O 1401-2 X 1420) were selected and test crossed,

fewer than the expected number of spotted kernels are seen in the

progenies (Table 4.19 and Figure 4.9). In addition, there are

several relevant features. Reciprocal crosses (versus straight crosses)

(1 1220 X 1338C-1t) and crosses with tillers (versus main plant)

(1 1338C-3t X 1228) gave higher frequency of spotted kernels among the

progeny. In one case, the main ear (1 1338C-1 X 1220) was completely

devoid of spotted kernels, while the tiller of the same plant yielded

2.4% spotted in straight cross (1 1338C-1t X 1227) and 24.7% spotted

in the reciprocal cross (1 1220 X 1338C-1t).

Secondly, the spotted kernels differ in spotting patterns (both in

size and frequency) among and within the test cross progenies (Table

4.19). In general, the spot size is smaller (finer) than the parental

kernels (c-d). These results show that the spotting patterns are not

consistent among test cross progenies and between these progenies and

the parental kernels.

Another aspect that is unique in one of the crosses (1 1338C-3t X

1228) is that the spotted kernels are localized in one area on the ear
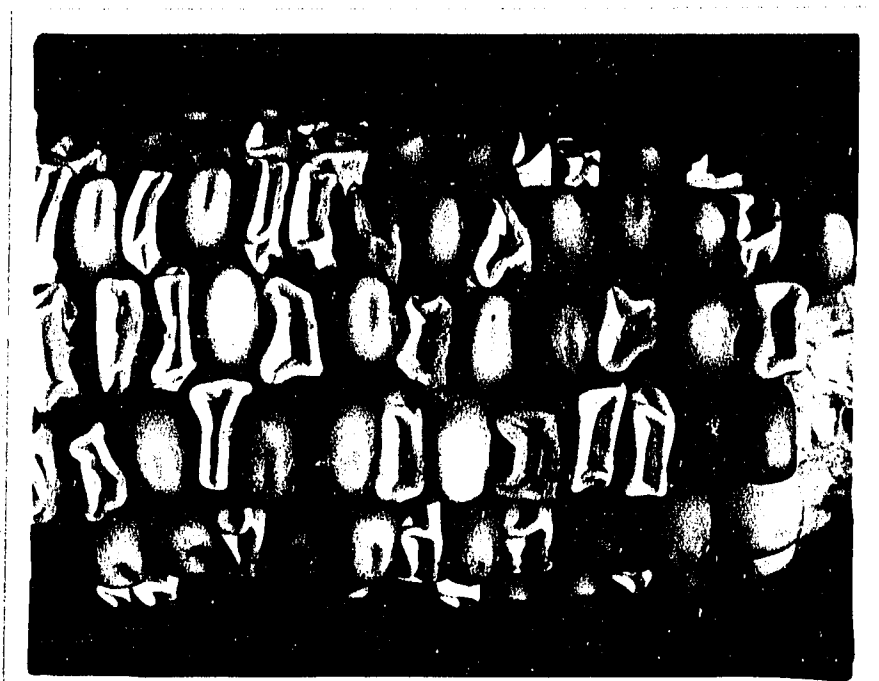
Figure 4.9. Test cross ear of 4a,c-d t1 spotted kernel (Table 4.19,
1 1338C-3 X 0529) showing the occurrence of only one
spotted (5a-c t1) kernel

Table 4.19. Heritability of 4a,c-d tl spotted kernels selected among test cross progenies with fewer than expected spotted kernels (from Table 4.15, 0 1401-2 X 1420)

$\underline{\text{a-m-1 5996-4 Sh2, En}}$ X $\underline{\text{a sh2}}$ $\underline{\text{a sh2}}$		Round						
$\underline{\text{a}}$ $\underline{\text{sh2}}$		Spotted	Pales			Color-less	%spotted	$\chi^2_{1:1}$ [a]
4a,c-d tl	X cl,sh		$\underline{\text{t6-7}}$	$\underline{\text{t4-5}}$	$\underline{\text{t2-3}}$	$\underline{\text{tl}}$		
1 1338C-1	X 1220	--	12	41	79	--	--	--
-1t	X 1227	2(4a-b tl) 1(3a tl) 1(2a t2)	--	30	132	--	2.4	S
-2	X 0533	1(3c tl)	--	--	4	186	0.50	S
-3	X 0529	1(5a-c tl) ·	--	9	--	163	0.57	S
-3t	X 1228	39 3c tl c̄ 3a specks	--	5	166	--	18.57	S
Reciprocal crosses								
1 1220	X 1338C-1t	33(3-4b-c tl) 1(1-2a-b tl)	--	--	--	103	24.8	S
1220	X 1338C-3t	71(3-4b-c tl)	--	--	--	116	37.96	S

[a] $\chi^2_{1:1}$ - test for 1:1 spotted to nonspotted; S is significant at 0.05 probability level.

Figure 4.10.   Two sides (A, B) of a single test cross ear of 4a,c-d t1
               spotted kernel (Table 4.19, 1 1338C-3t X 1228) showing
               the localization of spotted kernels on one side (B) of
               the ear

A
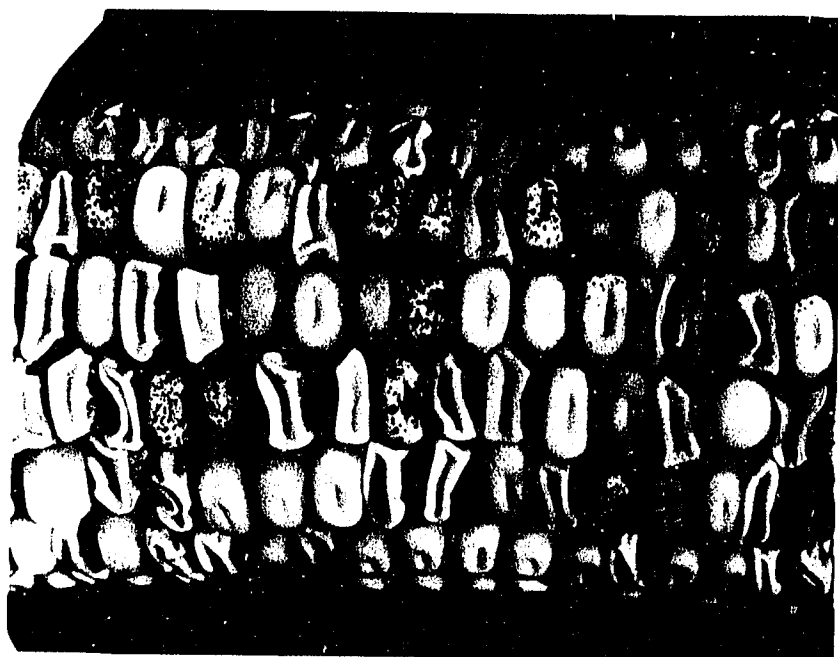
B

(Figure 4.10A). This suggests that the En is lost or changed in phase

of activity early in the development of the ear so that the kernels on

one side of the ear receive an active En whereas on the other side have

no En or receive an inactive En (Figure 4.10B).

The loss or change in phase of En would also explain the occur-

rence of fewer than the expected number of spotted kernels. An addi-

tional constraint in this case is that the loss or change in phase of

activity must occur prior to ear development.

3. Heritability of more than the expected
   number of spotted progeny

The cross 0 1401-22 X 1431 (Table 4.15) gave more than the expected

number of spotted kernels. When these spotted kernels were test

crossed, each of the progenies included more than the expected number

of spotted kernels (Table 4.20). Chi-square tests show that the ratio

of spotted to non-spotted kernels fit the expected 1:1 (independent En)

in only one of the crosses (1 1337 F-10 X 1226 and the reciprocal

1 1219 X 1337 F-10). In the other crosses, the spotted progeny com-

prised more than half of the total round kernels. When tested for 3:1

(2 independent En), the spotted to non-spotted fit the ratio in only

one of the reciprocal crosses (1 1219 X 1337 F-9). Since in the other

crosses the spotted kernels vary from 63.8% (1 1337 F-21 X 1228) to

84.6% (1 1337 F-20 X 1226), it is more likely that the En is linked to

the a-m-1 allele at varying distances on the chromosome. Because of

this possible linkage, more spotted than non-spotted would be seen in

successive test cross progenies of spotted kernels (Tables 4.15, 4.20).

Table 4.20. Heritability of 4a,c-d tl spotted kernels selected among test cross progenies with more than expected number of spotted kernels (from Table 4.15, 0 1401-22 X 1431)

| a-m-1 5996-4 Sh2, En X a sh2 | | | Round | | | | | |
a ___ sh2 ___ 4a,c-d tl	a sh2 ___ a sh2 ___ X cl,sh		Spotted 4-5a-c tl	Colorless- light pale tl-3	Exceptions	% spotted	$\chi^2_{1:1}$ [a]	$\chi^2_{3:1}$ [b]
1 1337F-2	X 1227		--	150	--	--	--	--
-9	X 1227		192	40	--	82.7	S	S
-10	X 1226		57	52	--	52.3	NS	S
-20	X 1226		182	33	--	84.6	S	S
-21	X 1228		127	72	--	63.8	S	S
Reciprocal crosses								
			3-4c tl-2	tl-2	--			
1 1220	X 1337F-2		--	1/2	--	--		
1219	X	-9	174	49	1(t7)	77.7	S	NS
1219	X	-10	33	34	--	50.0	NS	S
			1(5c-d tl)					

[a] $\chi^2_{1:1}$ - test for 1:1 spotted to non-spotted.

[b] $\chi^2_{3:1}$ - test for 3:1 spotted to non-spotted; S is significant at 0.05 probability level and NS is not significant.

In summary, both the fewer and more than the expected number of spotted progeny kernels are heritable, but for different reasons. Those progenies with fewer than the expected number of spotted kernels could be caused by a loss or change in phase of activity of En. But, since the fewer spotted than expected were also derived in crosses between different pale types (selected from progeny with fewer than expected spotted, Table 4.15), and a sh En sibs (selected from progeny with more than expected progeny, Table 4.15) (Tables 4.16, 4.17 and 4.18), this property of fewer spotted than expected is more likely caused by a lack of response of a-m-1 5996-4 to En. This lack of response either represents a change in state of the a-m-1 allele (non-responsive state) or is caused by some unknown factors blocking the allele's response to En or En expression itself.

The property of more than the expected number of spotted kernels among progenies can be due to the linkage of En to a-m-1 5996-4 allele. This can be distinguished from another possibility such as one independent En and one linked En by testing the a sh sibs for the occurrence of En.

4. Tests of exceptional spotted phenotypes

Among the test cross progenies of the parental 4a, c-d t1 spotted kernels (Table 4.15), five distinguishable exceptional spotted phenotypes were recovered. Three of these exceptional types were selected and they include: (1) 1c t2 mottled background (1 1336 C), (2) 3b t2 mottled background (1 1336 D), and (3) 1-2a t1-2 (1 1338 A). The t2 background of (1) and (2) exceptions appeared mottled under microscopic

examination.

(a) 1c t2 mottled background (1 1336 C)

Despite a 1c t2 mottled selection, the test cross progenies lacked spotted kernels. Only colorless kernels appeared in the progeny (Table 4.21A) confirming that the 1c t2 spotted phenotype is not heritable. This lack of heritability of a phenotypic selection could be due to several factors. The absence or loss of En from the embryonic cells is one possibility. Alternatively, the non-heritability of the spotted phenotype is that the changed expression could be a residual effect of the parental genotype, namely En on the a-m-1 5996-4 allele and without En, this expression is not propagaged in subsequent generations. The precise nature of this selection can be tested by crossing the colorless progeny with En.

(b) 3b t2 mottled background (1 1336 D)

As in the case of 1 1336 C, the test cross progenies contained only colorless kernels (Table 4.21B) in the straight cross, indicating that the 3b t2 spotted phenotype is not heritable. One of the reciprocal crosses (1 1354 X 1336 D), however, produced many faintly blotched kernels. The explanations for the non-heritability could be the same as described for 1c t2 exception in the preceding section. It will be necessary to test the colorless and faintly blotched kernels for the presence of En and for their response to an introduced En.

(c) 1-2a t1-2 (1 1338 A)

Spotted progenies of 1-2a t1-2 included mostly unexpected 3-4c t1-2 and a few parental type (in reciprocal crosses) (Table 4.21C).

Table 4.21. Tests of exceptional spotted kernels selected among test cross progenies of 4a,c-d t1
spotted kernels (from Table 4.15)

A. 1c t2 mottled background (1 1336C)

$\dfrac{\text{a-m-1 5996-4}}{a} \dfrac{\text{Sh2,}}{\text{sh2}} \dfrac{\text{En}}{} \text{X} \dfrac{\text{a sh2}}{\text{a sh2}}$	Round					Shrunken
	Spotted	Pales			Color-less t1	Color-less
1c t2 X cl,sh		t6-7	t4-5	t2-3		
1 1336C X 1226	--	--	--	--	160	165
Reciprocal crosses						
1 1217 X 1336C-t	--	--	--	--	109	112
1356 X 1336C-t	--	--	--	--	1/2	1/2
1225 X 1336C-t	--	--	--	--	1/2	1/2

B. 3b t2 mottled background (1 1336D)

$\dfrac{\text{a-m-1 5996-4}}{a} \dfrac{\text{Sh2,}}{\text{sh2}} \dfrac{\text{En}}{} \text{X} \dfrac{\text{a sh2}}{\text{a sh2}}$	Spotted	t6-7	t4-5	t2-3	Color-less t1	Color-less
3b t2 X cl,sh						
1 1336D X 1228	--	--	--	--	136	141
Reciprocal crosses						
1 1354 X 1336D	123[a]	--	--	--	34	1/2
1217 X 1336D-t	--	--	--	--	1/2	1/2
1356 X 1336D-t	--	--	--	--	1/2	1/2
1225 X 1336D-t	--	--	--	--	1/2	1/2

[a]Faintly blotched (spotted?).

Table 4.21. (Continued)

C. 1-2a tl-2 (1 1338A)

a-m-1 5996-4 Sh2, En	X a sh2 / a sh2	Round Spotted 3-4c tl-2	1-2a-b tl-2	Pale t6-7	t4-5	t2-3	Color-less tl	% spotted	$\chi^2_{1:1}$ [b]	Shrunken Color-less
a / 1-2a tl-2	X cl,sh									
1 1338A-1	X 0542	1	--	1	14	59	--	1.3	S	1/2
-1t	X 1227	1	--	3	50	88	--	3.4	S	1/2
Reciprocal crosses										
1 1220	X 1338A-1t	36	4	--	--	32	90	22.2	S	1/2
1227	X -1t	14	2	--	--	40	100	10.2	S	1/2

[b] $\chi^2_{1:1}$ - test for 1:1 spotted to non-spotted; S is significant at 0.05 probability level.

Figure 4.11. Test cross ear of 1-2a t1-2 spotted exception (Table
4.21C, 1 1338A-1t X 1227) containing only a few spotted
and variable pale (t2-3 to t6-7) non-spotted kernels

Moreover, the unexpected 3-4c t1-2 spotted are fewer than 50% of round

kernels (much fewer in straight crosses). The non-spotted progeny

ranged from colorless (t1) to light-pale (t2-3) in reciprocal crosses

and from light-pale (t2-3) to dark-pale (t6-7) in straight crosses

(Figure 4.11).

These results show that there are distinct differences in straight

and reciprocal crosses, both in the number of spotted and in the range

of pigmentation of -En progeny. The lighter basic allele phenotype

(-En) is associated with the occurrence of more spotted progeny (Table

4.21C reciprocal crosses) and the darker -En progeny with fewer spotted

(straight crosses). If this association between the number of spotted

progeny and the pigmentation of -En progeny is not coincidental, it

can be concluded that there are some factors that are responsible for

both the pale pigmentation and in the inhibition of En expression. The

same interpretation can be made for the non-heritability of 1-2a t1-2

in straight crosses and very little heritability (due to fewer pale

factors) in reciprocal crosses (Table 4.21C).

C. a-m-1 6078 State

The original state, obtained from Dr. B. McClintock, did not con-

tain Spm. In the absence of Spm, the state is light pale (t2-3)

(Figure 4.12A). In crosses with a sh En, the t2-3 pale yielded t7

colored (Figure 4.12B) with or without colorless areas (4.13 A, B) and

t2-3 pale progeny (Figure 4.14A, 1976, 1977 progeny). The t7 colored

were tested for their heritability and the selfed progeny consisted of
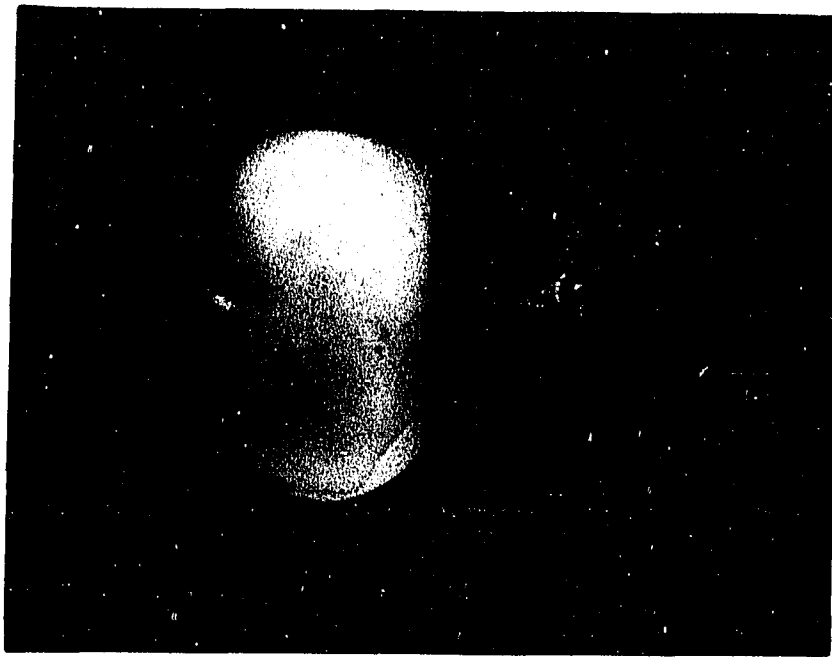
A   B

Figure 4.12. Original state of <u>a-m-1 6078</u>

A. Light-pale (t2-3) without <u>Spm</u>

B. Colored (t7) with <u>En</u>

A B
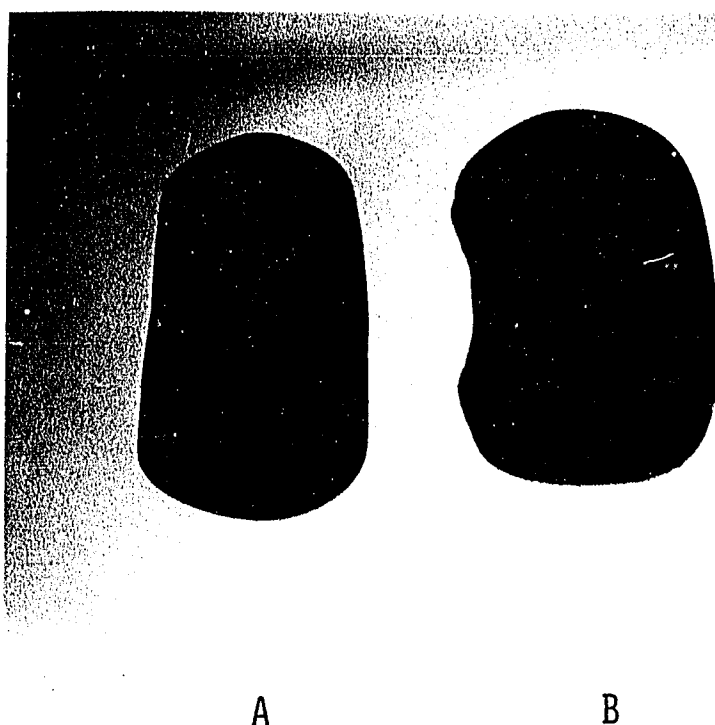
Figure 4.13. Colored phenotype of a-m-1 6078 + En

A. With colorless areas

B. Without colorless areas

Original   McC1 8615A ⑪(a-m-1 5719A-1 Sh2)/McC1 8615A ⑭(a-m-1 6078 Sh2)

x a sh

t2-3 (a-m-1 6078)   t7 (a-m-1 5719A-1)

1976   1601-2t
(t2-3)

x a sh En

t2-3, t7 c̄ or c̄ out colorless areas

1977   0957-7
(t2-3)

x a sh En

t2-3, t7 c̄ or c̄ out colorless areas

1979   1646
(t7)

⊗

t7 c̄ or c̄ out cl areas   variable spotted   t3-4   t1

1980   1307 1306   1310   1309   1308

1981   1121   1122   1123

x a sh   x a sh

t3-4, t1 (in two crosses) t1
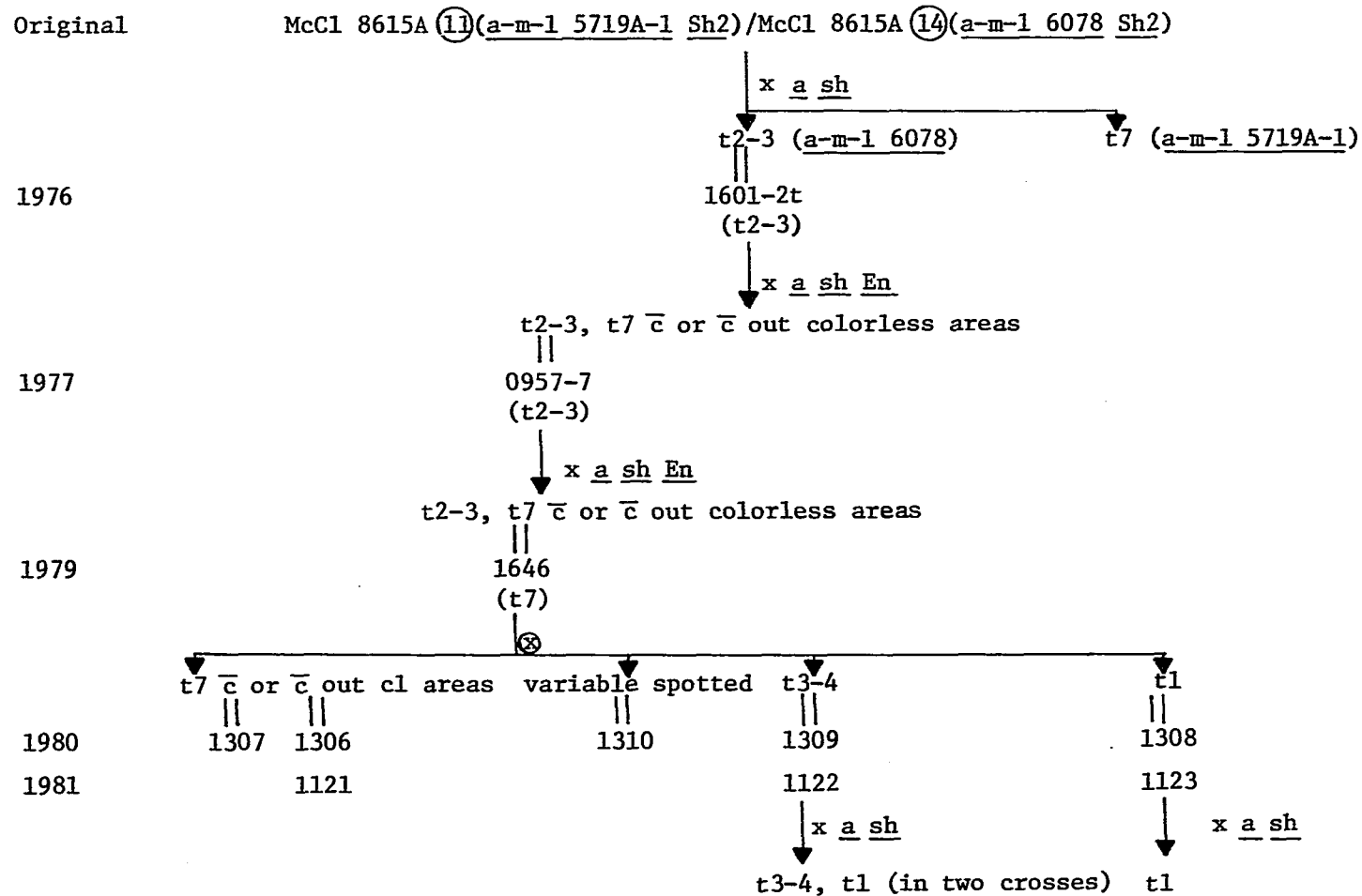
A

Figure 4.14. Flow diagram showing different derivatives from the original source of
a-m-1 6078 state

(from Figure 4.14A)

1980　　　1307
(t7 c̄ cl areas)

x a sh

t7 c̄ or c̄ out　　variable　　t2-3,　　t1
cl areas,　　spotted,
(1a-5c⁺)

1981　　　　　　　　　　　　　　　　　　1105A

B

(from Figure 4.14A)

1980　　　1306
(t7 c̄ out cl areas)

x a sh

t7 c̄ or c̄ out　　variable　　t2-3　t1
cl areas,　　spotted,
(1a-5c⁺)

1981　　　　　　　　　　　　　　　　1102E

C

(from Figure 4.14A)

1980　　　1310
(variable spotted)

1310W　　　　　　　　1310X　　　　　　　　1310Y
(1a-b t1)　　　　　　(3c t1)　　　　　　　(3b t3)

x a sh　　　　　　　　x a sh　　　　　　　x a sh

1a-b t1, t1　　3c t1 c̄　colored,　3b-c t1　t2-3,　　1-2a t1,　t2-3,　t1
specks,　(t7)　speckled　t4-5
spotted,

1981　　1109　　　　1112　　1110　　1114　　1113A　　1115

D

Figure 4.14.　(Continued)

the parental type (t7 with or without colorless areas), variable

spotted, light to medium pale (t3-4) and colorless (t1) kernels (Table

4.22). Each of these types were further tested to answer the following

questions:

(1) Do the colored kernels represent the phenotype of a-m-1 6078 + En?

(2) What is the heritability of colored with and without colorless

   areas?

(3) Do the variable spotted also represent the state a-m-1 6078 + En?

   How are they related to the colored kernels in terms of their

   heritability?

(4) Are the light to medium pale (t3-4) similar in behavior to the

   original light-pale (t2-3) a-m-1 6078 - En?

(5) Do the colorless kernels represent a change in state of the

   mutable allele?

1. Heritability and the En content of the
   colored (t7) kernels

   There are two types of colored kernels--those with and those without

colorless areas (Figure 4.13). Colorless areas are often not visible
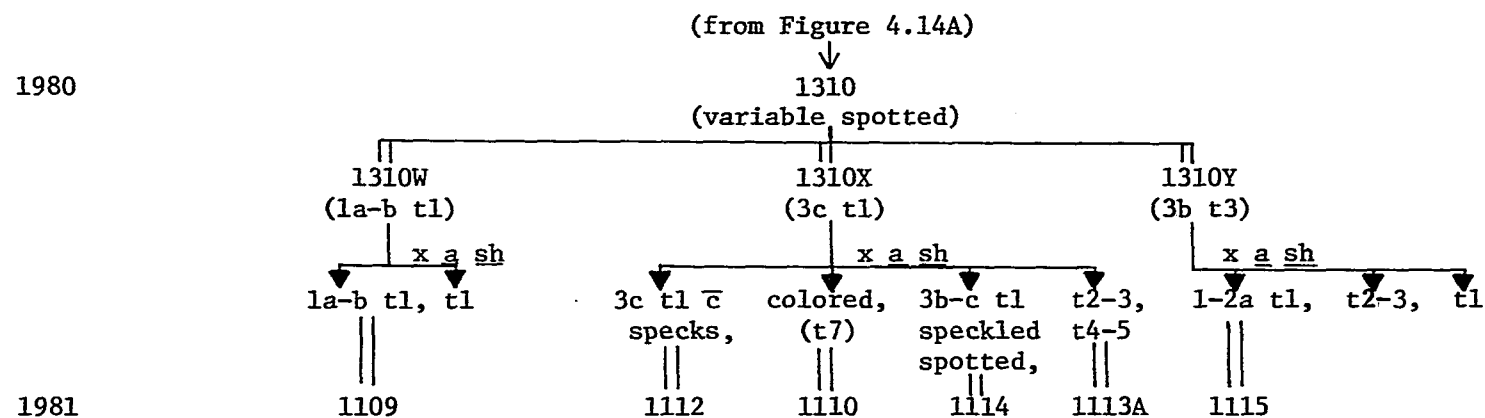
to the naked eye. These two types of colored kernels are distinguished

with the aid of a dissecting microscope.

   a. Colored (t7) kernels with colorless areas (Figure 4.14B, 1980

1307)   The test cross progenies include colored (t7) with and without

colorless areas, spotted, light-pales (t2-3) and colorless (t1)

kernels (Table 4.23). Though the colored (t7) progeny are present in

all the progenies, only three out of five have t7 types with colorless

Table 4.22. Heritability of colored (t7) with or without colorless areas (Figure 4.14A, 1979 1646)

a-m-1 6078 Sh2, En ⊗ a sh2 t7 c̄ or c̄ out cl areas	Round				Shrunken
	Colored (t7) c̄ and c̄ out cl areas	Spotted (variable) $1a-5c^+$	Pales $t3-4$	Colorless $t1$	Color- less $t1$
9 1646-10 ⊗	178	$3(1.3)^a$	26	$19(8.4)^a$	1/4
−11 ⊗	275	10(2.8)	50	18(5.0)	1/4
−12 ⊗	212*	10*(3.4)	48*	21*(7.2)	1/4
−13 ⊗	210	12(4.4)	37	15(5.4)	1/4
−14 ⊗	195	10(3.8)	40	15(5.8)	1/4
−15 ⊗	166	6(2.9)	−−	33(16.1)	1/4
1980 rows with selected kernels[b] ⟹	(1306*) (1307*)	(1310*)	(1309*)	(1308*)	
1981 rows with selected kernels[b] ⟹	(1121*)		(1122*)	(1123*)	

[a] Numbers in brackets are the percentages of respective phenotypes among non-shrunken progeny kernels.

[b] Selected kernels and their assigned row numbers in a column are identified by the same superscript.

Table 4.23. Heritability of colored (t7) with colorless areas (Figure 4.14B, 1980 1307)

$\dfrac{\text{a-m-1 6078 Sh2}}{\text{a-m-1 6078 Sh2/a sh2}}$, En X $\dfrac{\text{a sh2}}{\text{a sh2}}$		Round							Shrunken
		Colored (t7)		Spotted	Pale	Color-less			Color-less
t7 $\bar{c}$ cl areas	X cl,sh	$\bar{c}$ cl areas	$\bar{c}$ out cl areas	variable $(1a-5c^+)$	t2-3	t1	$\chi^2_{1:1}$ a	$\chi^2_{3:1}$ b	
0 1307-20	X 1420	15	46	—	57	10	NS	S	114
-21	X 1420	—	28*	51	25	—	S	NS	93
-22	X 1420	16	66	—	76	6*	NS	S	143*
-23	X 2160	—	43	—	15	64	S	NS	—
-24	X 0510	18	43	—	61	16	NS	S	133
1981 rows with selected kernels^c $\Longrightarrow$						(1105A*)			(1105B*)

$^a\chi^2_{1:1}$ - Test for 1:1 + En (colored + spotted--if present) to -En (t2-3 pale).

$^b\chi^2_{3:1}$ - Test for 3:1 + En (colored + spotted--if present) to -En (t2-3 pale).

^{c}Selected kernels and their assigned row numbers in a column are identified by the same superscript.

areas while all have t7 without colorless areas. Spotted kernels are present in only one of the progenies (0 1307-21 X 1420). The basic allele phenotype (-En) t2-3 pale appeared in all the progenies and in addition, colorless (t1) type appeared in four out of five progenies.

Since colored types appear only after the introduction of En, they must represent the +En expression of the t2-3 state. In three out of five progenies (Table 4.23, 0 1307-20, -22, -24), the colored (+En) to t2-3 pale (-En) ratios fit the expected 1:1 based on the independent segregation of En. In the two other progenies (0 1307-21, 23), the +En to -En types do not fit 1:1 ratio. In 0 1307-21 X 1420, there are spotted in addition to the colored (+En) progeny. The ratio of colored + spotted to t2-3 pale fit an expected 3:1 on the basis of two independent Ens. The 0 1307-23 X 2160 progeny represents an anomaly. While the ratio of colored to pale fit a 3:1 ratio, the t1 represents approximately one-half of the total progeny kernels. Because there are no shrunken progeny, these colorless kernels must represent a change to a non-responding type. The colorless kernels in the other progenies could also represent germinal changes in state that are not responsive to En. Tests confirming the state (responding or non-responding) of colorless kernels are presented in section 4.

These tests with colored kernels containing colorless areas indicate that the +En phenotype of a-m-1 6078 t2-3 state exists in three forms-- colored with colorless areas, colored without colorless areas and spotted. The colored without colorless areas is the most frequently represented phenotype among the progenies. That the colored kernels

Table 4.24. Heritability of colored (t7) without colorless areas (see Figure 4.14C, 1980, 1306; 1981 1121)

$\dfrac{\text{a-m-1 6078 Sh2}}{\text{a-m-1 6078 Sh2/a sh2}}$, En X $\dfrac{\text{a sh2}}{\text{a sh2}}$		Round						Shrunken
		Colored		Spotted	Pale	Color-less		Color-less
t7 $\bar{c}$ out cl areas	X cl,sh	$\bar{c}$ cl areas	$\bar{c}$ out cl areas	variable $1a\text{-}5c^+$	t2-3	t1	$\chi^2_{1:1}$ a	
0 1306-2	X 1409	--	all	--	--	--		--
-21	X 1420	--	38	--	26	--	S	--
1 1121-1	X 1220	33	191	$1(5c^+)$ 27(3-4b-c t1)	11	11	S	--
-3	X 1221	42	112	2(3-4b t1)	111	16	S	--
-4	X 1217	--	all	--	--	--	--	--
-11	X 1930	--	all	--	--	--	--	--
-8	X 1211	35	100	--	80	32	S	--
-10	X 1209	10	67	--	72	14	NS	1/2
-6	X 1222	--	1/2	--	--	--		1/2
-20	X 1207	--	1	--	97	73		--
Reciprocal cross								
0 1419	X 1306-10	24	51	1(5c $\bar{c}$ t2-3 sector) 1(5c $\bar{c}$ t7 sector)	5	21*	S	1/2*
1981 rows with selected kernels[b] ⟹					(1102E*)			(1103F*)

a $\chi^2_{1:1}$ – Test for 1:1 colored + spotted to t2-3 pales; S is significant at 0.05 probability level and NS is not significant.

[b] Selected kernels and their assigned row numbers in a column are identified by the same superscript.

Table 4.25. Test for the presence of En in colored (t7) kernels

$$\frac{\text{a-m(r) Sh2}}{\text{a-m(r) Sh2/a-m-1 sh2}} \times \frac{\text{a-m-1 6078 Sh2}}{\text{a-m-1 6078 Sh2/a sh2}} \pm \text{En}$$

Cl,rd	X t7 c̄ or c̄ out cl areas	Round Colored (t7) c out cl areas	Round Colored (t7) c̄ cl areas	Round Spotted 5c t1	Round Spotted 3b-c t1	Round Color-less to light pale t1-3	Shrunken Colored t7	Shrunken Spotted

a) Without colorless areas (Figure 4.14A, 1980 1306; 1981 1121)

Cl,rd	X t7 c̄ or c̄ out cl areas	c out cl areas	c̄ cl areas	5c t1	3b-c t1	Color-less to light pale t1-3	Colored t7	Spotted
0 1334	X 1306-2	all	--	--	--	--	--	--
1 1235	X 1121-4	210	1	--	--	--	--	--
1234	X -1	148	56	39	18	12	--	--
1237	X -3	135	100	14	--	81	--	--
1257	X -10	121	48	6	67	94	62	52

b) With colorless areas (Figure 4.14A, 1980 1307)

Cl,rd	X t7 c̄ or c̄ out cl areas	c out cl areas	c̄ cl areas	5c t1	3b-c t1	Color-less to light pale t1-3	Colored t7	Spotted
0 1335	X 1307-5	59	--	98	--	62(t1)	--	--
1335	X -13	√[a]	--	√	--	√	--	--

[a] Indicates the presence of kernels of the type in that column.

(with and without colorless areas) contain En is further confirmed by
the occurrence of spotted in tests on a-m(r)/a-m-1 (Table 4.25A, B).

    b. Colored (t7) kernels without colorless areas (Figure 4.14A,
1980 1306, 1981 1121)   With one exception, there are no differences
among the test cross progenies of the two types of colored (with and
without colorless areas) (Tables 4.23, 4.24). The exception is that
in a few progenies of colored without colorless areas, all the round
kernels are of parental type (Table 4.24, 0 1306-2, 1 1121-4, -11, -6).
Unlike in the rest of the progenies, these progenies do not contain
the t2-3 pale basic allele type and the t1 colorless germinal mutants
indicating that the colored parent kernels (without colorless areas)
represent En-triggered changes in the receptor element to a "locked"
type that would not respond any more to En.

These results on the heritability of colored with and without
colorless areas indicate the following:

    (1) The colored phenotype without colorless areas (with the
        exception of "locked" type) represents an En-responsive
        state of the t2-3 basic allele. The response may or may
        not involve excisions of the receptor element from the
        locus. If it does, the excisions must take place prior
        to first cell division of the endosperm (following
        fertilization), so that the kernel is fully-colored.

    (2) The colored with colorless areas also represent +En type.
        The following events can explain the formation of colorless

areas.

(a) Loss of $\underline{En}$ or a change in the suppressor($\underline{S}$) component

of $\underline{En}$ from $\underline{S}^+ \rightarrow S^-$ during the endosperm development.

(b) Somatic change in state of the receptor element from

a responsive (t7) to a non-responsive (t1) state.

(3) The spotted can either represent mutations (excisions of the

receptor) occurring only in a few kernels or delayed muta-

tions as compared to those in colored kernels (considering

that mutations in colored do occur at the one-cell stage of the

endosperm).

(4) The colorless kernels represent germinal changes in state

from t2-3 to t1. Their occurrence in many test cross

progenies indicate a high incidence of germinal mutations.

2. Heritability and the En content of light to medium
   pale (t3-4) kernels (Figure 4.14A, 1980 1309,
   1981 1122)

The t3-4 pales were derived from selfed progeny of colored (t7)

with or without colorless areas (Table 4.22, 9 1646-12) and are slightly

darker than the original t2-3 pale (a-m-1 6078 - $\underline{En}$). Therefore, the

t3-4 pales are analyzed to determine whether they also represent the

-En state of a-m-1 6078.

The t3-4 pales appear among all the progenies and are therefore

heritable (Table 4.26). However, in two of the test crosses (0 1309-20 X

1420, 0 1309-21 X 1420), an additional colorless (t1) phenotype

appeared. The t1 account for one-half of the total progeny kernels.

Since there are no shrunken kernels in these test cross progenies, the

Table 4.26. Heritability of light to medium-pale (t3-4) kernels (Figure 4.14A, 1980 1309; 1981 1122)

a-m-1 6078 Sh2 ⟋ a-m-1 6078 Sh2/a sh2 X a sh2 ⟋ a sh2 (t3-4 / X cl,sh)	Round				Shrunken
	Spotted	Colored $\underline{t7}$	Pales $\underline{t3\text{-}4}$	Colorless $\underline{t1}$	Colorless
0 1309-6 X 1420	--	--	60	--	63
-20 X 1420	--	--	174	193	--
-21 X 1420	--	--	115	123	--
1 1122-1 X 1208	--	--	all	--	--
-2 X 1229	--	--	1/2	--	1/2
-3 X 0550	--	--	1/2	--	1/2
-4 X 1207	--	--	all	--	--
-6 X 0535	--	--	1/2	--	1/2
-9 X 1223	--	--	1/2	--	1/2

Table 4.27. Test for the presence of En in light-medium pale (t3-4) kernels (Figure 4.14A, 1980 1309; 1981 1122)

$\dfrac{a\text{-}m(r)\ Sh2}{a\text{-}m(r)\ Sh2/a\text{-}m\text{-}1\ sh2}$ X $\dfrac{a\text{-}m\text{-}1\ 6078\ Sh2}{a\text{-}m\text{-}1\ 6078\ Sh2/a\ sh2}$ ± En		Round				Shrunken	
C1,rd X t3-4	Spotted	Colored t7	Pales t3-4	Color-less t1	Colored	Spotted
0 1336 X 1309-6	––	––	1/2	1/2	––	––
1 1347 X 1122-1	––	1/2	1/2	––	––	––
1234 X -2	––	1/4	1/4	1/4	1/4	––
1234 X -4	––	173	177	––	––	––
1347 X -6	––	70	77	74	50	––
1235 X -9	––	84	51	60	75	––

colorless must represent one of the a-m-1 6078 alleles of the t3-4

parent kernels that had changed in state in the selfed generation of

colored kernels (Table 4.22).

The heritability of t3-4 pales and the absence of spotted and

colored progeny (Table 4.26) indicate that the t3-4 represents a -En

state of a-m-1 6078. This is further confirmed in tests on a-m(r)/

a-m-1 where no spotted progeny were present (Table 4.27). These t3-4

pales are similar to the original t2-3 pale (-En) state of a-m-1 6078.

3. Heritability of spotted kernels

Spotted kernels are derived from selfed progenies of colored (t7)

with or without colorless areas (Figure 4.14A, 1979 1646, Table 4.22).

The following three spotted types are tested for their heritability:

    (a) 1a-b t1 = 0 1310W (Figure 4.14D, 1980).

    (b) 3c t1 = 0 1310X (Figure 4.14D, 1980).

    (c) 3b t3 = 0 1310Y (Figure 4.14D, 1980).

    (a) 1a-b t1 (0 1310W)

The 1a-b t1 spotted pattern is heritable (Table 4.28a). The

progenies include 1a-b t1 and colorless (t1) kernels and these two

phenotypes are combined because the spots are very fine. Spotted

were reselected and tested to determine their consistent heritability.

In test crosses, 1-2a-b t1-2 spotted and colorless to very light pale

(t1-2) appeared (Table 4.28a). The 1-2a-b t1-2 spotted are consistent

with the parental spotted (1a-b t1) and constitute a majority of the

progeny kernels. The preponderance of spotted progeny indicates the

presence of more than one En.

Table 4.28. Heritability of 1a-b t1 spotted kernels (Figure 4.14D, 1980 1310W)

$\dfrac{\text{a-m-1 6078 Sh2}}{a}$, $\dfrac{\text{En}}{}$ X $\dfrac{a\ sh2}{a\ sh2}$		Round		Shrunken
		Pale (t2-3)	Colorless (t1) plus fine-spotted (1a-b t1)	Colorless
1a-b t1	X cl,sh			

a) Progeny test

0 1310W-1	X 1420	--	83	80
-2	X 1420	1	111*	102
Reciprocal cross				
0 1406	X 1310W-4	--	1/2	1/2

1981 rows (selected only 1a-b t1 spotted)[a] ⟹ (1109*)

1a-b t1	X a sh	Spotted (1-2a-b t1-2)	Colorless—very light pale (t1-2)	cl,sh
1 1109-1	X 1218	163	22	189
-3	X 1230	203	14	205
-5	X 1217	141	14	144
-13	X 1230	181	13	182

$\dfrac{\text{a-m(r) Sh2}}{\text{a-m-1 sh2}}$ X $\dfrac{\text{a-m-1 6078 Sh2}}{a}$, $\dfrac{\text{En}}{sh2}$	Spotted pattern

b) Test for type of En in 1a-b t1 spotted

1 1231	X 1109	-1	4-5c t1 $\bar{c}$ or $\bar{c}$ out specks
1233	X	-3	"
1237	X	-5	"
1231	X	-13	"

[a]Selected kernels and their assigned row numbers in a column are identified by the same superscript.

In tests on a-m(r)/a-m-1, the 1a-b tl spotted produced 4-5c tl

spotted progeny (Table 4.28b) which is similar to that produced by the

original En in the same tests (Table 4.25). This similarity indicates

that the En in 1a-b tl spotted has not changed and it therefore follows

that the 1a-b tl spotted represent a change in state of the receptor,

I of a-m-1 6078 allele. This is further supported by the absence of

parental types—colored (original +En type) and t2-3 pales (original

-En type) in the progenies of 1a-b tl spotted (Table 4.28a).

(b)   3c tl (0 1310X)

Two out of three parents give spotted progeny kernels in either

straight or reciprocal crosses (Table 4.29). But the spotted are

distinguishable in the two progenies. In 0 1310X-2 X 1420, the spotting

pattern is 3c tl with 4a type specks and those in 0 1310X-3 X 1420 are

3b-c tl (speckled or mottled type spots). The non-spotted progeny types

also differ in background coloration. They are colored (t7) in

0 1310X-2 X 1420 and variable pale (t2-3, t4-5) in 0 1310X-3 X 1420. The

spotted and non-spotted types are further tested to determine if the

non-spotted represent the basic allele phenotype (-En) of the spotted

patterns in the respective crosses.

(i)   3c tl with 4a specks and colored (t7) kernels from

0 1310X-2 X 1420 (Table 4.29)

The 3c tl with 4a specks are tested for heritability and the

colored are tested for their response to the En in a sh sib kernels

(Table 4.30A, B). The expectation is that the progenies in these tests

should correspond to each other if the 3c tl with 4a specks and the

Table 4.29. Heritability of 3c tl spotted kernels (Figure 4.14D, 1980 1310X)

| $\dfrac{\text{a-m-1 6078 Sh2}}{\text{a-m-1 6078 Sh2/a sh2}}$, $\underline{\text{En}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$

 3c tl   X cl,sh | Round | | | | | Shrunken |
	Spotted	Colored t7	Pale t4-5	 t2-3	Colorless tl	Colorless
0 1310X-1 X 1421	--	1	45	47	--	--
-2 X 1420	80*(3c tl + 4a specks)	14*	--	--	--	83*
-3 X 1420	47**(3b-c tl speckled)	--	46	15*	--	110**
Reciprocal crosses						
0 1407 X 1310X-3	34(3b-c tl speckled)	--	36	1	--	77
1981 rows containing selected kernels[a] ⟹	(1112*) (1114**)	(1110*)	(1113A*)			(1111*) (1113B**)

[a] Selected kernels and their assigned row numbers in a column are identified by the same superscript.

Table 4.30. Heritability of 3c t1 with 4a specks and response of
colored (t7) to _En_ in _a sh_ sibs (from Table 4.29,
0 1310X-2 X 1420)

A. 3c t1 spotted with 4a specks (1 1112)

a-m-1 6078 Sh2, En X a sh2			Round					Shrunken
_a_ _sh2_		_a sh2_ / _a sh2_	Spotted	Colored	Colorless-very light			
3c t1 c̄ 4a specks		X cl,sh	(3c c̄ 4a specks)	_t7_	pale _t1-2_	$\chi^2_{1:1}$ [a]	$\chi^2_{3:1}$ [b]	Color-less
1 1112-1		X 1220	65	71	1	NS	S	150
-6		X 1220	50	44	–	NS	S	90
-8		X 0548	14	22	–	S	S	38
-10		X 1218	151	44	1	S	NS	193
-11t		X 0549	97	36	–	S	NS	123
-20		X 1218	95	95	1	NS	S	180

[a] $\chi^2_{1:1}$ – test for 1:1 spotted to colored.

[b] $\chi^2_{3:1}$ – test for 3:1 spotted to colored; S is significant at 0.05
probability level and NS is not significant.

Table 4.30. (Continued)

B. Colored-t7 (1 1110)

$\dfrac{\text{a-m-1 6078}}{\text{a}} \dfrac{\text{Sh2}}{\text{sh2}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$, En	Round				Shrunken
	Spotted	Colored			Color-
t7 X cl,sh	(3c t1 $\bar{c}$ 4a specks)	$\dfrac{t7}{}$	$X^2_{1:1}{}^a$	$X^2_{3:1}{}^b$	less

(1) Response of t7 to En

		Spotted	Colored	$X^2_{1:1}$	$X^2_{3:1}$	less
1 1110-1 X 1111-1 +[c]		41	22	S	S	1/2
-2 X -2 +		201	9	S	S	1/2
-3 X -3 +		17	31	S	S	1/2
-4 X -2 +		153	8	S	S	1/2
-5 X -2 +		71	2	S	S	1/2
-5t X -3 +		26	94	S	S	1/2
-6 X -5 +		114	125	NS	S	1/2

$\dfrac{\text{a-m(r)}}{\text{a-m(r)}} \dfrac{\text{Sh2}}{\text{Sh2}}$ X $\dfrac{\text{a-m-1 6078}}{\text{a}} \dfrac{\text{Sh2}}{\text{sh2}}$	Round		
	Spotted	Colored t7	Colorless t1

(2) Test for presence of En in t7

	Spotted	Colored t7	Colorless t1
1 1244 X 1110-1	--	1/2	1/2
1244 X -2	--	1/2	1/2
1248 X -3	--	1/2	1/2
1242 X -4	--	1/2	1/2

[c]Presence of En confirmed on a-m(r)/a-m(r).

colored represent + and -En types, respectively.

The test crosses of 3c t1 with 4a specks (1 1112) contain the parental spotting type and colored (t7) progeny (Table 4.30A). These progeny types are either in 1:1 or 3:1 ratio, indicating that they represent + and -En types. This indication is confirmed in crosses of colored (1 1110) (sibs of 3c t1 with 4a specks, Table 4.29, 0 1310X-2 X 1420) with a sh +En sibs (Table 4.30B(1)) where the progeny consist of 3c t1 with 4a specks and colored. However, the ratio of these progeny types suggests more than one En situation in a sh sibs.

The tests of colored (Table 4.30B(2)) on a-m(r)/a-m(r) did not yield spotted kernels and thus further confirmed the -En status of colored kernels. It is evident from these tests that the colored pheno-type constitutes a changed state of the receptor, I of a-m-1 6078 allele. In the presence of En, this new state exhibits 3c t1 with 4a specks.

(ii) 3b-c t1 speckled (faint) spotted and t2-3 pales from
       0 1310X-3 X 1420 (Table 4.29)

Among the progenies of the test crosses of the 3b-c t1 speckled spotted type, the same spotted and non-spotted ranging from t1 to t7 are included (Table 4.31A(1); Figure 4.15A). Since the 3b-c t1 speckled spotted is heritable and the non-spotted progeny types are different from the original t2-3 basic allele, the 3b-c t1 represents a change in state of the a-m-1 6078 allele. Further confirmation is obtained in crosses of t2-3 X a sh En sibs (sibs of 3b-c t1 speckled (faint) spotted from 0 1310X-3 X 1420 in Table 4.29) where the progeny included 3b-c t1 speckled (faint) spotted and non-spotted ranging from t1 to t6 (Table

Table 4.31. Heritability of 3b-c tl speckled (faint) spotted and response of t2-3 pales to <u>En</u> in <u>a</u> <u>sh</u> sibs (from Table 4.29, 0 1310X-3 X 1420)

A. 3b-c tl speckled (faint) spotted (1 1114)

$$\frac{\text{a-m-1 6078 Sh2, En}}{a \qquad sh2} \ \text{X} \ \frac{a \ sh2}{a \ sh2}$$

3b-c tl speckled spotted X cl,sh	Round				Shrunken
	Speckled (faint) spotted 3b-c tl	Colored t7	Pale t4-5	Colorless tl	Colorless

(1) Progeny test

1 1114-1	X 1928	84	98	33	34	1/2
-2	X 0548	112 (1-2a tl)	--	--	86	1/2
-3	X 1221	111	131	--	6	1/2
-4	X 0548	83	84	17	6	1/2
-5	X 0548	93	97	--	6	1/2
-6	X 1926	90	77	31	14	1/2
-7	X 0547	98 (1-2a tl)	--	--	26	1/2

$$\frac{\text{a-m(r) Sh2}}{\text{a-m-1 \ sh2}} \ \text{X} \ \frac{\text{a-m-1 6078 Sh2, En}}{a \qquad sh2} \qquad \text{Spotted pattern}$$

(2) Test for type of <u>En</u> in 3b-c tl speckled (faint) spotted

1 1249 X 1114-1		4-5 c tl $\bar{c}$ or $\bar{c}$ out pale areas
1242 X	-2	"
1242 X	-3	"
1250 X	-4	"

Table 4.31.   (Continued)

B.   t2-3 pales (1 1113A)

$\underline{a\ \underline{sh2},\ \underline{En}}$ X $\underline{a\text{-}m\text{-}1\ 6078\ \underline{Sh2}}$	Round			Shrunken
$\overline{a\ \underline{sh2}}$ $\qquad\underline{a}\qquad\ \underline{sh2}$	Speckled spotted 3b-c tl	Variable pales t2-6	Colorless tl	Colorless
cl,sh sibs X t2-3 pales				

(1) Response of t2-3 pales to En in a sh sibs

	Speckled spotted 3b-c tl	Variable pales t2-6	Colorless tl	Colorless
1 1113B-1- X 1113A-2t	--	1/2	--	1/2
-2- X -7t	--	1/2	--	1/2
-3- X -7t	--	1/2	--	1/2
-4+ X -7t	88	105	6	1/2
-5+ X -7t	80	171	--	1/2

$\underline{a\text{-}m(r)\ \underline{Sh2}}$ X $\underline{a\ \underline{sh2},\ \underline{En}}$	Spotted pattern
$\overline{a\text{-}m\text{-}1\ \underline{sh2}}$ $\quad\overline{a\ \underline{sh2}}$	

(2) Test for type of En in a sh sibs

1 1256 X 1113B-4	4-5c tl $\overline{c}$ or $\overline{c}$ out pale areas
4316 X -5	"

173

Figure 4.15. Progeny test ears of exceptional 3b-c t1 speckled (faint)
spotted and t2-3 pale kernels of a-m-1 6078 state

    A. Test cross ear of 3b-c t1 speckled (faint) spotted
(Table 4.31A(1), 1 114-5 X 0548) showing the same
parental spotted type and variable pales ranging
from t1 to t7

    B. Progeny ear of cross t2-3 pale X a sh En sibs con-
taining similar spotted and variable pales as in A

A
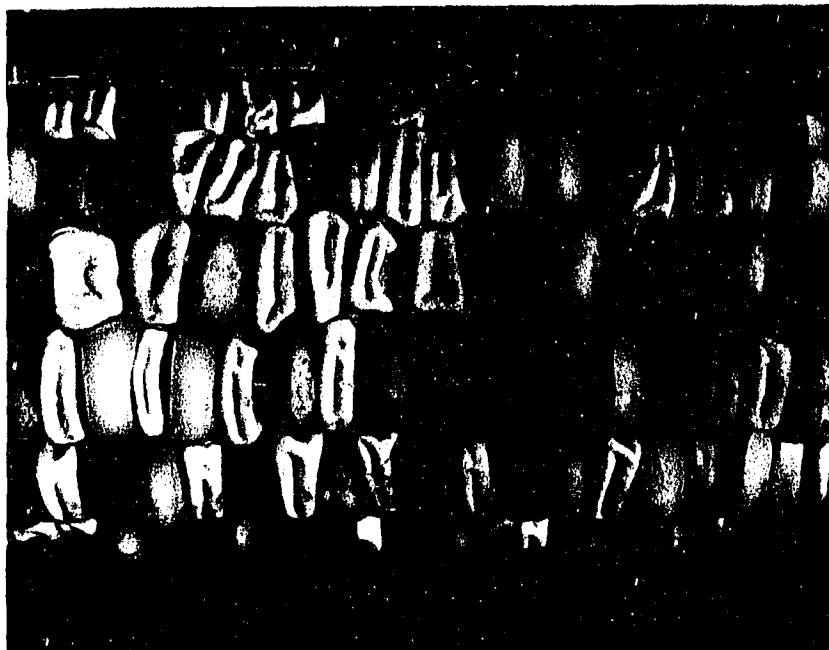
B

4.31B(1); Figure 4.15B). However, there is a deficiency in the number

of spotted among these progenies (Table 4.31B(1)) and also among the

test cross progenies of 3b-c tl spotted (Table 4.31A(1)). It needs to be

confirmed whether these aberrant ratios are a problem of En transmis-

sion or they occur as a result of influence of some factors that restrain

the expression of En.

In two of the test crosses of 3b-c tl spotted (Table 4.31A(1)

1 1114-2 X 0548, 1 1114-7 X 0547), the progenies included neither the

parental spotted (3b-c tl) nor the darker pigmented types (tl to t7) as

in other crosses. The progeny types include 1-2a tl spotted and color-

less (tl) indicating a change in state of the a-m-1 6078. Further tests

are required to confirm these results.

(c)   3b t3 (0 1310Y)

The test cross progeny of 3b t3 include 1-2a tl spotted and t2-3

pales (Table 4.32a). The spotted are fewer than the pales. In

reciprocal cross, the spotted types are absent among the progeny and

contain t2-3 pales and colorless (tl) in 1:1 ratio.

The results of these tests confirm that the 3b t3 phenotype is not

heritable. The occurrence of 1-2a tl spotted progeny could be due to a

change in state of En and not as a result of a change in the receptor

because the t2-3 pale phenotype (-En) of the original state appears in

the progeny (Table 4.32a). The presence of fewer spotted or lack of

spotted (in reciprocal cross) in the progenies indicate a problem with

En expression. To confirm this, the 1-2a tl spotted were further tested.

But in contrast to the expected (fewer spotted), the 1-2a tl spotted

Table 4.32. Heritability of 3b t3 spotted kernels (Figure 4.14D, 1980 1310Y)

$\dfrac{\text{a–m–1 6078 Sh2}}{\text{a–m–1 6078 Sh2/a sh2}}$, En X $\dfrac{\text{a sh2}}{\text{a sh2}}$	Round			Shrunken
	Spotted	Pale	Colorless	Colorless
3b t3 X cl,sh	1-2a tl	t2-3	tl	

a) Progeny test of 3b t3

	Round			Shrunken
	Spotted 1-2a tl	Pale t2-3	Colorless tl	Colorless
0 1310Y-1 X 1408	45	93	--	--
Reciprocal cross				
0 1407 X 1310Y-1	--	85	93	--

1-2a tl X a sh	Round			Shrunken
	Spotted 1-2a tl	Pale t2-3	Colorless tl	Colorless

b) Progeny test of 1-2a tl selected from 0 1310Y-1 X 1408 in (a) above

	Round			Shrunken
	Spotted	Pale	Colorless	Colorless
1 1115-4 X 0547	145	--	40	178
-9 X 1928	170	--	33	187
-10 X 0546	123	--	9	128

Table 4.33. Heritability and <u>En</u> content of colorless (tl) kernels (from Table 4.22, 9 1646-12)

$\dfrac{\text{a-m-1 6078 Sh2}}{\text{a-m-1 6078 Sh2/a sh2}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$		Round colorless	Shrunken colorless
tl	X cl,sh		

a) Progeny test of colorless (0 1308, 1 1123)

0 1308-2	X 1420	84	71
-5	X 1421	26	--
-10	X 1420	158	146
1 1123-2	X 0550	1/2	1/2
-3	X 1224	1/2	1/2
-5	X 0546	1/2	1/2

$\dfrac{\text{a-m(r) Sh2}}{\text{a-m(r) Sh2/a-m-1 sh2}}$ X $\dfrac{\text{a-m-1 6078 Sh2}}{\text{a-m-1 6078 Sh2/a sh2}}$		Round				Shrunken	
		Spotted	Colored (tl)		Color-less	Colored	Spotted
		5c tl	$\overline{c}$ out cl area	$\overline{c}$ cl area	tl	t7	

b) Test for presence of <u>En</u>

0 1335	X 1308-5	123	--	-	137	--	--
1 1233	X 1123-2	108	46	4	107	63	35
1233	X -3	--	71	-	30	67	--
1232	X -5	118	41	-	82.	38	46

now constitute a majority of the progeny (Table 4.32b). Further, the
basic allele phenotype is colorless (t1) rather than the t2-3 pale of
the original state. These results indicate that a change in state of
the receptor I of a-m-1 6078 occurred and there is more than one En
in these cultures.

3. Heritability and En content of colorless
    (t1) kernels

The colorless kernels were derived in the progenies of colored
(t7) with or without colorless areas (Tables 4.22, 4.23, 4.24). Since
they differ from the t2-3 basic allele phenotype of a-m-1 6078, tests
were made to determine a possible change in state of the receptor of
a-m-1 6078 allele.

(a) Colorless (t1) kernels (0 1308, 1 1123) derived from selfed
     progeny of 9 1646-12 (Table 4.22)

These colorless (t1) kernels were each tested for their herita-
bility and En content. In test crosses, the t1 phenotype is heritable
(Table 4.33a) and in tests with a-m(r)/a-m-1, it contains a highly
active En (Table 4.33b) indicating that the colorless phenotype repre-
sents a changed state of the receptor at a-m-1 6078 allele that is not
responsive to En.

(b) Colorless (t1) kernels (1 1105) derived from test cross
     0 1307-22 X 1422 (Table 4.23)

In crosses with a-m(r)/a-m(r) (Table 4.34b), the colorless kernels
yielded spotted progeny (Figure 4.16A). This test indicates that these
colorless are non-responsive type. However, in crosses with a sh En

Table 4.34. Response of colorless (tl) kernels (1 1105A) to the <u>En</u> in <u>a</u> <u>sh</u> sibs (from Table 4.23, 0 1307–22 X 1420; Figure 4.14B, 1981 1105A)

$\dfrac{\text{a-m-1 6078 Sh2}}{\underline{a} \qquad \underline{\text{sh2}}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$ ± En		Round				Shrunken
		Spotted	Colored (t–7)		Colorless & pales	Colorless
		1–5b–c	$\overline{c}$	$\overline{c}$ out		
tl	X cl,sh sibs	tl–2	cl areas	cl areas	tl–4	
a) Response of colorless to <u>En</u>						
1 1105A–1	X 1105B–3 +[a]	6	39	50	100	1/2
−5	X 1105B–3 +	4	14	70	97	1/2
−6	X 1105B–3 +	9	25	62	91	1/2
Reciprocal cross						
1 1105B–1 +	X 1105A–1t	43	––	26	80	1/2

$\dfrac{\text{a-m(r) Sh2}}{\text{a-m(r) Sh2}}$ X $\dfrac{\text{a-m-1 6078 Sh2}}{\underline{a} \qquad \underline{\text{sh2}}}$, En 3–5c tl

b) Test for presence of <u>En</u> in colorless kernels						
1 1239	X 1105A–1	86	––	––	310	––
1245	X −5	97	––	––	302	––
1245	X −6	134	––	––	315	––

[a]Presence of <u>En</u> confirmed in crosses with <u>a-m(r)</u>/<u>a-m-1</u>.

Figure 4.16. Progeny test ears of exceptional colorless (tl)
kernels of a-m-1 6078 state

    A. Progeny ear resulting from a test of colorless (tl)
       kernels on a-m(r)/a-m(r) (Table 4.34B, 1 1245 X
       1105-6)

    B. Progeny ear of colorless X a sh En sibs (Table 4.34A,
       1 1105B-1 X 1105A-1t) showing the responsiveness
       (presence of spotted) of colorless to En
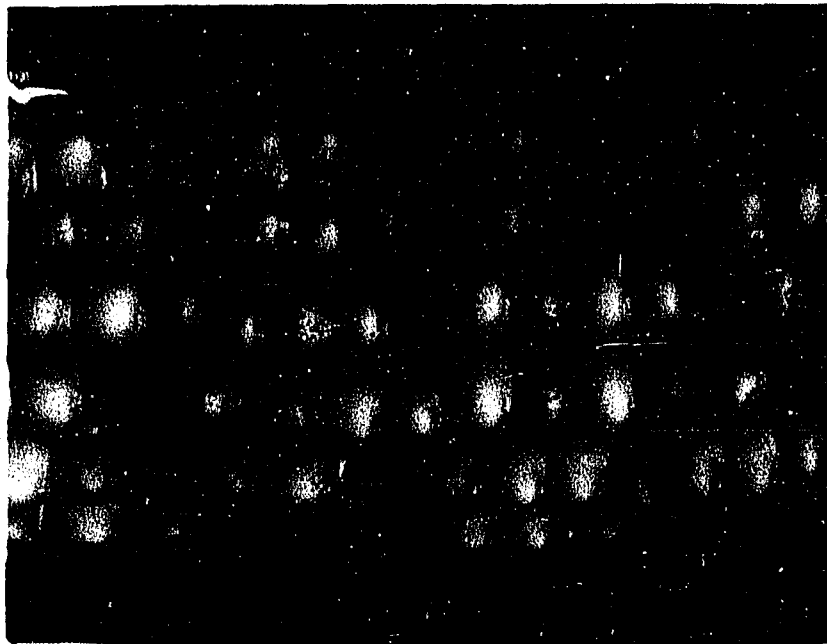
A

B

Table 4.35. Response of colorless (t1) kernels (1 1102E) to the $\underline{En}$ in $\underline{a}$ $\underline{sh}$ sibs (from Table 4.24, 0 1419 X 1306-10; Figure 4.14C, 1981 1102E)

$\dfrac{\text{a-m-1 6078}}{\underline{a}}\ \dfrac{\text{Sh2}}{\underline{sh2}}$ X $\dfrac{\underline{a}\ \underline{sh2}}{\underline{a}\ \underline{sh2}}$ ± $\underline{En}$		Round		Shrunken
		Spotted	Colorless	Colorless
t1	X cl,sh	1a-b t1	t1	
1 1102E-2[a]	X 1103F-8 -[b]	--	1/2	1/2
-3[a]	X -12 +[c]	--	1/2	1/2
-4[a]	X -6 +	--	1/2	1/2
-5[a]	X -7 +	--	1/2	1/2
-6	X -1 -	--	1/2	1/2
Reciprocal crosses				
1 1103F-4 ?[d]	X 1102E-3	--	1/2	1/2
-10 -	X -4t	7	128	1/2
-11 ?	X -5	7	150	1/2
-2 ?	X -5	4	115	1/2
-9 +	X -6t	11	205	1/2

[a] 1102E-2, 3, 4 and 5 contain $\underline{En}$ that gives 4-5c t-1 spotting pattern on $\underline{a\text{-}m(r)}$ $\underline{Sh2}/\underline{a\text{-}m\text{-}1}$ $\underline{sh2}$ tester.

[b] Absence of $\underline{En}$ confirmed in crosses with $\underline{a\text{-}m(r)}\ \underline{Sh2}/\underline{a\text{-}m(r)\,Sh2}/\underline{a\text{-}m\text{-}1}\ \underline{sh2}$.

[c] Presence of $\underline{En}$ confirmed in crosses with $\underline{a\text{-}m(r)}\ \underline{Sh2}/\underline{a\text{-}m(r)}\ \underline{Sh2}/\underline{a\text{-}m\text{-}1}\ \underline{sh2}$.

[d] Presence of $\underline{En}$ not confirmed.

sibs (Table 4.34a), the progeny of colorless included variable spotted (1a-5c), colored (t7 with and without colorless areas) and variable pales (t1-4) (Figure 4.16B). The presence of spotted and colored (+En types) progeny indicate that the colorless are responsive to the En in a sh sibs. These different responses of colorless indicate a possible difference between the En in colorless kernels and that in the a sh sib kernels. On the other hand, the presence of variable pales (t1-4) in the progeny of colorless X a sh + En sibs (Table 4.34a) suggests a change in state of the a-m-1 6078 allele. Further testing is required to confirm these possibilities.

(c)   Colorless (t1) kernels (1 1102E) derived from test cross

     0 1419 X 1306-10 (Table 4.24)

Colorless kernels of 1 1102E contain a highly active En in tests on a-m(r)/a-m-1 (Table 4.35 footnote). In crosses with a sh + En sibs, all the progenies are colorless (Table 4.35). However, in reciprocal crosses, a few 1a-b t1 spotted kernels appeared in the progeny, indicating that the colorless are weakly responsive to En. These results suggest that the colorless and 1a-b t1 spotted pattern represent -En and +En phenotypes, respectively, of changed state of the receptor I of a-m-1 6078 allele.

D.   a-m-2 7977B State

In the absence of an active Spm, the original state of a-m-2 7977B is colorless with or without faint or light pigmented areas. An active Spm induces some pigmentation (pale-t4) and spots (3-4c) (Figure 4.17A, B and C).

The original isolates in the present study included two kernels. One had an inactive Spm (colorless) and the other included an active Spm (3-4c t4) (Figure 4.18A, 1976 1609-1, 1609-2). The progenies derived from the colorless kernel are presented in Part I (Figure 4.18A, B) and those derived from the 3-4c t4 spotted kernel are presented in Part II (Figure 4.19A, B, C).

1. Part I

   a. Derivation of spotted kernels from the original colorless kernel   In test crosses, the original colorless (t1) kernel (Figure 4.18A, 1976 1609-1) yielded colorless (t1) (but some with faint pale areas) and also a few (6/180) spotted kernels. Because the single 1609-1 kernel was colorless, it is assumed that an active Spm was not present. But the appearance of a few spotted kernels among progeny of the 1609-1 plant would indicate that this plant contained an inactive Spm that had changed to an active state. In contrast to the pale t4 background of original spotted kernel (3-4c t4) in Part II (Figure 4.19A, 1976 1609-2), these spotted kernels had colorless to very light-pale background (3-4c t1-2). The changed background coloration (t1-2) of the spotted of the 1609-1 derivative would indicate that the new state of Spm activates the a-m-2 allele to a lower level (t1-2).

   b. Heritability of 3-4c t1-2 spotted kernels   From progeny ear of 1609-1, plants were grown from the selected spotted kernels with colorless to very light-pale background (3-4c t1-2) and selfed (Figure 4.18A, 1977 3405). The selfed progeny consisted of spotted,
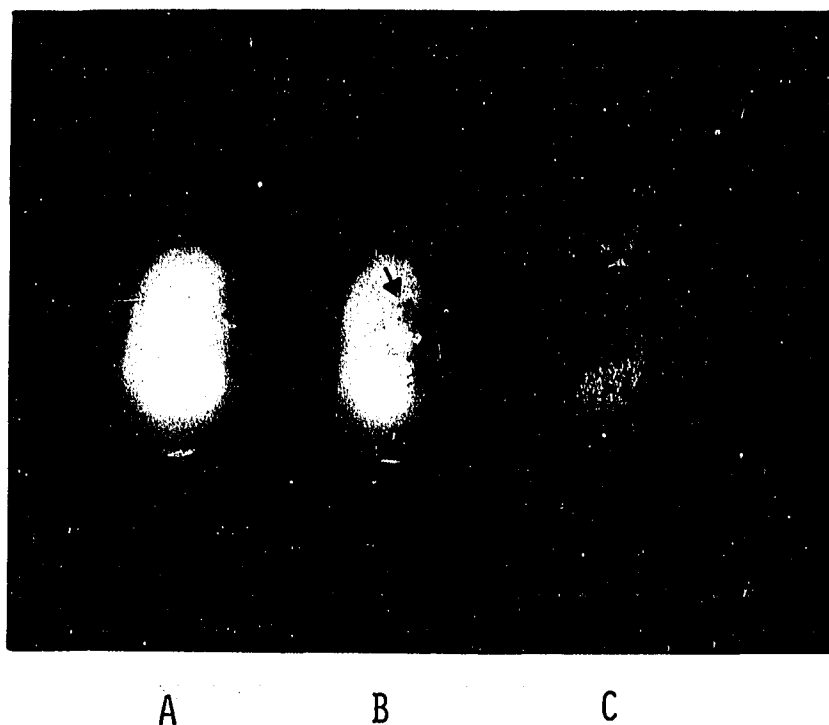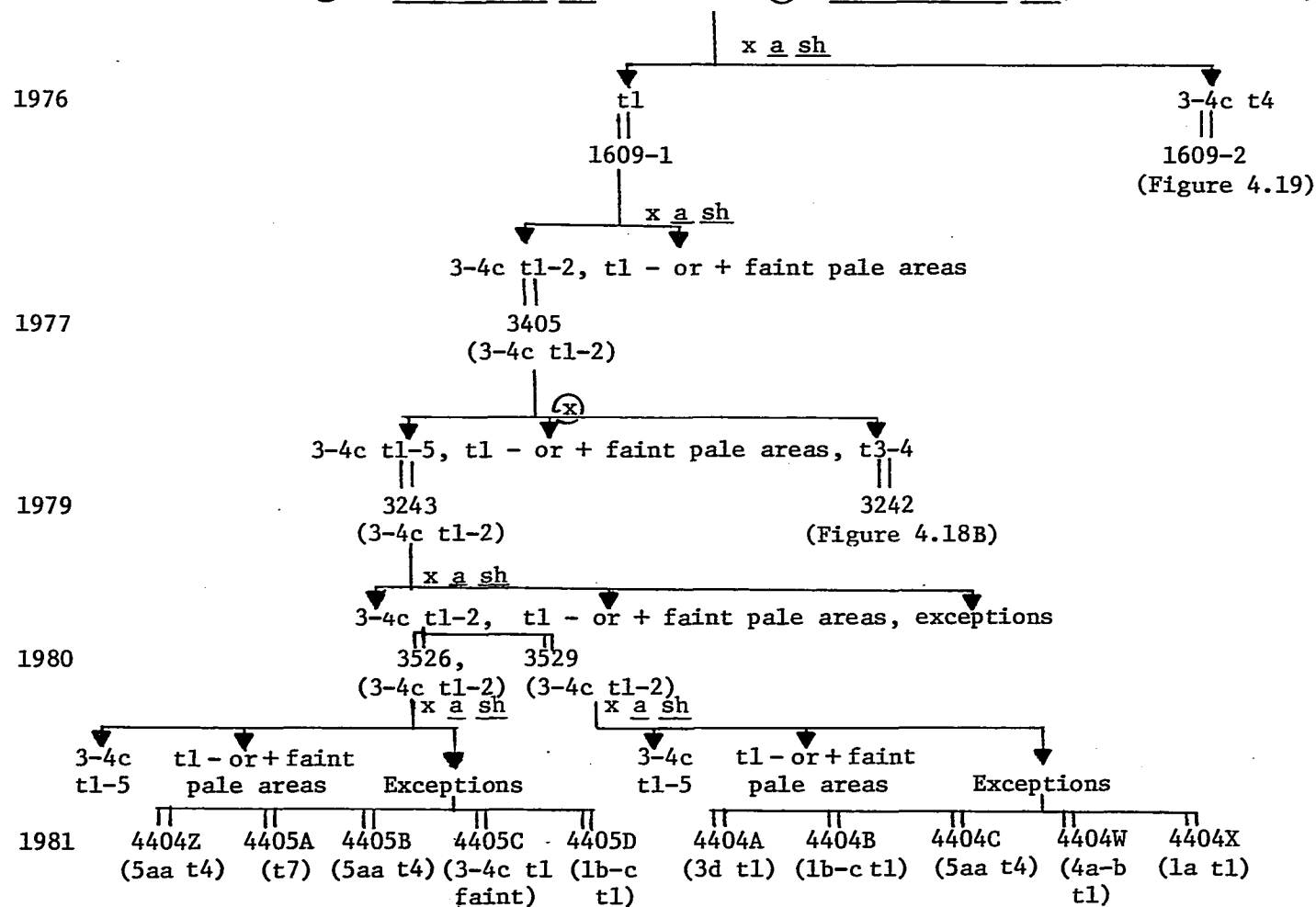
Figure 4.17. Original state of a-m-2 7977B

    A. Colorless (t1) without Spm

    B. Colorless (t1) (also without Spm) containing faint
       pale areas (arrow)

    C. 3-4c t4 spotted with Spm

Figure 4.18. Flow diagram showing different derivatives from the original source of
a-m-2 7977B state

Original   McCl 8516A② II(a-m-2 7977B Sh2)/McCl 8535 ⑪ (a-m-1 5719A-1 sh2)   (McClintock, 1967)

x a sh

1976   t1   3-4c t4

                               1609-1   1609-2
                                                                        (Figure 4.19)

x a sh

3-4c t1-2, t1 - or + faint pale areas

1977   3405
                            (3-4c t1-2)

Ⓧ

3-4c t1-5, t1 - or + faint pale areas, t3-4

1979   3243   3242
                    (3-4c t1-2)   (Figure 4.18B)

x a sh

3-4c t1-2, t1 - or + faint pale areas, exceptions

1980   3526,   3529
                (3-4c t1-2) (3-4c t1-2)
                  x a sh   x a sh

3-4c   t1 - or + faint   3-4c   t1 - or + faint
t1-5   pale areas   Exceptions   t1-5   pale areas   Exceptions

1981   4404Z   4405A   4405B   4405C · 4405D   4404A   4404B   4404C   4404W   4404X
      (5aa t4)   (t7)   (5aa t4) (3-4c t1 (1b-c   (3d t1) (1b-c t1) (5aa t4) (4a-b (1a t1)
                                   faint)   t1)   t1)

A

(from Figure 4.18A)

1979
          ↓
          3242
          (t3-4)

        x $\underline{a}$ $\underline{sh}$ $\underline{En}$ (high)   x $\underline{a}$ $\underline{sh}$ $\underline{En}$ (low)

     3c t2-5, t1-5, mottled (few)   1-2b t1-3, t1-3

1980
       3521   3522
       (t1)   (t2-3)

x $\underline{a}$ $\underline{sh}$ + $\underline{En}$ $\underline{a}$ $\underline{sh}$ - $\underline{En}$ x $\underline{a}$ $\underline{sh}$ + $\underline{En}$ x $\underline{a}$ $\underline{sh}$ - $\underline{En}$

4c t4, t1   single spotted, t1 3-4c t4-5, 1b t1-2, t1-2 1b t1-2, t1-2
          (in one cross)   (in one cross)

B

Figure 4.18. (Continued)

(from Figure 4.18A)

1976 | 1609-2
(3-4c t4)

x <u>a</u> <u>sh</u>

3-4c t1-4, t1 - or + faint pale areas, exceptions

1977 | 3402
(3-4c t4)

ⓧ

3-4c t1-4, t1 - or + faint pale areas, exceptions

1979 | 3238 | 3237 | 3234
(t1 + faint pale areas) | (t7) | (2b-c t1-3)

(Figures 4.19B, C)

ⓧ

t1 - or + faint pale areas

1980 | 3519
(t1 + faint pale areas)

x <u>a</u> <u>sh</u> - <u>En</u>

t1 - or + faint pale areas

1981
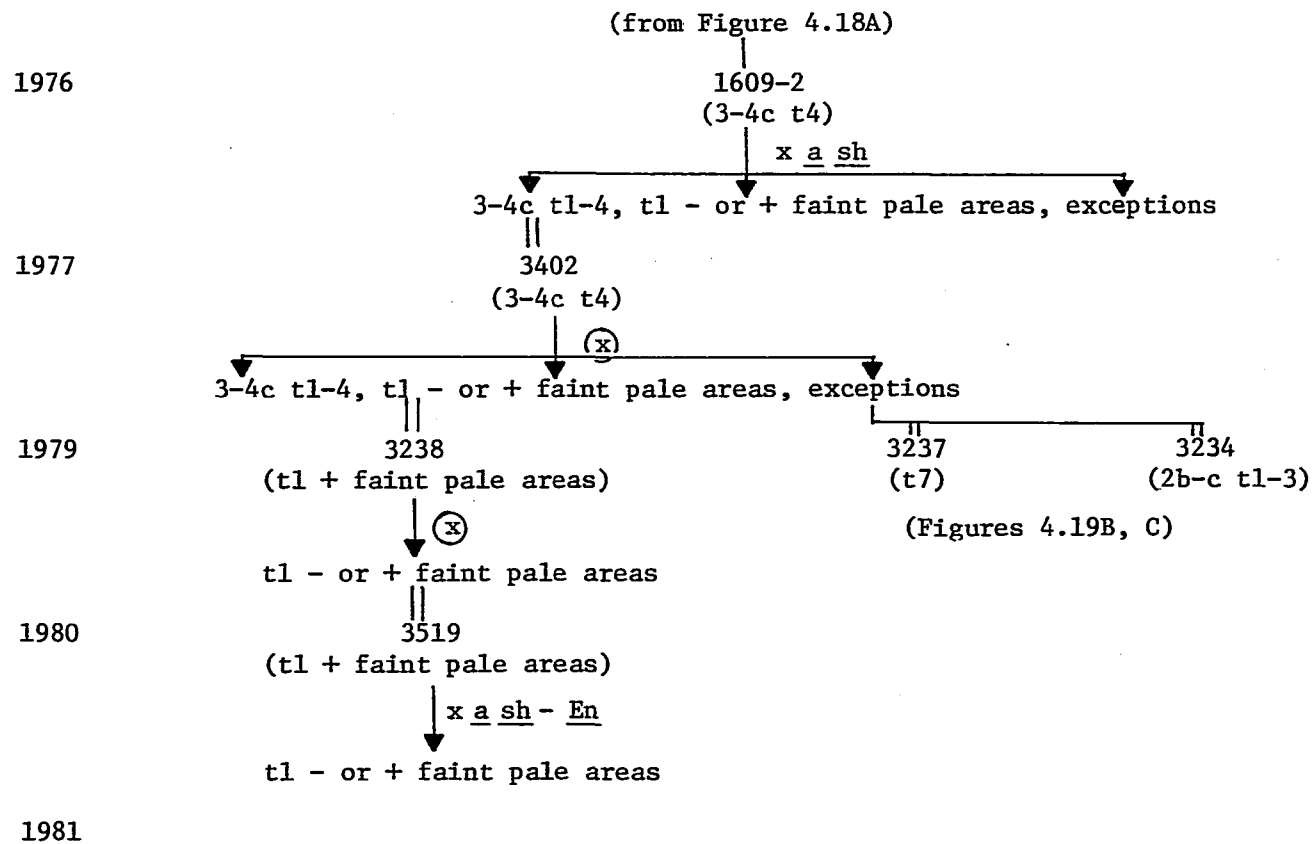
A

Figure 4.19. Flow diagram showing different derivatives from the original 3-4c t4 spotted state of <u>a-m-2 7977B</u>

B

1979 3237
(t7)

xa sh – En

t7,   t2-3
|| ||

1980 3517   3516

x a sh En   x a sh – En

t7   t2-3 ,   t5   , tl
(some 1 b   (not
spotted)   uniform)
|| ||

1981 4336   4335C   4334A

C

3234
(2b-c tl-3)

x a sh

2b-c tl-3,   tl,   mottled, 3-4b-c tl
(exceptions)

3509,   3510
(2b-c tl-3) (2b-c tl-3)

x a sh   x a sh

2b-c tl-3, tl, exceptions   1-2b tl-2, tl, exceptions

4326C 4327A   4328X   4328Y   4328A
(3-4c tl) (3-4c tl   (3c tl) (4-5c tl) (mottled)
all ker-
nels side
by side)
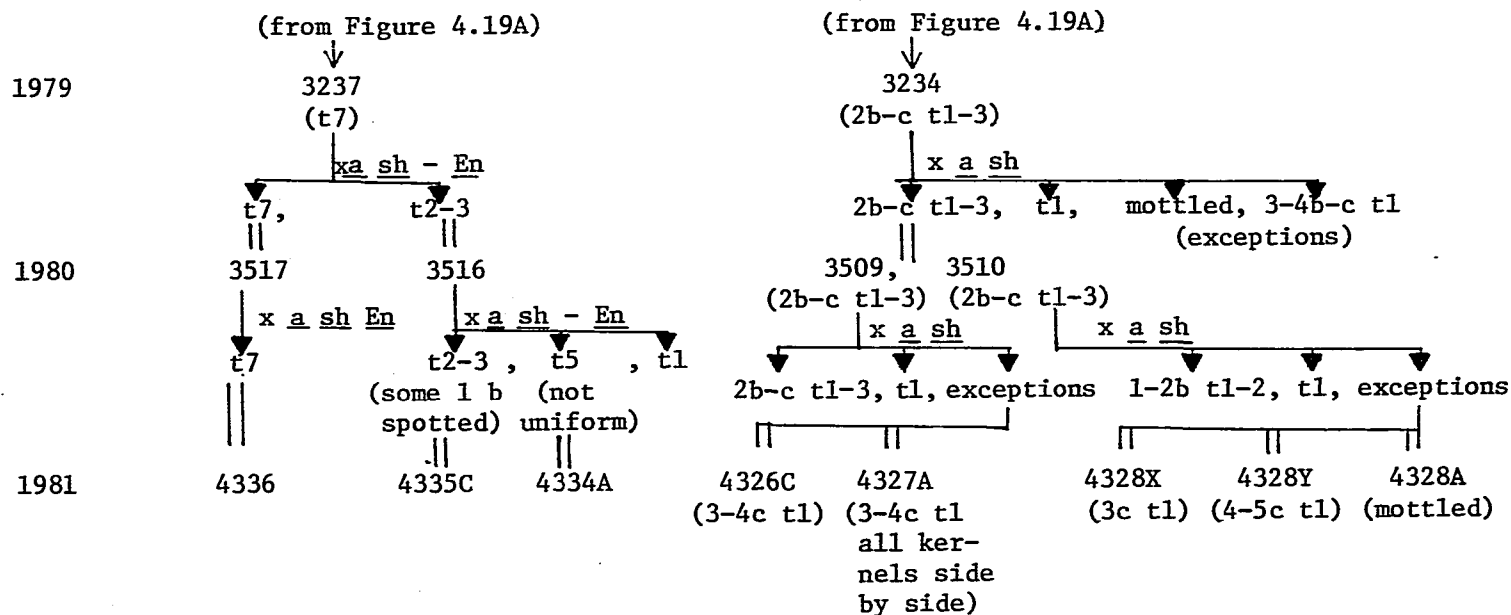
Figure 4.19.   (Continued)

colorless (with or without faint pale areas) and a few (4/323) pales

(t3-4). The spotted greatly varied in the background pigmentation

(t1-5), but they did include spots of the same frequency and size

(3-4c) as the parental kernels. Among these spotted (3-4c t1-5)

kernels, those with colorless to very-light pale background (3-4c

t1-2) were again selected to test if the variation in background pig-

mentation increases (t1-5) among the spotted progeny kernels (Figure

4.18A, 1979 3243). The progeny included the parental type spotted

(3-4c) but the background pigmentation among kernels did not vary

from t1-t5 as in the preceding generation. However, in further test

crosses of 3-4c t1-2 (Figure 4.18A and Table 4.36, 1980 3526, 3529),

the pigmentation among spotted progeny kernels varied from t1-t5.

These results indicate that there is an increase in the variation of

background pigmentation (t1-5) despite selection for t1-2 background.

Since this occurs only in spotted kernels and not in the basic allele

phenotype (t1), it can be concluded that the phase of activity of sup-

pressor component of Spm varies among spotted progeny kernels. This

means that the Spm changes to different levels of phase of activity

among the spotted kernels.

In addition to the spotted with varied background pigmentation

(3-4c t1-5), and colorless (with or without faint pale areas), several

exceptional phenotypes arose in the progeny of 3-4c t1-2 test crosses

(Figure 4.18A, 1980 3526, 3529 and Table 4.36). Most of these excep-

tional phenotypes are either not heritable or heritable only in a few

test crosses. Therefore, the tests of heritability of only a few

Table 4.36. Heritability of 3-4c t1-2 spotted kernels (Figure 4.18A, 1980 3526, 3529)

a-m-2 7977B Sh2 , Spm X a sh2 a sh2/a et a sh2 3-4c t1-2	X cl,sh	Round			Shrunken
		Spotted (3-4c t1-5)	Colorless c̄ or c̄ out pale areas (t1)	Exceptions	Color-less
0 3526-1	X 3503	68	83	2*(5aa t4); 2*(1b-c t1); 1*(3d t1)	1/2
-6	X 3505	38	43	3**(4a-b t1); 1**(1a t1)	1/2
-20	X 4127	115	125	--	1/2
-21	X 3505	112	116	2(2b t1)	1/2
0 3529-2	X 0351	101	334	3⁺(5aa t4)	--
-17	X 0355	103	320	5(3-4c faint); 1(t7)	--
-18	X 2151	90	255	9(3-4c faint); 3(t7)	--
-19	X 0352	134(4-5b-c)	372	5⁺⁺(3-4c faint); 1⁺⁺(1b-c t1); 4⁺⁺(t7); 5⁺⁺(5aa t4)	--
1981 rows containing selected kernels[a] ⟹				*4404A(3d t1-2), B(1b-c t1), C(5aa t4) **4404W(4a-b t1), X(1a t1) ⁺4404Z(5aa t4) ⁺⁺4405A(t7), B(5aa t4), C(3-4c t1 faint), D(1b-c t1)	

[a]Selected progeny kernels and their assigned rows are identified by the same superscript.

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exceptions are presented in the following section.

1) Tests of exceptional phenotypes derived among test

cross progenies of 3-4c t1-2 (Table A.36)

(a) 3d t1 spotted (1 4404A)

Kernel 1 4404A (3d t1) is characterized by larger spots than those

in parental kernels (3-4c t1-2) (Table 4.36, 0 3526-1 X 3503). But in

test crosses, the 3d t1 kernel yielded 3-4c t1-2 spotted (Table 4.37A),

indicating that this phenotype is not heritable. The non-heritability

can be explained by the following:

    (1) The 3d t1 spotted represents a change caused by the environ-

        ment;

    (2) Frequent changes in phase of activity of Spm.

Some other exceptional spotted phenotypes that were not heritable

in test crosses include 1b-c t1 (4404B, 4405D), 4a-b t1 (4404W),

3-4c t1 (faint) (4405C) and 1a t1 (4404X) (Figure 4.18A, 1981).

(b) 5aa t4 fine specked (1 4404C, 1 4404, 1 4405B)

The kernels of 5aa t4 type appear pale (t4) but under a microscope,

they reveal fine specks at high frequency (5aa). The 1 4404C, 1 4404Z,

and 1 4404B represent kernels of 5aa t4 phenotype derived from three

separate test crosses of 3-4c t1-2 spotted (Table 4.36, 0 3526-1 X 3503,

0 3529-2 X 0351 and 0 3529-19 X 0352).

(i) 1 4404C (from Table 4.36, 0 3526-1 X 3503)

In most test crosses, the progeny kernels of 1 4404C included 5aa

type but their appearance ranged from light-pale (t3) to dark-pale

(t5-6) (Table 4.37 B(1)). In one of the reciprocal crosses

(1 4342 X 4404C-2), however, there were only colorless (t1) progeny
kernels.

The test of a-m(r)/a-m-1 showed that the 5aa t4 type does not con-
tain an Spm that can trigger mutations of a-m(r) or a-m-1 alleles
(Table 4.37 B(2)). However, the 5aa t4 type appears in the progeny of
this test (a-m(r) Sh2/a-m-2 7977B Sh2) and is distinguishable from
colored (a-m-2 7977B Sh2/a-m-1 sh2) and colorless (a sh2/a-m(r) Sh2)
progeny types.

(ii) 1 4404Z (from Table 4.36, 0 3529-2 X 0351)

In test crosses, the 5aa t4 phenotype of 1 4404Z is not heritable
(Table 4.37 C(1)). The progenies include either colored (t7) or pale
(t3-4--in reciprocal crosses) kernels. In tests on a-m(r)/a-m-1, no
spotted kernels appeared (Table 4.37 C(2)).

(iii) 1 4405B (from Table 4.36 0 3529-19 X 0352)

The test cross progenies include 5aa type kernels and their appear-
ance ranges from pale (t3-4) to dark-pale (t5-6) (Table 4.37 D(1)).
Some of these progeny kernels contain single spots (Figure 4.20A). In
tests on a-m(r)/a-m-1 (Table 4.37 D(2)), the progenies include 3c-d with
specks and 4-5c t1 spotted kernels (Figure 4.20B), indicating that the
5aa t4 kernels of 1 4405B contain a highly active Spm. These results
are in contrast to that of the other two cultures of 5aa t4 (1 4404C,
1 4404Z) which do not contain Spm.

In summary, the heritability of 5aa t4 phenotype varied in three
independently isolated cultures. The 5aa t4 of 1 4404C is heritable in
most test crosses (Table 4.37 B(1)) but the appearance ranges from t3 to

Table 4.37. Tests of exceptional spotted phenotypes derived among test cross progenies of 3-4c tl-2 (Table A.36)

A. 3d tl = 1 4404A (1 kernel)

| a-m-2 7977B Sh2, Spm X a sh2 | | Round | | | Shrunken |
| a   sh2   a sh2 | | | | | |
3d tl X cl,sh	Spotted 3-4c tl-2	Colorless	Exceptions	Colorless
1 4404A X 4222	27	13	1(t7); 1(5aa t6)	1/2
A-t X 4416	32	17	3(5aa t4-5)	1/2
Reciprocal crosses				
1 4420 X 4404A	68	70	4(t7); 6(5aa t5)	1/2
4324 X 4404A	48	52	2(t7)	1/2
4350 X 4404A	47	52	1(t7); 1(5aa t4)	1/2

Table 4.37. (Continued)

B. 1 4404C = 5aa (appear t4)

a-m-2 7977B Sh2 Spm? X a sh2 / a sh2 sh2 / 5aa t4 X cl,sh		Round			Shrunken
	Spotted	Pale 5aa type	Colorless		Colorless
(1) Progeny test					
1 4404C-1 X 4223	--	1/2 (appear t5)	--		1/2
-2 X 4419	--	1/2 (appear t3)	--		1/2
Reciprocal crosses					
1 4341 X 4404C-1	--	1/2 (appear t4-5)	--		1/2
4343 X -1	--	1/2 (appear t5-6)	--		1/2
4342 X -2	--	--	1/2		1/2

a-m(r) Sh2 X a-m-2 7977B Sh2 / a-m-1 sh2 / a sh2		Round			Shrunken	
	Spotted	5aa t4	colored t7	colorless t1	colored t7	spotted
(2) Test for presence of Spm						
4426 X 4404C-1	--	69	76	73	81	--

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Table 4.37. (Continued)

C.   5aa t4 = 1 4404Z

a-m-2 7977B Sh2 Spm? ─── a ／ sh2 5aa t4 X a sh2 ─── a sh2 X cl,sh	Round					Shrunken
	Spotted	5aa t4	Colored t7	Pale t3-4	Colorless	Colorless
(1) Progeny test						
1 4404Z-1 X 4223	--	--	1/3	--	--	1/2
-2 X 4417	--	--	1/2	--	--	1/2
-3 X 4223	--	--	1/2	--	--	1/2
Reciprocal crosses						
1 4348 X 4404Z-1	--	--	--	1/2	--	1/2
4446 X -2	--	--	--	1/2	--	1/2
4341 X -3	--	--	--	1/2	--	1/2

a-m(r) Sh2 ─── a-m-1 sh2 X a-m-2 7977B Sh2 ─── a sh2	Shrunken Colored

(2) Test for presence of Spm in 5aa t4	Spotted	5aa t4	Colored t7	Pale t3-4	Colorless	Shrunken
1 4351 X 4404Z-1	--	--	√[a]	--	√	Somewhat mottled

[a]Indicates presence of kernels of the type in that column.

Table 4.37. (Continued)

D. 5aa t4 = 1 4405B

| $\dfrac{\text{a-m-2 7977B}}{\text{a}} \dfrac{\text{Sh2}}{\text{sh2}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$ | | Round | | | Shrunken |
t7 X cl,sh	Spotted	Colored	Pale 5aa type	Color- less
(1) Progeny test				
1 4405B-1 X 4321	--	--	1/2(t5-6)[b]	1/2
-2 X 4420	--	--	1/2(t3-4)	1/2
-3 X 4321	--	--	1/2(t5-6)[b]	1/2
-4 X 4324	12 (single spot t3 bkg)	--	70(t3-4)	1/2
-5 X 4321	--	--	1/2(t5-6)[b]	1/2
-6 X 4324	--	--	1/2(t5-6)[b]	1/2

$\dfrac{\text{a-m(r)}}{\text{a-m-1}} \dfrac{\text{Sh2}}{\text{sh2}}$ X $\dfrac{\text{a-m-2 7977B}}{\text{a}} \dfrac{\text{Sh2}}{\text{sh2}}$	Spotted

(2) Test for presence of Spm in 5aa t4

| 1 4240 X 4405B-3 | 3c-d with specks, 4-5c tl |
| 4354 X   -4 | 3c-d with specks, 4-5c tl |
4354 X -5	3c-d with specks, 4-5c tl

[b]Some kernels are single spotted.

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Figure 4.20. Progeny test ears of exceptional 5aa t4 kernels of
a-m-2 7977B state

    A. Test cross progeny ear of 5aa t4 (Table 4.37D(1),
       1 4405B-1 X 4321), containing a few single spotted
       (arrow) kernels

    B. Progeny ear resulting from a test of 5aa t4 on
       a-m(r)/a-m-1 (Table 4.37D(2), 1 4240 X 4405B-3)

A
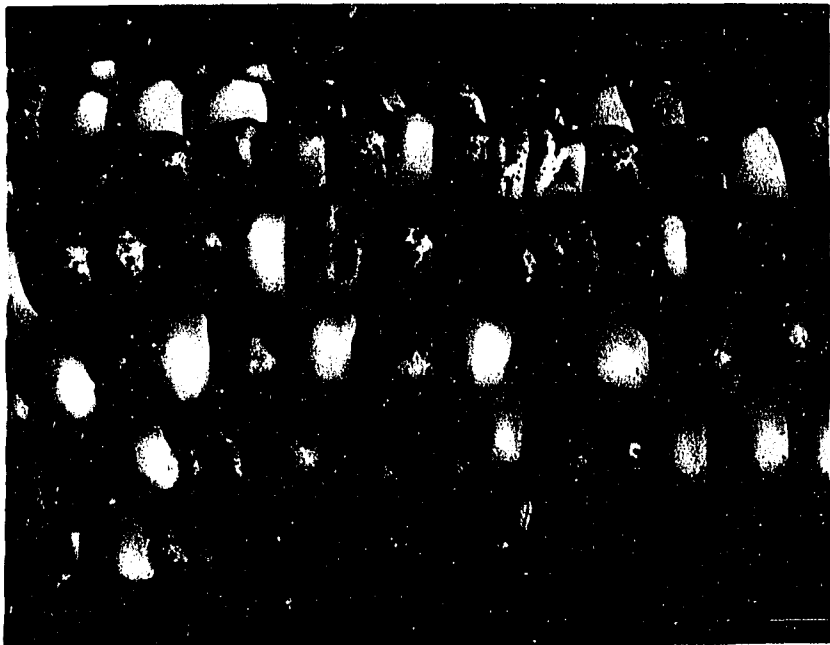
B

t5-6. Similar results are obtained with 1 4405B (Table 4.37 D(1)), but some progenies contained single spots. In contrast to these test results, the 5aa t4 of 1 4404Z is not heritable (Table 4.37 C(1)). The progeny included colored (t7) or pale (t3-4--in reciprocal crosses) kernels.

In tests on a-m(r)/a-m-1, while no Spm is detected in 5aa t4 of 1 4404C and 1 4404Z (Table 4.37 B(2), C(2)), the 5aa t4 of 1 4405B do contain a highly active Spm (Table 4.37 D(2)). The presence of Spm in 1 4405B coincided with the occurrence of single-spotted kernels in test cross progenies.

It can be concluded from these results that the 5aa t4 phenotype represents a change in state of the a-m-2 7977B allele. The expression of 5aa type fine specks and the background pigmentation are variable among test crosses and can be suggested to represent the basic allele phenotype (-Spm) of the new state. In the presence of Spm, this new state is single spotted.

c. Response of exceptional pale (t3-4) phenotype to En   The kernels with t3-4 pale phenotype (Figure 4.18A, 1979 3242) were derived among the selfed progeny of 3-4c t1-2 spotted kernels (1977 3405). These pales were tested against two Ens differing in mutator (M) activity--En-low and En-high. With En-high, the spotted response equalled 3c t2-5 spotted and rest of the progeny included colorless (t1) and variable pale types (t2-3, t4-5) (Table 4.38a). With En-low, 1-2b t1-3 spotted, colorless (t1) and t2-3 pales resulted.

With spotted among these progenies, the t3-4 exceptional pales are proven to be responsive to both En-high and En-low. But the non-spotted progeny kernels vary in pigmentation (Table 4.38a). To determine the factors associated with this unexpected variability, the colorless (t1) and the light-pale (t2-3) progeny kernels are further tested. These tests will be presented in section 1).

The t3-4 pales derived from 7 3405 are tested for their Spm content in crosses on a-m(r)/a-m-1. A highly active Spm is present in two out of four crosses indicating that the t3-4 pales represent a changed state of a-m-2 7977B that is not responsive to Spm. But this changed state (t3-4) was shown to be responsive to En (Table 4.38a). These differential responses of the t3-4 pale state to Spm and En suggest an unprecedented difference between the Spm and the En modes of action or the receptivity of the allele to one and not the other.

1) Analyses of t1 colorless (1 3521) and t2-3 pales (1 3522 derived from t3-4 X a sh En cross (Table 4.38a, 9 3242-45 X 3311-8)

The colorless and the light-pales were tested for their response to En and also for their heritability (Tables 4.39 and 4.40). Both responded to En in crosses with a sh + En plants. The colorless phenotype is heritable in crosses with a sh - En plants, except that in one of the crosses (Table 4.39, 0 3521-18 X 3507-10) several colorless kernels contained single spots. The light-pales (t2-3) in most crosses with a sh - En plants contained colorless to very light pale (t1-2) and several 1b t1-2 spotted progeny kernels (Table 4.40).

The occurrence of low spotted kernels (single spotted or 1b t1-2)

Table 4.38. Analysis of t3-4 pales derived in selfed progeny of 3-4c t1-2 spotted kernels (Figure 4.18B, 1979 3242)

$\dfrac{\text{a-m-2 7977B Sh2}}{\text{a-m-2 7977B Sh2/a sh2}}$, Spm? X $\dfrac{\text{a sh2, En}}{\text{a sh2}}$

a) Response of t3-4 pales to En

t3-4	X cl,sh ± En	Spotted 3c t2-5	Spotted 1-2b t1-3	Colorless t1	Pales t2-3	Pales t4-5	Mottled	Shrunken Colorless
9 3242-4t	X 3311-8 +[a](hi)[b]	143	7	47*	29*	18	5	—
-8	X 3310-2 +(hi)	15	—	5	10	6	—	1/2
-20	X 1722-4 -[c]	—	—	1	—	120	—	1/2
-21	X -9 +(lo)[d]	—	35	63	10	—	—	1/2
-22	X -7 +(lo)	—	32	20	64	—	—	1/2
-23	X -8 +(lo)	—	39	67	10	—	—	1/2
1981 rows containing selected kernels[e] ⟹				(3521*)	(3522*)			

$\dfrac{\text{a-m(r) Sh2}}{\text{a-m(r) Sh2}}$ X $\dfrac{\text{a-m-2 7977B Sh2}}{\text{a-m-2 7977B Sh2/a sh2}}$ ± Spm?

b) Test for Spm content of t3-4 pales

		Round Spotted 5b t1-3	Round Colorless t1	Round Pales t2-3	Round Pales t4-5
3324	X 3242-12	—	1/2	1/2	—
3324	X -13	—	all	—	—
3324	X -9	211	4	6	—
3324	X -8	86	69	56	—

[a]Presence of En confirmed in tests on a-m(r)/a-m-1.
[b]Hi – En with high mutator activity.
[c]Absence of En confirmed in tests on a-m(r)/a-m-1.
[d]Lo – En with low mutator activity.
[e]Selected progeny kernels and their assigned rows in a column are identified by the same superscript.

Table 4.39. Analysis of colorless (tl) derived in the progeny of t3-4 pales following the reactivation with $\underline{En}$ (from Table 4.38a, 9 3242-4t X 3311-8)

$\underline{\text{a-m-2 7977B}}$ $\underline{\text{Sh2}}$ X $\underline{\text{a sh2}}$ ± $\underline{En}$		Round		Shrunken
$\underline{\quad\text{a}\quad}$ $\underline{\text{sh2}}$ a sh2				
		Spotted	Colorless	Colorless
tl	X cl,sh ± $\underline{En}$	(4c tl-4)	(tl)	
D 3521-15	X 3507-1 +[a]	10	17	1/2
-16	X -2 -[b]	--	1/2	1/2
-17	X -6 -	--	1/2	1/2
-18	X -10 -	--	1/2[c]	1/2
-19	X 1431 -	--	1/2	1/2
-3	X 1433 -	--	1/2	1/2

[a]Presence of $\underline{En}$ confirmed in tests on $\underline{\text{a-m(r)}}/\underline{\text{a-m-1}}$.

[b]Absence of $\underline{En}$ confirmed in tests on $\underline{\text{a-m(r)}}/\underline{\text{a-m-1}}$

[c]Several single spotted.

Table 4.40.   Analysis of t2-3 pales derived in the progeny of t3-4
following the reactivation with $\underline{En}$ (from Table 4.38a
9 3242-4t X 3311-8)

a-m-2 7977B $\frac{Sh2}{sh2}$ X $\frac{a\ sh2}{a\ sh2}$ $\pm$ $\underline{En}$		Round			Shrunken
		Spotted		Colorless-very light pale t1-2	Color-less
t2-3	X cl,sh $\pm$ $\underline{En}$	3-4c t4-5	1b t1-2		
0 3522-15	X 1528-10t $+^a$	19	--	42	1/2
-16	X 3507-4 +	14	2	15	1/2
-17	X -6 $-^b$	--	--	1/2	1/2
-18	X 3508-3 +	64	--	50	1/2
-19	X -4 -	--	27	106	1/2
-20	X 2160 -	--	12	235	1/2
-21	X 2156 -	--	12	61	1/2
-22	X 2155 -	--	9	94	1/2

[a]Presence of $\underline{En}$ confirmed in tests on $\underline{a\text{-}m(r)}/\underline{a\text{-}m\text{-}1}$.

[b]Absence of $\underline{En}$ confirmed in tests on $\underline{a\text{-}m(r)}/\underline{a\text{-}m\text{-}1}$.

among the progenies of colorless (t1) and light-pales (t2-3) indicates

that these cultures either contain a low active Spm or they represent

a changed state of a-m-2 7977B that exhibits a low response to an

otherwise highly active Spm. It is most likely that the low spotting

of t1 and t2-3 is in response to a highly active Spm whose presence

has already been detected in the parental t3-4 kernels (Table 4.38b).

Since the t1 and t2-3 are derived from the original t3-4 pale excep-

tion, it can be deduced that all these variable phenotypes represent a

new state of a-m-2 7977B that exhibits only a sporadic low response to

Spm. Further tests are required to confirm the presence of an active

Spm in t1 and t2-3 (similar to that in parental t3-4).

2. Part 2

    a. Heritability of 3-4c t4 spotted phenotype   Among the

test cross progeny of the original 3-4c t4 spotted kernel of a-m-2 7977B

(Figure 4.19A, 1976 1609-2 progeny), there were 3-4c spotted with

t1 and t4 background pigmentation, colorless (t1) with or without

faint pale areas and a few exceptions. The appearance of two types

of background pigmentation (t1 and t4) of the 3-4c spotted was

further confirmed in the selfed progeny of 3-4c t4 spotted kernels

(1977 3402). These results show that despite selection for 3-4c t4

spotted, some of the 3-4c spotted progeny kernels have colorless (t1)

background. This change in the background pigmentation of spotted

kernels can be attributed either to a change in phase of the suppressor

(S) component of Spm or the allele is not fully suppressed in some cells.

In addition to the 3-4c t4 and 3-4c t1 spotted, the selfed

progeny of 1977 3402 (Figure 4.19A) included colorless with or with-

out faint areas (the basic allele phenotype), and the exceptions

colored (t7) and 2b-c t1-3 spotted. Tests were made to determine the

heritability of colorless with faint pale areas, and of the exceptional

phenotypes.

   b. Heritability of colorless with faint pale areas   The

progeny of colorless with faint pale areas included the parental type

and colorless without the faint pale areas (Table 4.41a). These two

types did not occur in any consistent ratio among the progenies indi-

cating that the a-m-2 7977B allele randomly exhibits faint pale areas

in colorless background.

   In crosses with a-m(r)/a-m(r), there were only colorless kernels

in the progeny (Table 4.41b), confirming that the colorless with pale

areas do not contain Spm. Also, the absence of pale areas on the

colorless progeny kernels indicates that the a-m(r) allele inhibits

the faint pale area expression of a-m-2 7977 allele.

   c. Analysis of colored (t7) exceptional kernel   The colored

exceptional kernel (Figure 4.19B, 1977 3402 progeny) in a cross

with a sh - En (Table 4.42, 9 3237-4 X 3310-5) produced colored

and light-pale (t2-3) progeny kernels in 1:1 ratio. The colored

progeny in turn in crosses with a sh En yielded the same type (Table

4.42, 1980, 1981), whereas the t2-3 pales in crosses with a sh - En

produced the parental type (t2-3), non-uniform pale (t5) and colorless

(t1) (Table 4.43). The t2-3 pale progeny upon microscopic examination

Table 4.41. Heritability of colorless (t1) with faint pale areas
derived from selfed progeny of 3-4c t4 spotted kernels
(Figure 4.19A, 1977 3402)

$\dfrac{\text{a-m-2 7977B Sh2}}{\text{a-m-2 7977B Sh2/a sh2}}$ ⓧ or X $\dfrac{\text{a sh2}}{\text{a sh2}}$	Colorless (t1) c̄ faint pale areas (2b type)	Colorless (t1)
t1 c̄ faint pale areas X cl,sh		

a) Progeny test

		Colorless (t1) c̄ faint pale areas (2b type)	Colorless (t1)
9 3238-6 ⓧ		83*	145
-7 ⓧ		48	17
-15 ⓧ		15	115
-20	X 3308	73	16

1980 rows with selected kernels ⟹ ⟶ (3519*)

		Colorless (t1) c̄ faint pale areas (2b type)	Colorless
0 3519-2	X 3507-12 – En	31	8
-3	X 3508-12 – En	52	14

$\dfrac{\text{a-m(r) Sh2}}{\text{a-m(r) Sh2}}$ X $\dfrac{\text{a-m-2 7977B Sh2}}{\text{a}\qquad\text{sh2}}$	Spotted	Colorless

b) Test for presence of Spm

		Spotted	Colorless
0 3459	X 3519-2	--	all
3460	X 3519-3	--	all

Table 4.42. Heritability and response of colored (t7) kernels to En;
the colored kernels are derived in the selfed progeny of
3-4c t4 spotted kernels (Figure 4.19A, 1977 3402 progeny)

a-m-2 7977B Sh2	X a sh2 ± En		Round		Shrunken
a-m-2 7977B Sh2/a sh2	a sh2	Spotted	Colored	Light pale	Colorless
t7	X cl,sh ± En		(t7)	(t2-3)	
9 3237-1 +[a]	X 3310-5 -[b]	--	30*	24*	--
1980 rows with selected kernels[c] ⟹			(3517*)	(3516*)	
0 3517-1 -	X 3507-8 +	--	1/2	--	1/2
-2 -	X -2 +	--	1/2*	--	1/2
1981 rows with selected kernels ⟹			(4336*)		
1 4336-1 -	X 4220-1 +	--	1/2	--	1/2
-3 -	X -7 +	--	1/2	--	1/2
-7 -	X -6 +	--	1/2	--	1/2
-10 -	X -3 +	--	1/2	--	1/2

[a]Indicates presence of En or Spm in tests on a-m(r).

[b]Indicates absence of En or Spm in tests on a-m(r).

[c]Selected kernels and their assigned rows in a column are identified by the same superscript.

Table 4.43. Heritability of t2-3 pales (O 3516) derived in the progeny of colored (from Table 4.42, 9 3237-1 X 3310-5)

a-m-2 7977B Sh2 $\quad$ a sh2 - En			Round			Shrunken
			Pales			
$\dfrac{\text{a}}{\text{t2-3}}$ $\dfrac{\text{sh2}}{}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$ X cl,sh - En			t2-3 (some single spotted)	t5 (not uni-form)	Color-less (t1)	Color-less
O 3516-1	X 3508-3		96	54	3	138
-5	X	-10	124*	36*	10	155
-15	X	-4	135	44	9	173
-20	X	-2	144	6	3	160
-3	X 1434		54	3	32	110
1981 rows containing selected kernels[a] $\Longrightarrow$			(4335C*) (selected only spot-ted 1b t2-3)	(4334A*)		

[a]Selected kernels and their assigned rows in a column are identified by the same superscript.

revealed fine, single spots (1b t2-3). These 1b t2-3 (1 4335C) and the
non-uniform t5 pale (1 4334A) progenies were further test crossed
(Table 4.44 A(1), B(1)). The 1b t2-3 spotted produced colorless to very
light pales (t1-2--some are single spotted), non-uniform pales (t4-5--
some contain 5aa type specks), 3c t1-2 spotted (1 kernel) and colored
(one kernel) (Table 4.44 A(1)). The non-uniform pales (t5) produced
variable pales (t1-5) (Table 4.44 B(1)). In crosses on a-m(r)/a-m-1,
both 1b t2-3 and non-uniform t5 pales (not all t5 pales) contain a
highly active Spm (Table 4.44 A(2), B(2)).

In summary, these series of tests demonstrate the following:

(1) The original exceptional colored kernel (Table 4.42, 9 3237-1)
    contains two changed allelic states of a-m-2 7977B. They are
    represented by colored (0 3517) and t2-3 pale (0 3516) pheno-
    types (Table 4.42, 9 3237-1 X 3310-5 progeny).

(2) The colored phenotype is not responsive to Spm or En (Table
    4.42, 1980, 1981 tests).

(3) The t2-3 pales are responsive (some progeny kernels have
    single spots) to Spm, but their progeny in test crosses
    ranges from t1 to t5 (Table 4.43, 4.44 A(1)). The t5 pale
    progeny kernels are non-uniform and their progeny kernels
    in turn range from t1-5 (Table 4.44 B(1)).

There is an indication that the colored phenotype represents a
change in state from mutable (original t1) to non-mutable wild-type
allele, whereas the t2-3 pale appears to be a changed Spm-responding
state (fine-single spots) and the phenotype of this state ranges from

Table 4.44. Heritability and Spm content of (A) 1b t2-3 spotted
(1 4335C) and (B) non-uniform t5 pales (1 4334A) (from
Table 4.43, O 3516-5 X 3508-10)

A. 1b t2-3 spotted (1 4335C)

$\dfrac{\text{a-m-2 7977B}}{\text{a}} \dfrac{\text{Sh2}}{\text{sh2}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$		Round			Shrunken
1b t2-3 X cl,sh	t1-2 (some single spotted)	Some non-uniform some 5aa type	Exceptions		Color- less
(1) Progeny test					
1 4335-1 X 1211t	23	49	--		1/2
-2 X 1720	115	26	1(3c t1-2), 1(t7)		1/2
-3 X 1720	14	66	--		1/2
-4 X 4308	64	54	--		1/2

$\dfrac{\text{a-m(r)}}{\text{a-m-1}} \dfrac{\text{Sh2}}{\text{sh2}}$ X $\dfrac{\text{a-m-2 7977B}}{\text{a}} \dfrac{\text{Sh2}}{\text{sh2}}$	Spotted

(2) Test for presence of Spm

1 4316 X 4335-1	3c-d $\overline{c}$ specks, 4-5b-c t1
4316 X -2	3c-d $\overline{c}$ specks, 4-5b-c t1
4316 X -3	3c-d $\overline{c}$ specks, 4-5b-c t1
4354 X -4	3c-d $\overline{c}$ specks, 4-5b-c t1

Table 4.44.　(Continued)

B.　Non-uniform t5 pales (1 4334A)

$\dfrac{\text{a-m-2 7977B}}{\text{a}}\dfrac{\text{Sh2}}{\text{sh2}}$ X $\dfrac{\text{a}}{\text{a}}\dfrac{\text{sh2}}{\text{sh2}}$	Round		Shrunken
	Variable pales (t1-5)	Spotted	Color-less
1b t2-3　　　　X cl,sh			

(1) Progeny test

	Variable pales (t1-5)	Spotted	Color-less
1 4334A-1　X 4227	1/2	--	1/2
-2　X 4227	1/2	--	1/2
-3　X 1721	1/2	--	1/2
-4　X 4308	1/2	--	1/2
-5　X 1721	1/2	--	1/2

$\dfrac{\text{a-m(r)}}{\text{a-m-1}}\dfrac{\text{Sh2}}{\text{sh2}}$ X $\dfrac{\text{a-m-2 7977B}}{\text{a}}\dfrac{\text{Sh2}}{\text{sh2}}$	Spotted

(2) Test for presence of Spm

1 4335 X 4334A-1	3c-d $\bar{\text{c}}$ specks, 4-5b-c t1
4353 X -3	3c-d $\bar{\text{c}}$ specks, 4-5b-c t1
4352 X -5	3c-d $\bar{\text{c}}$ specks, 4-5b-c t1

t1-t5.

    d. <u>Analyses</u> <u>of</u> <u>2b-c</u> <u>t1-3</u> <u>spotted</u> <u>exceptions</u>   The 2b-c t1-3

spotted were derived in the selfed progeny of 3-4c t4 spotted (Figure

4.19A, 1977 3402 progeny). In test crosses, the 2b-c t1-3 spotted

produced the parental type (2b-c t1-3), colorless (t1), and several

exceptions that include mottled and 3-4b-c t1 spotted types (Figure

4.19C, 1979 3234 progeny). At this point of study, the appearance of

3-4b-c t1 spotted exceptions in the progeny of 2b-c t1-3 suggested that

the mutator (<u>M</u>) component of <u>Spm</u> reverted to its original state trig-

gering high frequency (3-4c) mutations.

    The <u>Spm</u> was further analyzed in test crosses of 2b-c t1-3 spotted

kernels (Table 4.45, 1 3509, 1 3510). Parental types, colorless (t1)

and several exceptional types were included in the progeny. The six

exceptional types are given below:

<pre>
                        2b-c t1-3

  3-4c t1   4-5c t1   4c t1 with   3-4c t1 with   mottled   t7
                        2c t1 sector t1-2 sector
</pre>
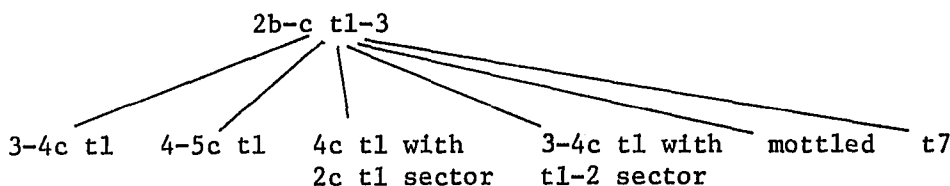
What is clear is that the spot size and the frequency of the

spotted exceptions agree closely with original state (3-4c t4) of

<u>a-m-2 7977B</u> (Figure 4.19A, 1976 1609-2) confirming a reversion in the

mutator (<u>M</u>) component of <u>Spm</u>. Certain instabilities of the spotted

derivatives are expressed in some of the 3-4c t1 spotted exceptions

where sectors of the 2c t1 and t1-2 occur. The 2c t1 sector is due to

Table 4.45. Heritability of 2b-c t1-3 spotted kernels that were
originally derived in selfed progeny of 3-4c t4 spotted
kernels (Figure 4.19A, 1977 3402 progeny)

$\dfrac{\text{a-m-2 7977B Sh2}}{a}$, $\dfrac{\text{Spm}}{\text{sh2}}$ X $\dfrac{a\ sh2}{a\ sh2}$		Spotted (2b-c t1-3)	Color-less (t1)	Exceptions
2b-c t1-3	X cl,sh			
1 3509-1	X 0353	135	142	2*(3-4c t1); 1(4-5c t1)
-15	X 0353	84	80	6**(3-4c t1 side by side in a row)
-20	X 0353	102	126	1(4c t1 c̄ 2c t1 sector)
-21	X 0352	153	135	1(3-4c t1)
1 3510-1	X 0353	142	100	12⁺(large mottles)
-20	X 3503	115	93	1(t7)
-21	X 0353	119	127	1⁺⁺(4-5c t1); 1(4c t1 c̄ 2c t1 sector)
-22	X 0353	95	90	15 (mottled)
-23	X 4127	101	96	4 (mottled)
Reciprocal crosses				
1 3504	X 3509-1t	27	57	1(3-4c t1 c̄ t1-2 sector)
3505	X 3509-3t	54	70	--
3506	X 3510-12t	67	83	8(mottled); 1*(3c t1)
1981 rows containing the selected kernels[a] ⟹				(4326C*) (4328X*) (4327A**) (4328A⁺) (4328Y⁺⁺)

[a]Selected kernels and their assigned rows in a column are identified by the same superscript.

reversion in M component of Spm to that of low mutating type and the

tl-2 sector can be attributed to a loss or change in phase of activity

of Spm from an active to an inactive state.

The spotted exceptions are further analyzed for their heritability

and the results of only those with significant differences are presented

here.

1) Heritability of spotted exceptions derived from 2b-c

tl-3 spotted (Figure 4.19C, 1980 3509, 3510 progeny)

(a) 3-4c tl (1 4326C)

The 3-4c tl phenotype is not heritable (Table 4.46 A(1)). The test

cross progeny included spotted type (1-2a-b tl) that is similar to

2b-c tl-3 from which the 3-4c tl exception was derived. In tests with

a-m(r)/a-m-1, the same low spotted (1-2a-b tl) progeny appeared (Table

4.46 A(2)) indicating that the 3-4c tl exceptional phenotype is not

transmissible and the expression is confined to the endosperm.

(b) 3-4c tl (6 kernels all in a row side by side) 1 4327A

The 3-4c tl spotted phenotype is heritable among test cross

progenies (Table 4.46 B(1)). However, one of the crosses (1 4327A-2 X

4225) contains a new expression (1c tl) that confirms an additional

change in state of Spm.

(c) 3c tl (1 4328X)

The 3c tl phenotype is only heritable in the reciprocal cross

(Table 4.46C). In the straight cross (1 4328X X 4224), the spotted

progeny are 5c tl-2 type, also deviant from the parental. Reciprocal

differences of this type emphasize the nature of the subtle changes in

Table 4.46. Analyses of the exceptional phenotypes that were selected in test cross progenies of 2b-c tl-3 spotted kernels (from Table 4.45)

A. 3-4c tl spotted (1 4326C)

$\dfrac{\text{a-m-2 7977B}}{\text{a}}\ \dfrac{\text{Sh2,}}{\text{sh2}}\ \text{Spm}\ \ X\ \dfrac{\text{a}}{\text{a}}\ \dfrac{\text{sh2}}{\text{sh2}}$	Round	
3-4c tl ⎯⎯⎯⎯⎯⎯ X cl,sh	Spotted (1-2a-b tl)	Colorless (tl)

(1) Progeny test

1 4326C	X 4221	93	100
Reciprocal cross			
1 4311	X 4326C	42	68

$\dfrac{\text{a-m(r)}}{\text{a-m-1}}\ \dfrac{\text{Sh2}}{\text{sh2}}\ X\ \dfrac{\text{a-m-2 7977B}}{\text{a}}\ \dfrac{\text{Sh2,}}{\text{sh2}}\ \text{Spm}$	Round			Shrunken	
	Spotted (1-2a-b tl)	Color-less	Colored	Colored	Spotted (1-2b tl)

(2) Test for type of Spm in 3-4c tl

1 4434 X 4326C	83	91	30	34	41

B. 3-4c tl spotted (1 4327A)

$\dfrac{\text{a-m-2 7977B}}{\text{a}}\ \dfrac{\text{Sh2,}}{\text{sh2}}\ \text{Spm}\ X\ \dfrac{\text{a}}{\text{a}}\ \dfrac{\text{sh2}}{\text{sh2}}$	Round		Colorless or very light pale (tl-2)
	Spotted		
3-4c tl ⎯⎯⎯⎯⎯⎯ X cl,sh	(3-4c tl-2)	(1c tl)	

(1) Progeny test

1 4327A-1	X 4224	30	--	39
-2	X 4225	58	23	31
Reciprocal cross				
1 4308	X 4327A-1	56	--	133

$\dfrac{\text{a-m(r)}}{\text{a-m-1}}\ \dfrac{\text{Sh2}}{\text{sh2}}\ X\ \dfrac{\text{a-m-2 7977B}}{\text{a}}\ \dfrac{\text{Sh2,}}{\text{sh2}}\ \text{Spm}$	Round			Shrunken	
	Spotted (3-4c tl)	Color-less	Colored	Colored	Spotted

(2) Test for type of Spm in 3-4c tl

1 4356 X 4327A-1	121	87	45	48	36

Table 4.46. (Continued)

C. 3c tl (1 4328X)

a-m-2 7977B Sh2, Spm X a sh2		a sh2	Round			Shrunken
a	sh2	a sh2				
			Spotted	Colorless	Light	Color-
3c tl		X cl,sh	(3c tl-2)	(tl)	mottled	less

		Spotted (3c tl-2)	Colorless (tl)	Light mottled	Color-less
1 4328X	X 4224	67 (5c tl-2)	56	14	1/2
Reciprocal crosses					
1 4309	X 4328X	78	43	23	1/2
4344	X 4328X	86	83	5	1/2

D. 4-5c tl (1 4328Y)

a-m-2 7977B Sh2, Spm X a sh2		a sh2	Round		Shrunken
a	sh2	a sh2	Spotted	Colorless	Color-
4-5c tl		X cl,sh	4-5b-c		less
			tl-2	tl	

		Spotted 4-5b-c tl-2	Colorless tl	Color-less
1 4328Y	X 4224	57	86	1/2
Reciprocal cross				
1 4350	X 4328Y	26	45	1/2

phase of activity of Spm.

(d)   4-5c tl (1 4328Y)

The 4-5c tl spotted pattern is heritable in both straight and reciprocal crosses (Table 4.46D). All the spotted progeny kernels exhibited the 4-5c tl phenotype.

The general conclusions of these analyses of heritability of the exceptional spotted confirm that the mutator (M) component of Spm undergoes frequent changes in phase of activity and these changes occur in both directions--that is to a lower or a higher activity level. All these changes in phase of activity of Spm starting from the original 3c t4 spotted kernel are summarized in the following diagram:
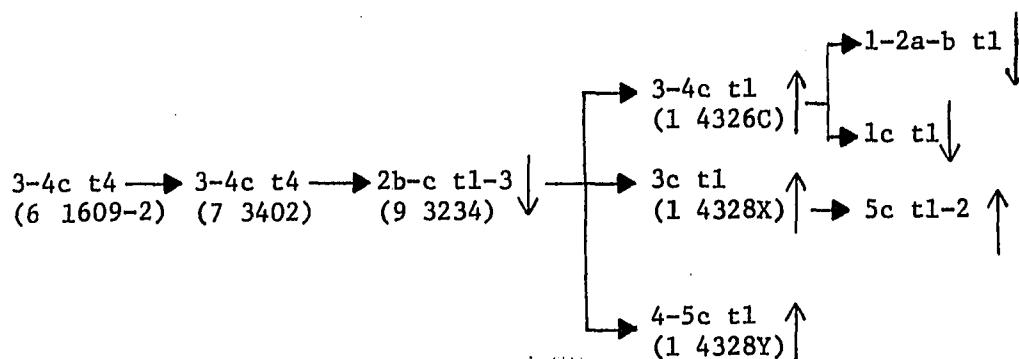
Numbers in parentheses refer to the row numbers given in Figures 419A and 419C. Vertical arrows indicate changes in phase to high (↑) or low (↓) mutator (M) activities of Spm.

2)   Heritability of mottled exceptions   The mottled exceptions that arose among the test cross progenies of 2b-c tl-3 spotted (Table 4.45) contain large pale areas on a colorless background (no spots) when compared to the basic allele phenotype (colorless with small faint pale areas) of a-m-2 7977B (Figure 4.17B). Since the basic

Table 4.47. Response to Spm and Spm content of mottled (1 4328A)
derived in test cross progenies of 2b-c tl-3 spotted
(Figure 4.19C, 1980 3510 progeny)

a-m-2 7977B Sh2 X a sh2 ± Spm sibs	Round			Shrunken
a sh2 a sh2 mottled X cl,sh ± Spm sibs	Spotted (1-2b tl)	Color-less	Light mottled	Color-less

a) Response of mottled to Spm in a sh sibs

1 4328A-3	X	4328B-4	+[a]	64	84	10	1/2
-4	X	-1	-[b]	--	1/2	few	1/2
-5	X	-5	+	61	55	5	1/2
-7	X	-3	+	62	50	2	1/2

Reciprocal crosses

1 4328B-3 +	X	4328A-5		52	57	4	1/2
-1 -	X	-7		--	1/2	--	1/2
-6 -	X	-1		--	1/2	--	1/2

$$\frac{a\text{-}m(r)\ Sh2}{a\text{-}m\text{-}1\ \ sh2} \quad X \quad \frac{a\text{-}m\text{-}2\ 7977B\ Sh2}{a\qquad\quad sh2}$$

b) Test for presence of Spm in mottled

1 4353	X	4328A-1	no spotted
4354	X	-3	no spotted
4429	X	-4	no spotted

[a]Indicates presence of Spm in tests on a-m(r).

[b]Indicates absence of Spm in tests on a-m(r).

allele phenotype with small faint pale areas was not represented among

the test cross progenies of 2b-c tl-3 spotted (Table 4.45), the

mottled phenotype could represent another expression of the same basic

allele. The mottled could be further characterized by testing for

heritability, Spm content and response to an introduced Spm.

In crosses of the mottled with a sh sibs + Spm (Table 4.47a),

the spotted response (1-2b tl) is similar to the parental (2-bc tl-3).

Also, the non-spotted progeny are similarly parental (colorless - tl)

and a few mottled types again appeared. When the crosses were made with

a sh sibs without Spm, the same parental type (tl) and a few mottled

(none in reciprocal crosses) occurred. No Spm could be detected when

the mottled kernels were tested on a-m(r)/a-m-1 (Table 4.47b).

The mottled are no different from the colorless basic allele in

their response to Spm. However, there are differences in the incon-

sistent transmission and control of expression of the mottled phenotype.

Whether these differences are related to the type of Spm in cultures

needs to be determined.

E. a-m-2 8004 State

The Spm with the original a-m-2 8004 induces colored spots in

colorless background (2b-c tl) (Figure 4.21). In most kernels, one or

two rimmed areas (R areas) are evident and these are encircled by

dark rims or peripheries. These R areas are devoid of spots and are

lighter than the rims. McClintock's interpretation (1967a) is that the

R areas consist of cells in which Spm became inactive early in the

development of the endosperm. She considered the dark rims to result
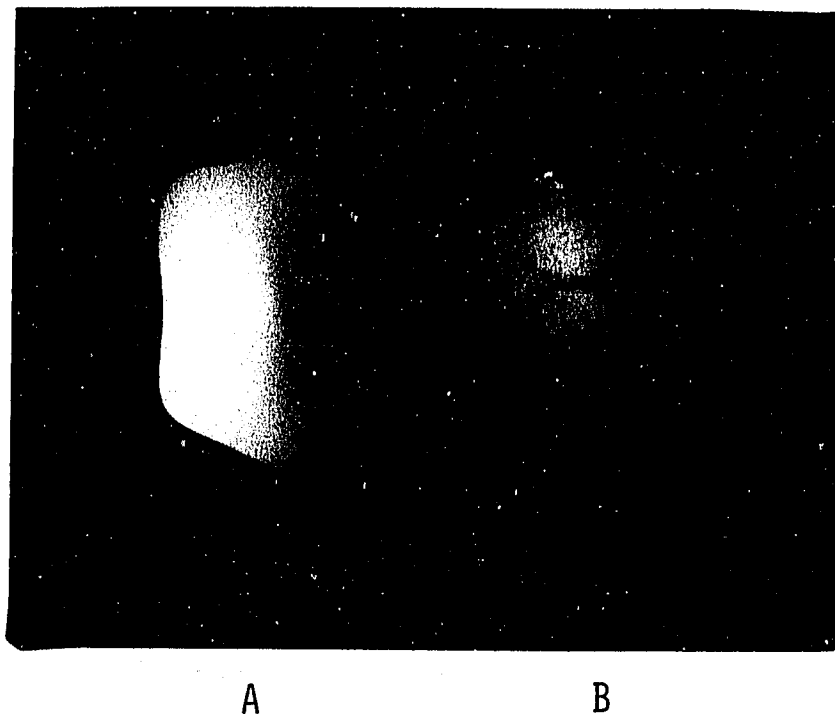
Figure 4.21. Original state of a-m-2 8004

       A. Without Spm colorless (t1)

       B. With Spm 2b-c t1 spotted and rimmed areas. Note the
          absence of spots within the rimmed area (arrow)

from a complementation reaction between the gene products of the R area

and the spotted area.

A single spotted kernel characterized by 2b-c tl spotted pattern

and R areas was the initial start of a-m-2 8004 state. In test crosses

of the plant originating from this kernel (Figure 4.22A, 1976 1614),

the progeny included 2b-c tl spotted with (+) or without (-) R areas,

and colorless (tl) kernels. The progeny kernels containing Spm

(2b-c tl + and -R) are used in Part 1 (Figure 4.22A, B, C) and those

without Spm form Part 2 of a-m-2 8004 study (Figure 4.23).

1. Part 1

    a. Heritability of 2b-c tl spotted + R   The heritability of

2b-c tl spotted + R was studied in three successive generations

(Figure 4.22A and Table 4.48, 1976, 1979 and 1980). The 2b-c tl

spotted + R progeny in each generation was selected and used for

the heritability study in the next generation.

The 2b-c tl spotted + R are either selfed (Table 4.48, 9 3250) or

crossed by an a sh tester (Table 4.48, 6 1614, 0 3452). The eight

progenies from both the self and cross included spotted and also

colorless and 18 kernels of exceptional phenotypes (Table 4.48). Two

types of spotted kernels occur in the progenies—those with (+ R)

and those without (- R) R areas. There is no consistent ratio between

the two types among the progenies.

The presence of R areas must be random because selection for + R

type does not increase the probability of occurrence of R areas in the

Original   McCl 8767②I (a-m-2 7995 Sh2)/McCl 8753⑤ (a-m-2 8004 Sh2)   (McClintock, 1967)

x a sh

a-m-2 8004   a-m-2 7995

1976   1614
                    (2b-c t1 + R areas)

x a sh

2b-c t1 + or - R areas, t1

1979   3250   3252
        (2b-c t1+R)   (2b-c t1-R)

                ⊗   ⊗

2b-c t1 + or - R areas, t1, exceptions   2b-c t1 + or -R, t1, exceptions

1980 3452   3454   3453D
      (2b-c   (2b-c t1-R)   (2-3c t3)
      t1+R)

            x a sh   x a sh   x a sh

      2b-c + or -R, t1, except. 2b-c + or -R, t1, except. 1-2b-c t1-3, t7
      t1   t1

1981 4408B, 4407B   4412A
      (2b-c t1-R)
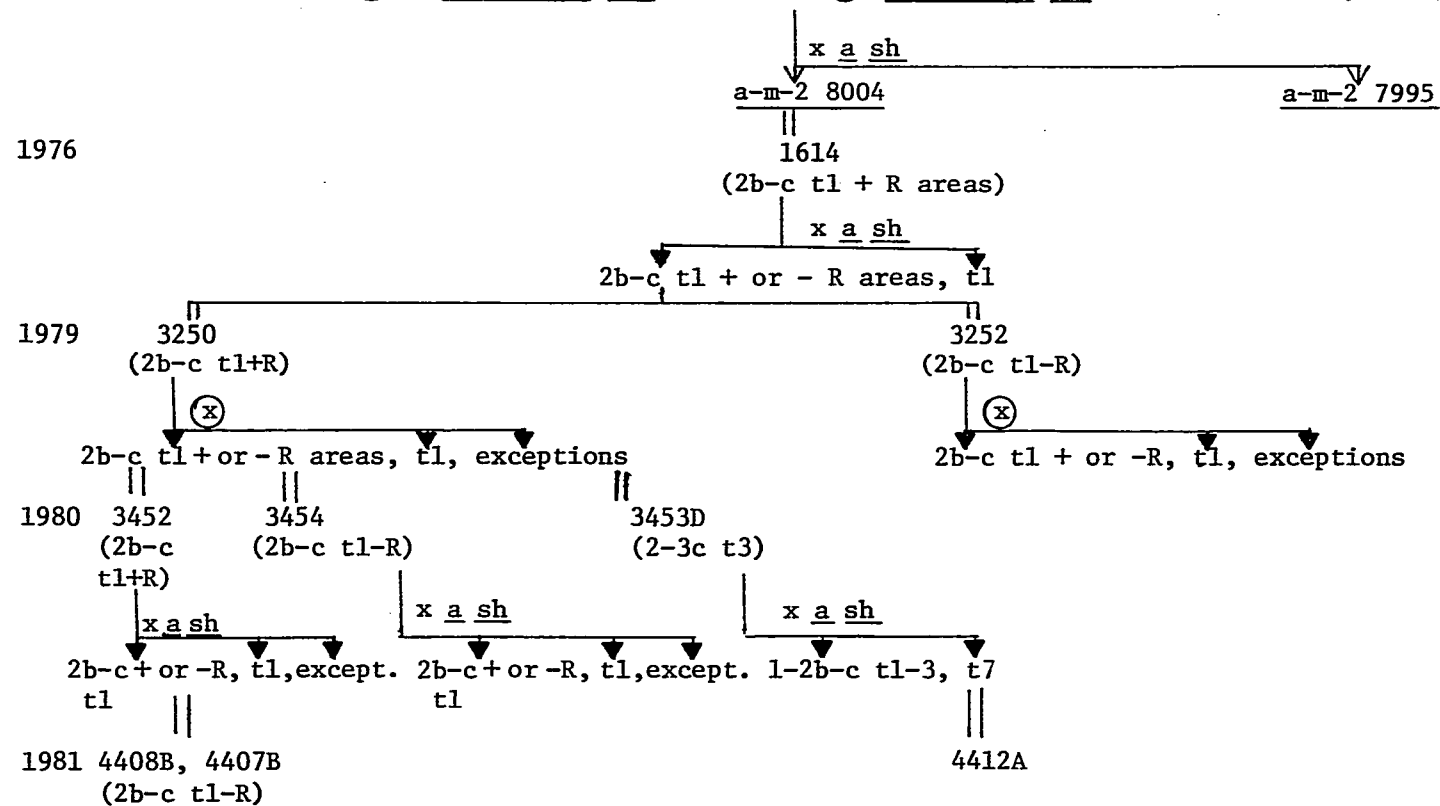
A

Figure 4.22.   Flow diagram showing different derivatives from the original 2b-c t1 spotted
               with rimmed (+ R) areas of a-m-2 8004 state

(from Figure 4.22A) (from Figure 4.22A)

1980 3452 3454
(2b-c t1+R) (2b-c t1-R)

x a sh x a sh

2b-c t1 - or +R, t1, exceptions 2b-c t1 - or +R, t1, exceptions

1981 4408D 4410A 4410B 4413A 4413C
(t3-4) (2-3a-b t1 (2-3a-b t4) (3-4c t1-2) (5b-c t2)
c̄ t4 mottles)

B C

Figure 4.22. (Continued)

(from Figure 4.22A)

1976   1614
(2b-c tl + R areas)

x $\underline{a}$ $\underline{sh}$

2b-c tl + or -R   tl
‖   ‖
1979   3250, 3252   3251
(Figure 4.22A)   (tl)

x $\underline{a}$ $\underline{sh}$ $\underline{En-hi}$ x $\underline{a}$ $\underline{sh}$ $\underline{En-lo}$

tl, 3c tl   1-2b tl, tl
‖   ‖
1980   3456   3455
(3c tl)   (1-2b tl)

x $\underline{a}$ $\underline{sh}$ x $\underline{a}$ $\underline{sh}$

tl, 3b-c tl-5, Exceptions   tl, 1-2b tl, Exceptions
‖

1981   4438B   4438A   4439B   4439A   4436B   4436A   4435Y   4435X
(3b-c   (3b-c   (3a-b   (5a-b tl)   (1-2b   (1-2b   (3b   (mot-
tl-3)   t4-5)   tl)   speckle   tl)   t2-3)   tl-2)   tled)
speckle spots
spots

Figure 4.23.   Flow diagram showing different derivatives obtained following reactivation of colorless (tl) state of $\underline{a-m-2}$ $\underline{8004}$ with $\underline{En}$

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Table 4.48. Heritability of 2b-c t1 spotted +R areas (Figure 4.22A, 1976 1614; 1979 3250; 1980 3452)

$\dfrac{\text{a-m-2 8004 Sh2}}{\text{a-m-2 8004 Sh2/a sh2}}$, Spm(x) or X $\dfrac{\text{a sh2}}{\text{a sh2}}$		Round				Shrunken
		Spotted (2b-c t1)		Color-less	Exceptions	Color-less
2b-c t1 + R (x) or X c1,sh		+R	−R	(t1)		
6 1614-4	X 1362	25	23	42	—	—
-8	X 1362	60*	61*	111*	—	—
1979 rows with selected kernels[a] ⟹		(3250*)	(3252*)	(3251*)		
9 3250-1(x)		13	97	44	3(t4)	1/4
-2(x)		100*	175*	104	2*(2-3c t3)	1/4
1980 rows with selected kernels ⟹		(3452)*	(3454)*	—	(3453D*)	
0 3452-1	X 3506	50	13*	80	1(t3-4)*;2(2b-c t1 speckle spotted)	1/2
-3	X 3505	145	18	147	—	
-20	X 3505	58	19	88	8(2-3a-b t1 + t4 mottles**) 1(2-3a-b t4)[+]	—
-21	X 3505	78	34**	126	1(t7)	—
1981 rows with selected kernels ⟹		(4408B*) (4407B*)			(4408D*) (4410A**) (4410B[+])	

[a]Selected progeny kernels in each generation and their assigned row numbers in a column are identified by the same superscript.

spotted progeny kernels. It can be concluded that this change of <u>Spm</u>

from an active to an inactive state early in the endosperm development

must be autonomously controlled, and not governed by a second gene.

Additional exceptional phenotypes appeared among the test cross

progenies of the 2b-c tl spotted +R and included spotted, pale and

colored types. The analyses of these exceptions are presented in sec-

tion c.

b. <u>Heritability</u> of <u>2b-c tl spotted − R</u>   The 2b-c tl spotted

− R, that were derived in the progenies of 2b-c tl + R (Table 4.48,

1976, 1979, 1980 − R column), are either selfed or test crossed

(Table 4.49, 9 3252, 0 3454, 1 4407B, 1 4408B). Like the tests with

+ R types, all the progenies included spotted, colorless and a few

exceptional phenotypes. But the spotted types differed among the

progenies. The spotted progenies of 9 3252 and 0 3454 consisted

of 2b-c tl + R or − R, whereas those of 1 4407B and 1 4408B included

2b-c tl with mottles (small pale areas) and 2b-c tl − R. Again, like

the progeny of + R types, the ratios of 2b-c tl + R and − R types

are not consistent (9 3254, 0 3454), but there are fewer 2b-c tl

with mottles than the 2b-c tl − R (1 4407B, 1 4408B).

These results indicate the following with respect to the herita-

bility of 2b-c tl − R phenotype:

(i)   The absence (− R) of R areas in the spotted progeny of 9 3252

      and 0 3454 (Table 4.49) is random (lack of consistency in

      the ratios of − R, + R types.

(ii) The spotted progenies of 1 4407B and 1 4408B (Table 4.49)

Table 4.49. Heritability of 2b-c tl spotted -R areas (Figure 4.22A, 1979 1352; 1980 3454; 1981 4408B, 4407B)

a-m-2 8004 $\frac{Sh2,}{sh2}$ 2b-c tl-R	Spm (X) or X $\frac{a\ sh2}{a\ sh2}$ (X) or X cl,sh	Round Spotted 2b-c tl $\bar{c}$ R areas	$\bar{c}$ out R areas	Color-less (tl)	Exceptions	Shrunken Color-less
9 3252-3	(X)	80*	85	56	--	1/4
-4t	(X)	25	128	54	6(5c tl)	1/4
0 3454-20	X 3503	49	1	--	--	1/2
-21	X 0354	2	57	80	6*(3c tl-2) 1^{**}(5b-c t2); 4(t7)	1/2*
-6	X 3504	--	--	120	--	1/2
1981 rows with selected kernels[a] ⟹					(4413A*) (4413C**)	(4414E*)
1 4407B-1	X 4422	--	58	68	--	1/2
-3	X 4422	1[b]	53	69	--	1/2
-4	X 422	4[b]	78	--	1(3c-d tl)	1/2
-5	X 4419	1[b]	100	40	1(3a-b tl)[b]	1/2
1 4408B-1	X 4419	--	65	30	1(3-4d-e mottles?)	1/2
-20	X 4222	23[b]	51	76	1(t7)	1/2

[a] Selected progeny kernels and their assigned row numbers in a column are identified by the same superscript.

[b] With mottles.

do not contain 2b-c tl + R type kernels. Instead, there

are kernels of 2b-c tl + mottles among the progenies.

This coincidence of presence of mottles and the absence

of R areas can be attributed to a change in state of Spm

from that undergoing early changes in phase (= R areas)

to that with late changes in phase (= mottles) of activity

during the endosperm development.

c. Analyses of the exceptional phenotypes derived in the

test cross progenies of 2b-c tl spotted + R and - R   Spotted, pale

and colored exceptional kernels (in all 39 kernels) appeared among

the + R and - R spotted progenies (Tables 4.48, 4.49). There are

several different spotted exceptions that differ from the original

2b-c tl + R and - R spotted in their pattern (size and frequency of

spots) and also in the background pigmentation. The pale and the

colored kernels differ from the original basic allele phenotype

(colorless) and tests were made to determine if they are new states

of the a-m-2 8004 allele.

1) Analyses of colored and pale exceptions

(a) Colored (t7) (1 4412A)

The 1 4412A colored (t7) exceptions were derived from 2-3c t3

spotted exception (Figure 4.22A, 1980 3453D progeny). They are tested

for their response to Spm in a sh sib kernels. The progeny of two

(Table 4.50 A(1), 1 4412A-1 and 1 4412A-3) out of three of these colored

kernels included spotted, colorless and colored but no shrunken kernels.

The absence of shrunken kernels in the progeny indicates that the colored kernels 1 4412A-1 and 1 4412A-3 are contaminants and are thus dismissed from further consideration.

The other colored kernel (Table 4.50 A(1), 1 4412A-2) in crosses with a sh sibs without Spm yielded 1-2b-c t5 spotted and colorless to very light pale (t1-2) kernels in 1:1 ratio but produced no colored kernels in the progeny (Figure 4.24), indicating that the parental kernel (1 4412A-2) must have been a 1-2b-c t5 but was misclassified as colored due to unrecognized spots on a dark pale background.

The 1 1412 A-2 kernel on a-m(r)/a-m-1 produced 3-4b-c t1 and 3-4b-c t4-5 spotted kernels (Table 4.50 A(2), 1 4430 X 4412A-2). In a comparative test, the a sh Spm sibs on a-m(r)/a-m-1 yielded 3-4b-c t1 (with or without pale areas) spotted progeny (Table 4.50 A(3)). The similar spotting patterns of progeny kernels in all these tests indicate that the Spm in 1 1412A-2 kernel and in a sh sibs is the same. But the t4-5 pale background of some of the spotted progeny (3-4b-c t4-5) of a-m(r)/a-m-1 X 1 1412A-2 cross suggests that this background is due to the presence of a-m-2 8004 allele and the background is comparable to that of 1-2b-c t5 progeny of 1 1412A-2 X a sh sib - Spm (Table 4.50A(3)).

These results confirm that the t5 background of 1-2b-c spotted exceptional kernel (1 4412A-2) is due to a change in state of the a-m-2 8004 allele. Since the spot size and the frequency (1-2b-c) are similar to that of the original state (2b-c t1), the change is most likely in that part of the receptor that responds to the suppressor
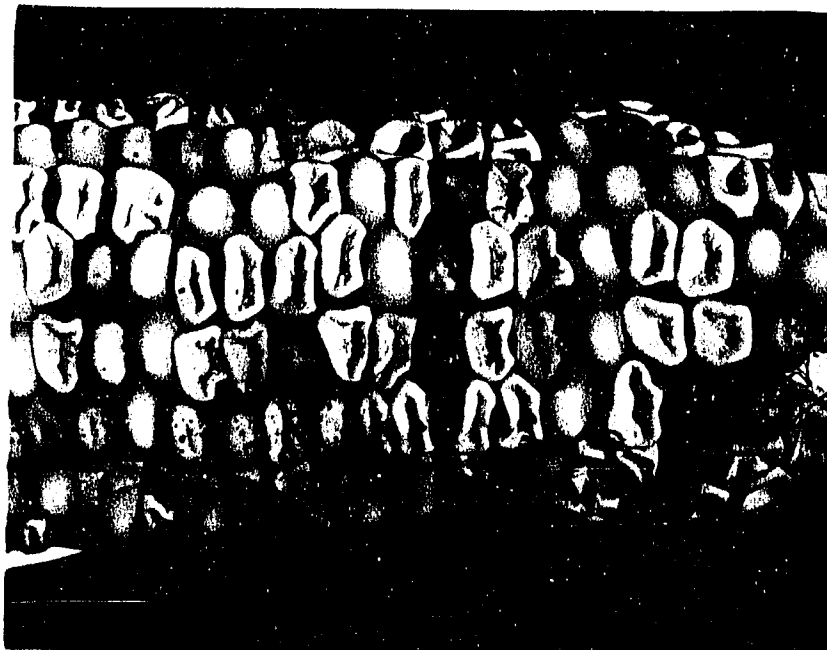
Figure 4.24. Progeny ear of cross colored (t7) X a sh - En (1 4414-11 X
4412A-2) containing 1-2b-c t5 spotted and colorless to
very light pale (t1-2) kernels

Table 4.50. Analyses of colored (t7) and pale (t3-4) exceptional phenotypes

A. Colored (t7)-1 4412A (Figure 4.22A, 1980 3453D progeny)

$\dfrac{\text{a-m-2 8004 Sh2}}{\text{a} \quad \text{sh2}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$ ± Spm t7 X cl,sh sibs		Round				Shrunken
		Spotted		Color-less t1	Colored t7	Color-less
		1-2b-c t1 - or + mottles	1-2b-c t5			
(1) Progeny test						
1 4412A-1	X 4414E-8 +[a]	103	—	27	138	—
-2	X -2 -[b]	—	59	62(t1-2)	—	1/2
Reciprocal crosses						
1 4414E-2 -	X 4412A-1	9	—	10	9	—
-5 ?[c]	X -1	126	—	42	159	—
-11 -	X -2	—	81	88(t1-2)	—	1/2
-8 +	X -3	72	—	111	190	—
-6 -	X -3	—	—	1/2	1/2	—

$\dfrac{\text{a-m(r)} \quad \text{Sh2}}{\text{a-m(r)} \quad \text{Sh2/a-m-1 sh2}}$ X $\dfrac{\text{a-m-2 8004 Sh2}}{\text{a} \quad \text{sh2}}$		Round					Shrunken	
		Spotted			tl	t7	t7	tl
		3-4b-c t1	3-4c-d t1 c 4a specks	3-4b-c t4-5				
(2) Test for type of Spm in t7								
1 4445	X 4412A-1	69	46	—	28	145	—	—
4430	X -2	75	—	137	132 (t1-2)	69	60	63

[a] Presence of Spm confirmed in tests on a-m(r)/a-m-1.

[b] Absence of Spm confirmed in tests on a-m(r)/a-m-1.

[c] Not confirmed.

Table 4.50. (Continued)

$\dfrac{\text{a-m(r)} \ \underline{\text{Sh2}}}{\text{a-m-l} \ \underline{\text{sh2}}}$ X $\dfrac{\text{a sh2, } \underline{\text{Spm}}}{\text{a sh2}}$	Spotted types

3) Test for type of <u>Spm</u> in <u>a</u> <u>sh</u> sibs

1 4444	X 4414–9		3–4b–c t1, 3–4c–d t1 $\overline{c}$ 4a specks
4443	X	–13	3–4b–c t1, 3–4c–d t1 $\overline{c}$ 4a specks

B. Pale (t3–4); 1 4408D (Figure 4.22B)

$\dfrac{\text{a-m-2 8004 } \underline{\text{Sh2}}}{\underline{\text{a}} \qquad \underline{\text{sh2}}}$ X $\dfrac{\text{a sh2, } \underline{\text{Spm}} \text{ sibs}}{\text{a sh2}}$ t3–4 X cl,sh	Round			Shrunken
	Spotted	Pale t3–4	Colored t7	Color- less
1 4408D X 4409–6 +[a]	––	––	1/2	1/2
D–t X 4416	––	––	1/2	1/2

component of <u>Spm</u>.

(b) Pale (t3-4) (1 4408D)

In crosses with <u>a</u> <u>sh</u> <u>Spm</u> sibs and with <u>a</u> <u>sh</u>, the t3-4 pales

yielded only colored progeny kernels (Table 4.50B), indicating that

the parental phenotype was most likely a colored rather than a t3-4

pale. Since this exceptional phenotype did not respond to the <u>Spm</u> in

<u>a</u> <u>sh</u> sibs, it represents a germinal change from responsive <u>a-m-2 8004</u>

to a non-responsive wild type allele.

2) <u>Analyses</u> <u>of</u> <u>spotted</u> <u>exceptions</u>

(a) 2-3a-b t1 with t4 mottles (1 4410A)

The 2-3a-b t1 + t4 mottle exceptional kernels were selected among

the test cross progeny of 2b-c t1 spotted + R (Table 4.48, 0 3452-20 X

3505). The t4 mottles on these spotted kernels appear as small pale

areas and are similar to those found in the progeny of 2-bc t1 - R

(Table 4.49, 1 4407B, 1 4408B).

In test crosses, the 2-3a-b t1 + t4 mottle spotted produced

1c t1 + or - t4 mottles (Table 4.51 A(1)). In reciprocal crosses, how-

ever, the progeny consisted of mostly 2b-c t1 without any t4 mottles.

The different spotted pattern in the reciprocal progeny can be attributed

either to the tissue differences (cytoplasm of <u>a</u> <u>sh</u> tester in reciprocal

crosses) or to a change in phase of <u>Spm</u>. The latter possibility is more

likely because in crosses of the exceptional kernels on <u>a-m(r)</u>/<u>a-m-1</u>

the spotted progeny were different among two separate crosses (Table

4.51 A(2) 1 4430 X 4410A-5 and 1 4427 X 4410A-7). Also, the non-

heritability of parental spotted phenotype (2-3a-b t1 + t4 mottles -

Table 4.51 A(1)) indicates a change in phase of activity of _Spm_.

(b) 2-3a-b t4 (1 4410B)

Kernel 1 4410B (2-3a-b t4) is another exception derived in

O 3452-20 X 3505 progeny (Table 4.48). The colored spots in this kernel

are present on a uniform pale (t4) background and this is in contrast

to the 1 4410A kernels (Table 4.51A) that have similar spotting pattern

but the pale pigmentation is limited to a few areas.

The 1 4410B spotted kernel (2-3a-b t4) yielded the same spotted

type in the progeny of a test cross (Table 4.51 B(1), 1 4410B-1 X 4420

and Figure 4.25). A slightly different spotting pattern (2b-c t3-4) in

reciprocal crosses can be attributed to the influence of cytoplasm.

Thus, these test crosses demonstrate that the change from 2b-c t1

(Figure 4.22B, 1980 3452) to 2-3a-b t4 is heritable.

In order to determine whether this change is in the receptor _I_ or

in the _Spm_, the 2-3a-b t4 exception and the parental type 2b-c t1

spotted were tested on a-m(r)/a-m-1. The spotted progeny in both these

crosses included 4c t1 (a-m(r) Sh2/a sh2) and 3c-d with pale speckles

(a-m-1 sh2/a-m-2 Sh2) (Table 4.51 B(2), B(3), 1 4444 X 4410B-1 and

1 4357 X 4411-2). But, in addition, the test with 2-3a-b t4 (Table

4.51 B(2)) yielded 4c t3-4 spotted progeny. The t3-4 background of

these spotted kernels corresponds to that of the exceptional parent

kernel (1 4410B) confirming that it is due to a change in state of the

receptor of a-m-2 8004 allele.

Figure 4.25. Test cross ear of 2-3a-b t4 spotted exception (Table
4.51B(1), 1 4410B-1 X 4420)

(c) 3-4c t1-2 (1 4413A)

The 3-4c t1-2 exceptions were derived from a test cross of 2b-c
t1 - R spotted (Table 4.49, 0 3454-21 X 0354). In test crosses of
3-4c t1-2, the spotted progeny included the parental type (3-4c t1-2)
and 2b-c t1 + or - t4 mottles (Table 4.51C, Figure 4.26). The
reappearance of many 2b-c t1 spotted in the progeny indicates that the
Spm is reverting to its original phase of activity at a high frequency.

(d) 5b-c t2 (1 4413C)

Kernel 1 4413C also arose in the 0 3454-21X 0354 test cross
progeny (Table 4.49). In test crosses, this exceptional 5b-c t2
spotting pattern is heritable (Table 4.51 D(1) and Figure 4.27).
Therefore, the change from 2b-c t1 to 5b-c t2 should involve a change
in state of either the receptor I or the Spm. In order to distinguish
between these possibilities, the Spm in 5b-c t2 spotted and in a sh
sib kernels is tested on a-m(r)/a-m-1. The spotted progeny of 5b-c t2
on a-m(r)/a-m-1 included 3-4c t1-2 kernels (Table 4.51 D(2)), whereas
the a sh sibs on a-m(r)/a-m-1 produced several different spotted pat-
terns in separate crosses (Table 4.51D(3)). This latter test indicates
that the Spm in a sh sibs is represented by different states and thus
provides evidence for frequent changes in state of Spm. This evidence
would, therefore, indicate that the Spm in 5b-c t2 spotted exception
represents a new state.

2. Part 2

The part 2 study is related to the reactivation of a-m-2 8004
basic allele (colorless) with En (Figure 4.23). Two types of En

Table 4.51. Analyses of spotted exceptional phenotypes that were derived in the test cross progenies of 2b-c t1 spotted + or -R (Figure 4.22B, 0 3452; 4.22C, 0 3454 progeny)

A.   2-3a-b t1 with t4 mottles (1 4410A)

$\dfrac{\text{a-m-2 8004 Sh2, Spm}}{\text{a} \qquad \text{sh2}} \times \dfrac{\text{a sh2}}{\text{a sh2}}$ 2-3a-b t1 + t4 mottles X cl,sh	Round				
	Spotted				Colorless
	1c t1 c̄ t4 mottles	1c t1 c̄ out t4 mottles	2b-c t1 c̄ t4 mottles	2b-c t1 c̄ out t4 mottles	
(1) Progeny test					
1 4410A-3 X 4420	14	67	--	--	111
-5 X 4420	12	60	--	--	100
-7 X 4228	24	71	--	--	111
Reciprocal cross					
1 4345 X 4410A-7	--	--	--	74	84
4348 X -3	--	--	3	86	124

$\dfrac{\text{a-m(r) Sh2}}{\text{a-m-1 sh2}} \times \dfrac{\text{a-m-2 8004 Sh2, Spm}}{\text{a} \qquad \text{sh2}}$	Round		Colorless	Colored	Shrunken	
	Spotted				Colored	Spotted
	4b-c t1	3c-d c̄ speckles				
(2) Test for type of Spm in 2-3a-b t1 + t4 mottles						
1 4430 X 4410A-5	116	50	118	58	43	36
4427 X -7	111(2-3b-c)	44	104	59	40	47

Table 4.51. (Continued)

B. 2-3a-b t4 = 1 4410B

$\dfrac{\text{a-m-2 8004}}{\text{a}}\ \dfrac{\text{Sh2}}{\text{sh2}},\ \text{Spm}$ X $\dfrac{\text{a}}{\text{a}}\ \dfrac{\text{sh2}}{\text{sh2}}$	Round		Colorless (t1)	Exceptions
	Spotted			
2-3a-b t4 X cl,sh	2b-c t3-4	2-3a-b t4		

(1) Progeny test

	2b-c t3-4	2-3a-b t4	Colorless (t1)	Exceptions
1 4410B-1 X 4420	--	107	94	1(t7)
Reciprocal crosses				
1 4344 X 4410B-1	44	--	35	--
4345 X -1	13	--	23	--

$\dfrac{\text{a-m(r)}}{\text{a-m-1}}\ \dfrac{\text{Sh2}}{\text{sh2}}$ X $\dfrac{\text{a-m-2 8004}}{\text{a}}\ \dfrac{\text{Sh2}}{\text{sh2}},\ \text{Spm}$	Round					Shrunken	
	4c t1	4c t3-4	3c-d $\overline{\text{c}}$ speckles	Color-less	Colored	Colored	Spotted
Cl,rd X 2-3a-b t4							

(2) Test for type of Spm in 2-3a-b t4

	4c t1	4c t3-4	3c-d c speckles	Color-less	Colored	Colored	Spotted
1 4444 X 4410B-1	9	10	6(t3-4)	15	6	2	7

$\dfrac{\text{a-m(r)}}{\text{a-m-1}}\ \dfrac{\text{Sh2}}{\text{sh2}}$ X $\dfrac{\text{a-m-2 8004}}{\text{a}}\ \dfrac{\text{Sh2}}{\text{sh2}},\ \text{Spm}$							
Cl,rd X 2b-c t1							

(3) Test for type of Spm in 2b-c t1 sibs

	4c t1	4c t3-4	3c-d c speckles	Color-less	Colored	Colored	Spotted
1 4357 X 4411-2	173	--	118	30	22	13	103

Table 4.51. (Continued)

C. 3-4c t1-2 = 1 4413A

a-m-2 8004 Sh2 , Spm X a sh2		Round					Shrunken
a-m-2 8004 Sh2/a sh2 a sh2			Spotted			Only mottles or R areas	Color-less	Color-less
3-4c t1-2 X cl,sh		3-4c t1-2	2b-c t1				
			c̄ mottles	c̄ out mottles			
1 4413A-20	X 4222	103	39	83	8	105	--
-21	X 4221	57	10	48	15	112	--
-22	X 4421	60	--	--	16	61	1/2
-23	X 4221	41	--	23	--	31	1/2

D. 5b-c t2 = 1 4413C

a-m-2 8004 Sh2, Spm X a sh2		Round		Exceptions	Shrunken
a sh2 a sh2		Spotted 5b-c t1-2	Colorless		Colorless
5b-c t2 X cl,sh					
(1) Progeny test					
1 4413C-1	X 4416	53	53	--	1/2
-1t	X 4423	115	112	--	1/2
Reciprocal cross					
1 4345	X 4413C-1	63	72	2(t7)	1/2

a-m(r) Sh2 X a-m-2 8004 Sh2, Spm		Round			Shrunken	
a-m-1 a sh2		Spotted	Colorless	Colored	Colored	Spotted
(2) Test for type of Spm in 5b-c t2						
1 4334	X 4413C-1	111 (3-4c t1-2)	77	50	39	30

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Table 4.51. (Continued)

$\dfrac{a\text{-}m(r)}{a\text{-}m\text{-}l}\ \dfrac{Sh2}{sh2}$ X $\dfrac{a\ sh2,\ Spm}{a\ sh2}$	Round			Shrunken	
	Spotted	Colorless	Colored	Colored	Spotted

(3) Test for type of <u>Spm</u> in a sh sibs

		Spotted	Colorless	Colored	Colored	Spotted
1 4445 X 4414	-4, 10	2c + 3a speckles	√[a]	--	√	2c+3a tl
4442 X	-7	2b-c tl	√	--	√	2b-c tl
4444 X	-9, 13	4-5c tl	√	--	√	4-5c tl

[a]Indicates the presence of kernels of the type in that column.
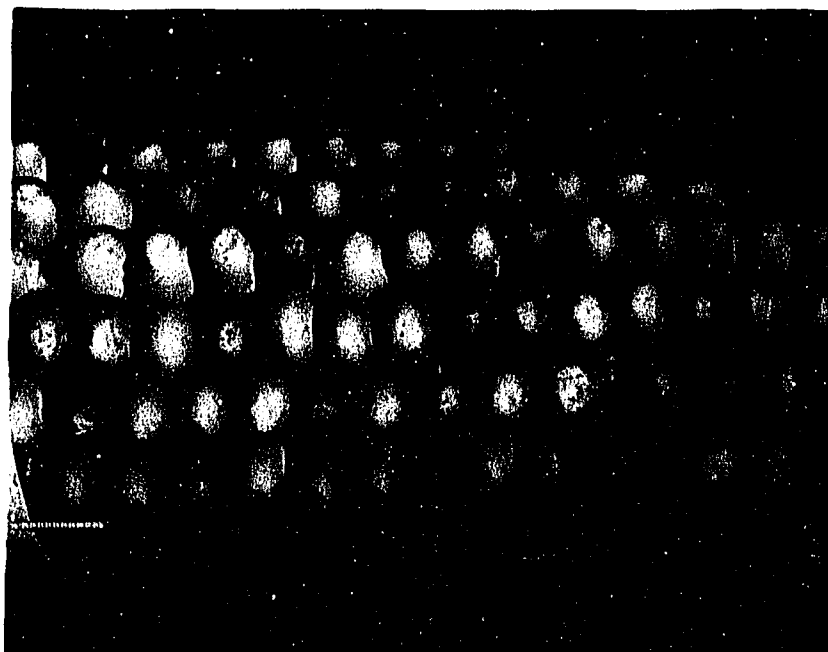
Figure 4.26. Test cross ear of 3-4c tl-2 spotted (Table 4.51C,
1 4413A-20 X 4222). The presence of several 2b-c tl
spotted indicates a reversion of _Spm_ to the original
phase of activity
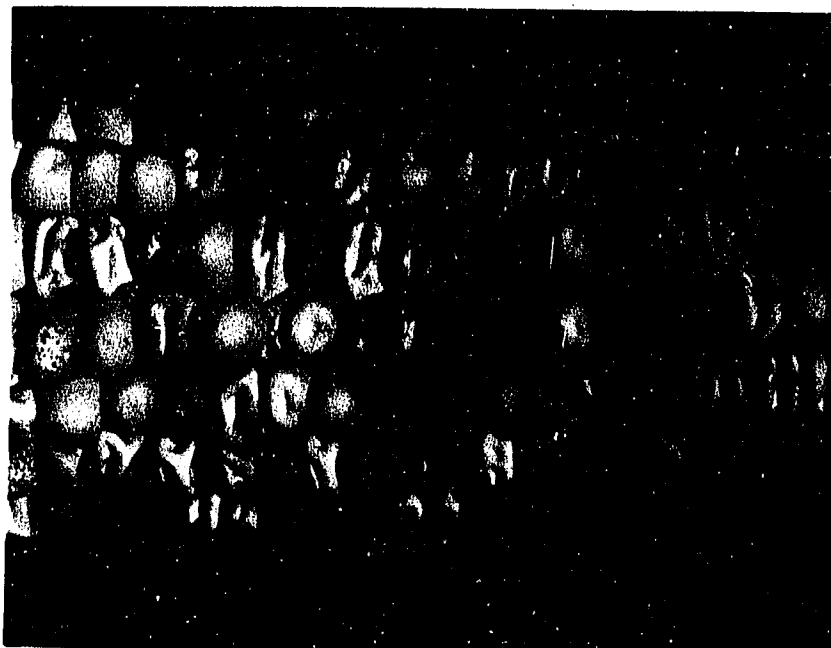
Figure 4.27. Test cross ear of 5b-c t2 spotted (Table 4.51D(1),
1 4413C-1t X 4423) showing the heritability of this
high spotted pattern

differing in mutator activities, En-high and En-low, were used. With

En-high, the spotted progeny included 3c tl, with En-low 1-2b tl

resulted (Table 4.52). Spotted patterns from both progenies were

further analyzed to determine their heritability and to derive any

exceptional phenotypes that may arise in the progenies.

a. <u>Heritability</u> <u>of</u> <u>3c</u> <u>tl</u> <u>spotted</u> <u>kernels</u>   Among the test

cross progenies of 3c tl, mostly the parental type, colorless and

two exceptional spotted appeared (Table 4.53). Although the majority

of the spotted progeny kernels exhibited pattern similar to the

parental type (3b-c vs 3c), the kernels varied in background pigmenta-

tion from colorless to pale (tl-5). The 3b-c spotted kernels were

separated into two classes--tl-3 versus t4-5 and tested for their

heritability.

1) <u>Heritability</u> <u>of</u> <u>3b-c</u> <u>tl-3</u> <u>spotted</u>   Among the test

crosses (x <u>a</u> <u>sh</u>) of 3b-c tl-3 spotted (Table 4.54A), only two of the

progenies (1 4438B-1, B-7) included spotted kernels that are similar

(2-3b-c t2-3, 2-3a-b tl-2) to the parental type. All the rest (6) of

the progenies contained 1-2b-c t2-3 spotted kernels. The basic allele

phenotype (-<u>En</u>) in all the progenies is colorless (tl) except a few

kernels with very light pale (t2) pigmentation.

These results show that the exceptions incurred a reduction in

the frequency of spots from 3b-c (parental) to 1-2b-c in most of the

test cross progenies. This change in spot frequency can be attributed to

a change in phase of activity of <u>En</u>. The presence of a few very light

Table 4.52. Reactivation of colorless basic allele (-Spm) of a-m-2
8004 with En (Figure 4.23, 1979 3251)

a-m-2 8004 Sh2 - Spm X a sh2, En		Round		
		Spotted		Color-less
a sh2 tl	a sh2 a sh2 X cl,sh	3c tl	1-2b tl	
9 3251-20	X 5024-9(+hi)[a]	58*	––	67
-4	X 1723-11(+lo)[b]	––	49	52
-21	X -12(+lo)	––	78	67
-22	X -11(+lo)	––	65	72
-23	X -3 (+lo)	––	58*	65
1980 rows containing the selected kernels[c] ⟹		(3456*)	(3455*)	

[a]Indicates the presence of En with highly active mutator function.

[b]Indicates the presence of En with weakly active mutator function.

[c]Selected kernels and their assigned rows in a column are identified by the same superscript.

Table 4.53. Heritability of 3c tl spotted kernels that were derived in the progeny of colorless X a sh En-hi crosses (from Table 4.52, 9 3251-20 X 5024-9; Figure 4.23, 1980 3456)

a-m-2 8004 Sh2, En-hi / a / sh2 — 3c tl	X a sh2 / a sh2 — X cl,sh	Round Spotted 3b-c tl-5	Round Color-less (tl)	Exceptions
0 3456-3	X 0355	73(tl-5*)	88	--
-20	X 0352	107(tl-4)	50	--
-21	X 0352	111(tl-3)	121	--
-22	X 0351	45(tl-2)	56	--
-23	X 0353	64(tl-3)	59·	1(5a-b tl speckled spots)* 1(3a-b tl speckled spots)**
-24	X 0355	101(tl)	70	--
1981 rows containing the selected kernels[a] ⟹		(4438A,B*)		(4439A*) (4439B**)

[a]Selected kernels and their assigned rows in a column are identified by the same superscript.

pale (t2) (-$\underline{En}$) kernels is possibly due to the influence of $\underline{En}$ on

$\underline{a\text{-}m\text{-}2\ 8004}$ allele when they both are together in the plant (1 4438A)

and the effect is transmitted to the progeny even in the absence of $\underline{En}$.

2) <u>Heritability of 3b-c t4-5 spotted</u>   The 3b-c t4-5 are

the sibs of 3b-c t1-3 spotted (preceding section) and both originated

among the test cross progenies of 3c t1 spotted (Table 4.53). The

3b-c t4-5 in test crosses produced spotted progenies with reduced

(1-2c-d t3-5) spot frequency (Table 4.54B). This reduction is similar

to that in the progeny of 3b-c t1-3 (Table 4.54A) and can be attributed

to a change in phase of activity of $\underline{En}$.

3) <u>Analyses of exceptional phenotypes</u>   There were two

exceptional spotted kernels (5a-b t1 and 3a-b t1) among the test cross

progeny of 3c t1 spotted kernels (Table 4.53, 0 3456-23 X 0353). Unlike

the parental type, the spots on these exceptional kernels are not

regular and round but are small and irregular (speckled type). These

speckle spotted kernels were further characterized.

(a)   5a-b t1 (1 4439A)

The 5a-b t1 spotted kernel in test crosses produced no shrunken

progeny (Table 4.55A). This unexpected (because the exceptional kernel

is heterozygous for $\underline{Sh2}$) lack of shrunken progeny indicates that the

5a-b t1 spotted exception is a contaminant and thus not considered

further for discussion.

(b)   3a-b t1 (1 4439B)

Among the test cross progeny of 3a-b t1, there appeared 1c t2-3,

2b-c t2-3 spotted and colorless kernels (Table 4.55B). Both the

Table 4.54. Analysis of 3b-c spotted kernels with dark (t4-5) and light (t1-3) pigmented backgrounds; both types are derived in the test cross progeny of 3c t1 spotted (from Table 4.53, 0 3456-3 X 0355)

A. 3b-c t1-3 spotted = 1 4338B

$\dfrac{\text{a-m-2 8004 Sh2}}{\dfrac{a}{3b\text{-}c\ t1\text{-}3}\quad sh2}$ X $\dfrac{a\ sh2}{\dfrac{a\ sh2}{X\ cl,sh}}$	Round	
	Spotted 1-2b-c t2-3	Colorless (t1, few t2)
1 4438B-1 X 4323	109 (2-3b-c t2-3)	91
-2 X 4323	87	82
-3 X 4322	31	118
-4 X 4322	96	80
-5 X 4226	49 (2-3a-b t1-2)	58
-6 X 4322	115	96
-7 X 4221	78	53
-8 X 4226	107	117

B. 3b-c t4-5 spotted = 1 4338A

$\dfrac{\text{a-m-2 8004 Sh2}}{\dfrac{a\qquad sh2}{3b\text{-}c\ t4\text{-}5}}$ X $\dfrac{a\ sh2}{\dfrac{a\ sh2}{X\ cl,sh}}$	Round	
	Spotted 1-2b-c t2-3	Colorless (t1, few t2)
1 4438A-1 X 4310	59	45
-2 X 4324	88	79
-3 X 4323	41	43

Table 4.55. Analyses of the exceptional kernels that were derived
in test cross progenies of 3b-c tl ($\underline{En}$-hi) spotted kernels
(from Table 4.53, O 3456-23 X 0353)

A. 5a-b tl speckled type of spots = 1 4439A

a-m-2 8004 $\frac{Sh2,}{sh2}$ $\frac{En}{}$ X $\frac{a}{a}$ $\frac{sh2}{sh2}$	Round		Shrunken
	Spotted		
	$\frac{5b-5b^+}{\text{speckled}}$ $\frac{2c\ t2-3}{\overline{c}}$	Color-less	Color-less
5a-b tl X cl,sh	speckled type speckles		
1 4439A-1 X 4221 62	101	179	—
Reciprocal cross			
1 4331 X 4439A-1 33(5a	36(2a-b t2)	73	—
t1-2)			

B. 3a-b tl speckled type of spots = 1 4439B

a-m-2 8004 $\frac{Sh2,}{sh2}$ $\frac{En}{}$ X $\frac{a}{a}$ $\frac{sh2}{sh2}$	Round		Color-less	Shrunken
	Spotted			Color-less
3a-b tl X cl,sh	2b-c t2-3	1c t2-3		
1 4439B-1t X 4423 86	—		64	1/2
Reciprocal cross				
1 4323 X 4439B-1 —	88		120	1/2

1c t2-3 and 2b-c t2-3 spotted represent changed patterns (reduction in the frequency of spots) from the parental type (3a-b t1) and thus indicate changes in state of En. Since the changed patterns appeared on all the progeny kernels, the changes in state of En should occur very early in the development of the ear.

b. <u>Heritability of 1-2b t1 spotted kernel</u>   In tests of a-m-2 8004 basic allele (t1) with En-low, 1-2b t1 spotted kernels appeared among the progeny (Table 4.52). The progeny of 1-2b t1 in test crosses included the parental type spotted, colorless and a few exceptional kernels (Table 4.56). The exceptional phenotypes 1-2b t2-3 spotted (1 4436A), 3b-c t1 spotted (1 4435Y) and mottled (1 4435X) are further analyzed to determine the changes in state of En or the receptor, I.

1) <u>Analyses of the exceptional phenotypes</u>

(a) 1-2b t2-3 mottled background (1 4436A)

The parental type spotted (1-2b t2-3) mottled background, color-less and a few light mottled appeared among the test cross progeny of 1-2b t2-3 confirming the heritability of the change (Table 4.57 A(1)). In a comparative test cross, the progeny of 1-2b t1 sibs included mostly the parental type spotted, colorless and a few mottled kernels (Table 4.57 A(2)). These results indicate that both the 1-2b t2-3 mottled exception and the 1-2b t1 sib spotted patterns are heritable.

The 1-2b t2-3 and 1-2b t1 sibs were also tested on <u>a-m(r)/a-m-1</u> (Table 4.57 A(3), A(4)). Both yielded 1-2a-b t1-2 spotted progeny,

Table 4.56. Heritability of 1-2b tl spotted kernels that were derived in the progeny of colorless X <u>a</u> <u>sh</u> <u>En</u>-1o crosses (Figure 4.23, 1979 3251 progeny)

<u>a-m-2 8004 Sh2</u>, <u>En</u>(1o)	X <u>a sh2</u>	Round		Exceptions
<u>a</u> <u>sh2</u> 1-2b tl	<u>a sh2</u> X cl,sh	Spotted 1-2b tl	Color- less	
0 3455-2	X 4134	40*	99	8*(1-2b t2-3 mottled bkg)
-10	X 4132	26	25	11** (mottled)
-20	X 0351	81	72	1 (3c tl)
-21	X 0353	37	18	15 (mottled)
-22	X 0355	35	80	8+ (3b-c tl)
-23	X 1435	40	44	--
1981 rows containing the selected kernels[a] ⟹(4436B*)				(4436A*) (4435X**) (4435Y+)

[a]Selected kernels and their assigned rows in a column are identified by the same superscript.

indicating that the state of En is the same in both spotted patterns.
Thus, the evidence for lack of change in En plus the heritability of
the 1-2b t2-3 exceptional spotted phenotype confirm a change in state
of the a-m-2 8004 allele.

(b)   3b-c t1 (1 4435Y)

The 3b-c t1 spotted exception is not heritable in test crosses
(Table 4.57B). The spotted progeny include 1b t1-2 type which is simi-
lar to the original spotted (1-2b t1, Table 4.56), indicating a rever-
sion in phase of activity of Spm.

(c)   Mottled (1 4435X)

The mottled exceptions are tested for their response to the En in
a sh sib kernels (Table 4.57C). The progeny included 1-2b t1, color-
less and several mottled kernels. The 1-2b t1 spotted response is the
same as that of the original basic allele (t1), indicating that there
is no change in the receptor of mottled exceptions in terms of its
response to En. However, since the mottled phenotype is represented
in crosses with a sh + En, it can represent a change in the receptor
that alters control of gene action of a-m-2 8004 allele but not its
regulation (response) by En.

Table 4.57. Analyses of the exceptional kernels that were derived in test cross progenies of 1-2b tl (En-lo) spotted kernels (from Table 4.56, 0 3455-2 X 4134)

A. 1-2b t2-3 (t2-3 background is light mottled type) = 1 4436A

$\dfrac{\text{a-m-2 8004 Sh2, En}}{\dfrac{a}{\text{1-2b t2-3}} \quad \dfrac{\text{sh2}}{\quad}}$ X $\dfrac{\text{a sh2}}{\text{a sh2}}$ X cl,sh	Round		
	Spotted (1b t2-3 mottled)	Colorless	Light pale type mottled

(1) Progeny test of 1-2b t2-3

		Spotted	Colorless	Light pale
1 4436A-1	X 4322	60	68	10
-3	X 4421	34	89	11

	Spotted 1-2b tl (few 1-2b t2 mottled)	Colorless tl (few t2)	Mottled	Exceptions

(2) Comparative progeny test of 1-2b tl sibs (Table 4.57, 0 3455-2 X 4134)

		Spotted 1-2b tl (few 1-2b t2 mottled)	Colorless tl (few t2)	Mottled	Exceptions
1 4436B-1	X 1725	45	54	7	
-4	X 4321	64	117	6	4 3-4b-c tl (all at one place)
-5	X 1725	52	71	5	

$\dfrac{\text{a-m(r)}}{\text{a-m-1}} \dfrac{\text{Sh2}}{\text{sh2}}$ X $\dfrac{\text{a-m-2 8004 Sh2, En}}{\dfrac{a}{\quad} \quad \dfrac{\text{sh}}{\quad}}$	Round			Shrunken	
	Spotted 1-2a-b tl-2 mostly speckled type	Color-less (tl-2)	Colored	Colored	Spotted

(3) Test for type of En in 1-2b t2-3

1 4428 X 4436A-3	101	90	28	23	47

(4) Test for type of En in 1-2b tl sibs

1 4428 X 4436B-1	158	118	58	41	60

Table 4.57. (Continued)

B. 3b–c tl spotted = 1 4435Y

$\underline{\text{a–m–2 8004}}\ \dfrac{\text{Sh2}}{}$, $\underline{\text{En}}(\text{lo})$ $\quad\times\ \dfrac{\text{a sh2}}{\text{a sh2}}$	Round	
$\dfrac{\text{a}\qquad\qquad \text{sh2}}{\text{3b–c tl}}$ $\qquad\qquad\qquad$ X cl,sh	Spotted (1b tl–2)	Colorless or very light pale (tl–2)
1 4435Y–1 $\qquad$ X 4321	48	113
–3 $\qquad$ X 4321	36	52

C. Mottled, no spots = 1 4435X

$\underline{\text{a–m–2 8004}}\ \dfrac{\text{Sh2}}{}$ $\ \times\ \dfrac{\text{a sh2}}{\text{a sh2}}\ \pm\ \text{En}$	Round		
$\dfrac{\text{a}\qquad\quad \text{sh2}}{\text{mottled}}$ $\qquad\quad$ X cl,sh	Spotted (1–2b tl)	Mottled	Colorless
1 4435X–2 $\quad$ X 4437–13 –	––	6	64
–6 $\quad$ X $\quad$ –2 –	––	7	145
–20 $\quad$ X $\quad$ –1 +	41	5	62
	1(3b–c t2)		
–22 $\quad$ X $\quad$ –5 +	62	25	104

Tests on <u>a–m(r)</u> <u>Sh2</u>/<u>a–m–1</u> <u>sh2</u> $\qquad$ no spotted kernels

V. DISCUSSION

A. States of En(Spm)-I Controlling Element System

This study is an evaluation at the genetic level on the kinds of changes that can occur at a specific locus under the influence of a controlling element system. Five independently originated states of the En(Spm)-I controlling element system were used in this analysis. Spotting (or mutability) in the aleurone of maize kernels was the assay used to evaluate these changes.

The current understanding that the spotted kernel phenotype is associated with the operation of a controlling element system was initially established by McClintock (1952a) following her discovery of Ac-Ds system (McClintock, 1947). The same type of spotted phenotype is identified with four other established two-element systems which include: En(Spm)-I (Peterson, 1953, 1960; McClintock, 1954, 1968); Dt-a-dt (Rhoades, 1936); Fcu-rcu (Gonella and Peterson, 1977) and Uq-ruq (Friedemann and Peterson, 1982).

These controlling element systems include two elements that interact to produce spotting. One, a receptor element (such as Ds, I, dt, cu, ruq) responds to a second element, the regulatory element (such as Ac(Mp), En(Spm), Dt, Fcu and Uq), respectively (Fincham and Sastry, 1974). The receptor element in cis position to a locus (for example, A = colored aleurone), completely or partially suppresses the gene activity at that locus (A → a = colorless or pale), and the regulatory element located elsewhere in the genome functions in trans to relieve the gene from

suppression (a → A) in some cells of the endosperm. Generally, the resulting phenotype is colored spots on a colorless background yielding a spotted aleurone. Each colored spot represents a mutation caused by an excision of the receptor element from the suppressed locus (McClintock, 1949; Brink and Nilan, 1952). The size and the number of spots reflect the time (early or late) and the frequency of mutations during endosperm development. The combined effect of timing and frequency results in a specific pattern of mutability.

In the En(Spm)-I controlling element system, the independently discovered regulatory elements En (Peterson, 1953) and Spm (McClintock, 1954) are functionally identical (Peterson, 1965). The mutable alleles of En also respond to Spm and vice versa. With certain mutable alleles such as a-m-1, both En and Spm exhibit two components of action. Component-1 (Suppressor-S) suppresses the pale aleurone pigmentation of a-m-1 allele and component-2 (Mutator-M) causes mutations by excising the receptor element and leading to full expression of the allele (McClintock, 1954; Peterson, 1965).

Changes in both the receptor (I) and/or the regulatory (En or Spm) elements occur and are reflected in the time (spot size) and the frequency (spot number) of mutations during the endosperm development. These changes yield diverse patterns of mutability ranging from early (coarse spots) to late (fine spots) and one (single spot) to frequent (numerous spots) mutations. Each pattern of mutability is referred to as a state of a receptor (mutable allele) or of a regulatory element (McClintock, 1951, 1968).

A

a-m-1 5719A-1

+En   -En

3-4c-d t1   t1, t2-3

① | x a sh   ②

exceptions→ 5c⁺(3-4c-d +   1b t1
3-4a spots)

· x a sh   x a sh

+En   -En   +En   -En

5c⁺   t1, t2-3   non-   t1, t2-3
heritable

Δ in I

B

a-m-1 5719A-1

+En   -En

3-4c-d t1 c̄   t4-5, t7
pale areas

① | x a sh   ②   ③

3c t4   t1   t2-3

x a sh   x a sh   x a sh En

t7   t1   +En   -En
non-   non-
heritable   responsive
to En

Δ in I   no Δ   4b-c t1   t4-5, t7

259
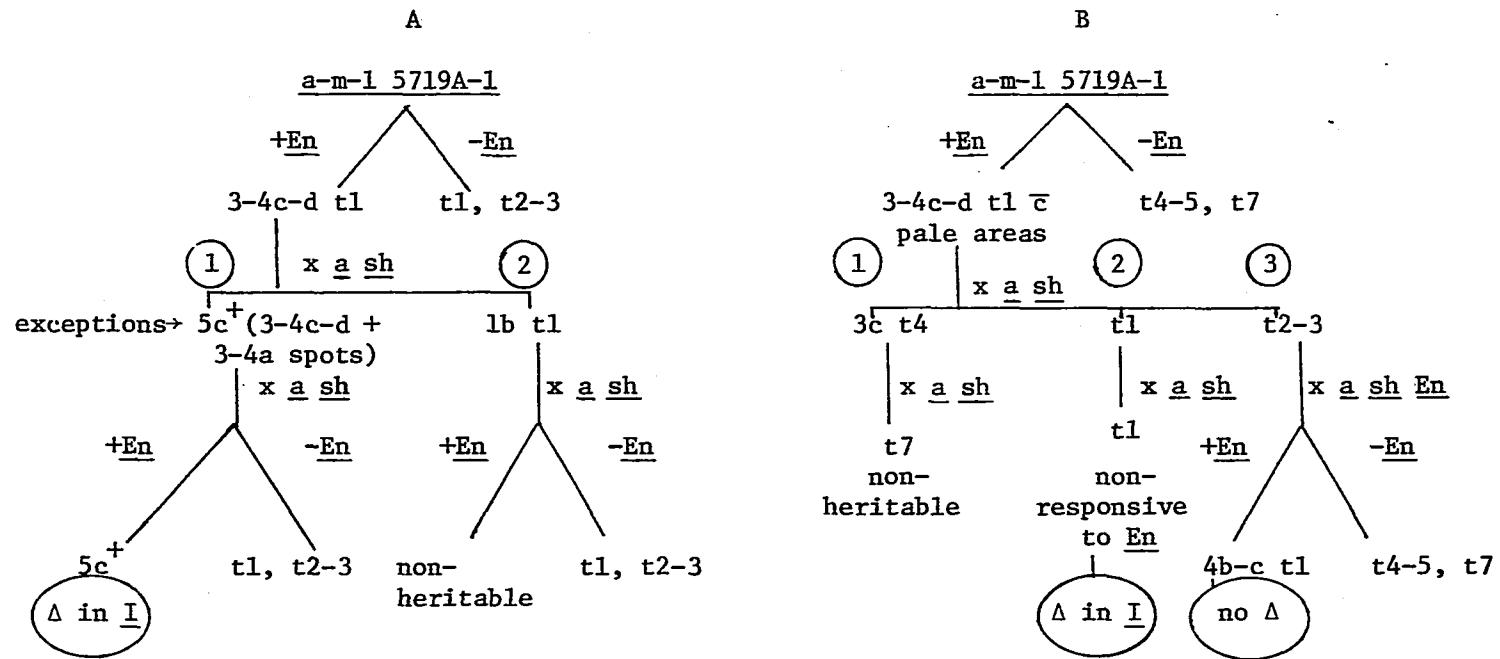
Figure 5.1. Exceptional phenotypes in a-m-1 5719A-1 cultures representing changes in I or En (Δ = change)

C

a-m-1 5719A-1

+En   -En

2-3b t1   t2-3 - t7

① | x a sh   ②

exceptions→   1-2a-b t1   1a-b t1

x a sh

+En   -En   +En   -En

1a t1,   t1-7   1-2a-b t1   t1-7
few 1a t6

(Δ in En)   (Δ in En)
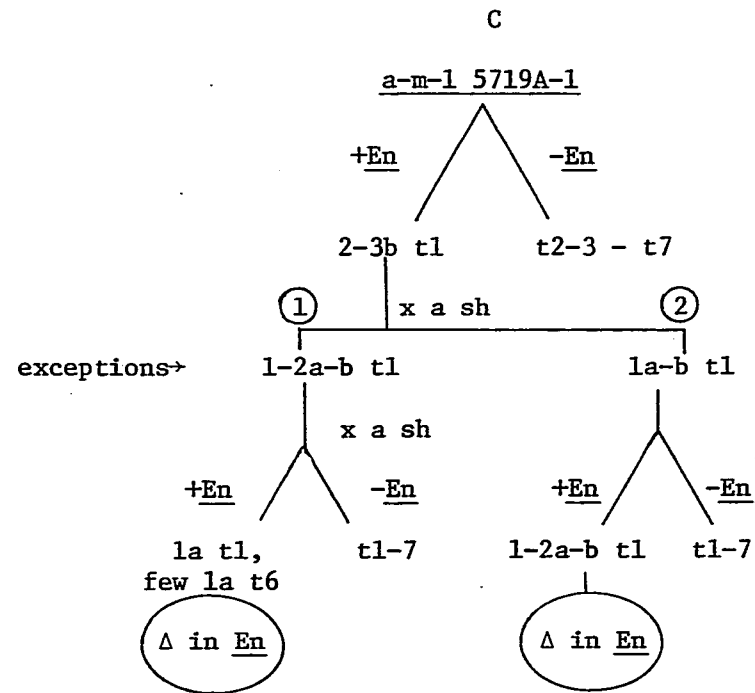
Figure 5.1.   (Continued)

A particular state of an element can be characterized by other criteria in addition to the mutability pattern resulting from differential timing and frequency of mutations. A change in the receptor element is recognized also by a change in the basic allele phenotype (-En or Spm); i.e., in the degree of inhibition of gene activity. With the En or Spm, a change in state of the suppressor (S) component results in a change in the background pigmentation of a mutability pattern. Another unique feature of a change in state of En or Spm is that the changes can occur from an active to an inactive and back to the original active state and are referred to as changes in phase of activity (McClintock, 1957, 1961, 1964; Peterson, 1981).

In the present study, changes in state of both the receptor (I) and the regulatory element (En or Spm) are confirmed in each of the five states of a-m-1 5719A-1, a-m-1 5996-4, a-m-1 6078, a-m-2 7977B and a-m-2 8004 mutable alleles.

1. Changes in state of the receptor element (I)

Several changes in state of receptor (I) are identified. These changes represent phenotypic differences in the absence of the regulatory element En or Spm, responsiveness or non-responsiveness to En or Spm, and if responsive, the kind of response to En or Spm. These changes are classified into the following categories based on the phenotype.

a. Change in the spotting pattern (+En or Spm) without a change in the basic allele phenotype (-En or Spm) (Figure 5.1A- ①)   In a-m-1 5719A-1 cultures, a change in spotting pattern from 3-4c-d tl to 5c$^+$ represents a change in state of the receptor I (compare Figures
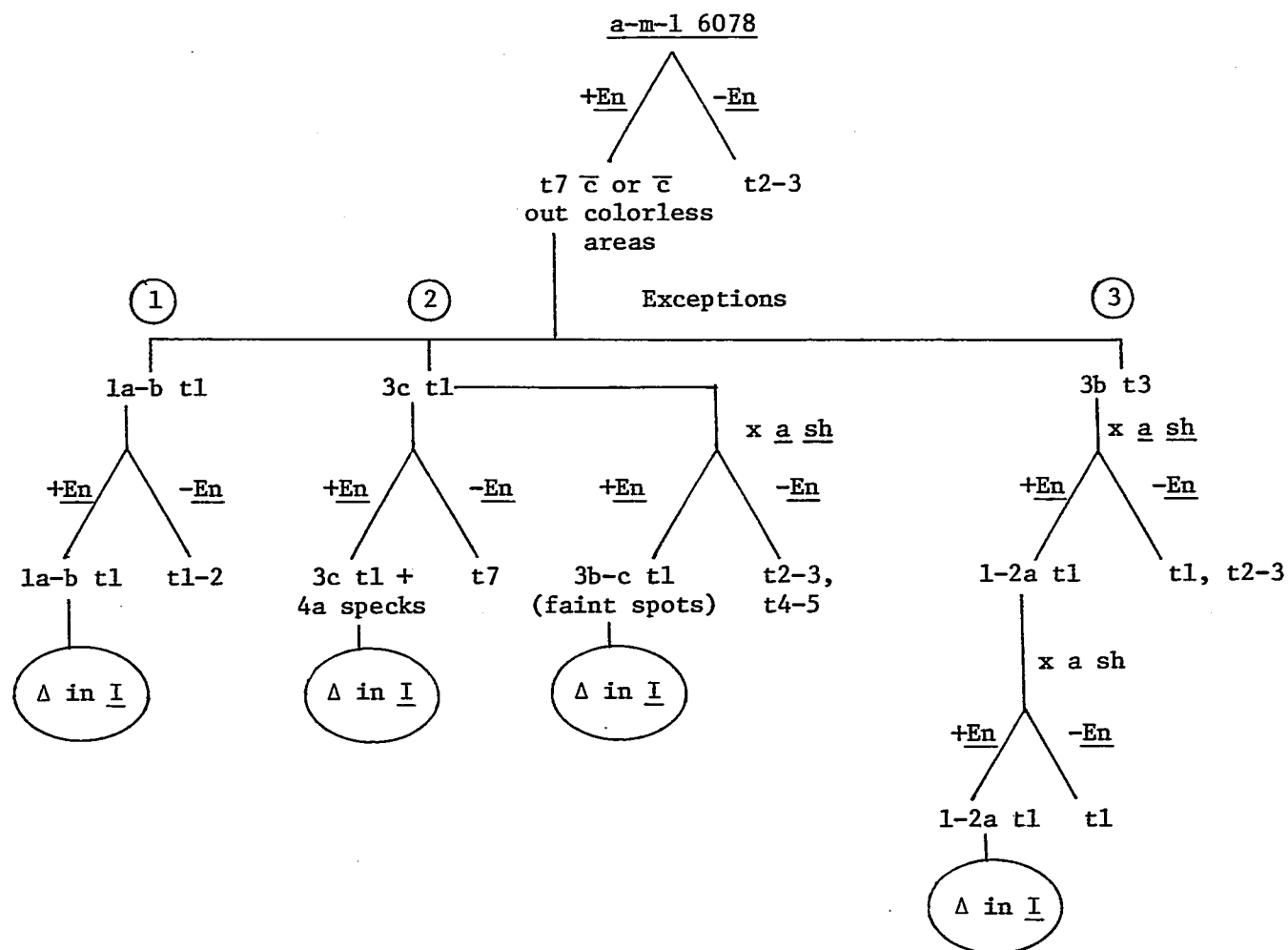
Figure 5.2. Exceptional phenotypes in a-m-1 6078 cultures representing changes in I or En (Δ = change)
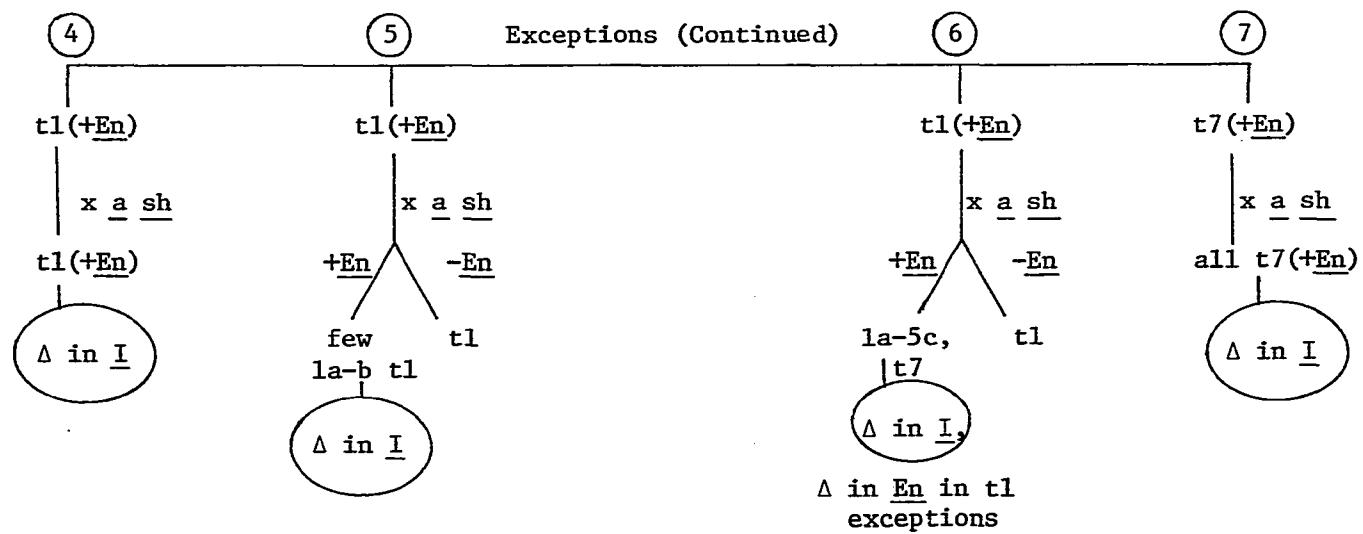
Figure 5.2. (Continued)

4.1B and 4.2). With the aid of a microscope (also in Figure 4.2), the

$5c^+$ spotted pattern reveals 3-4a spots in addition to the parental type

3-4c-d spots, indicating that the change (3-4c-d t1 to $5c^+$) involves

neither a change in size nor a change in frequency of the parental

spotted (3-4c-d) but does result from the occurrence of additional

mutations early in the development of endosperm (3-4a). The combination

of these additional mutant spots and the parental type spots (3-4a +

3-4c-d) yields a $5c^+$ spotted appearance to the new state of I (Figure

5.1A,①). Thus, the changes in spot size (presence of additional 3-4a

spots) and in spot frequency (3-4c-d + 3-4a spots = $5c^+$) are due to an

increased duration of time in which the changed state of I responds to

En. In other words, the new state of I of a-m-1 5719A-1 precociously

undergoes excisions in response to En.

b. _Changes in both spotting pattern (+En) and basic allele pheno-_
type (-En)   From the original a-m-1 6078 state (+En = t7 with or with-

out colorless areas, -En = t2-3), several spotted and basic allele ex-

ceptional phenotypes representing a change in the receptor element (I)

are derived (Figure 5.2). The spot size and frequency ranged from very

low (1a-b) to medium (3c) and the basic allele phenotypes varied from

completely colorless (t1) to full-colored (t7). These diverse pheno-

types represent changes in the receptor element (I) with respect to its

response to En and also in terms of degree of suppression of gene

activity at the A locus.

Non-responsive exceptional types, both colorless (t1) and colored

(t7) are also derived (Figure 5.2, ④ and ⑦) in addition to those

derivatives that respond to En. The tl non-responsive types appeared

in most progenies of +En colored kernels with or without colorless

areas (Tables 4.23, 4.24 and 4.25), indicating that there is a high

frequency of germinal mutations from responsive to non-responsive types.

The t7 non-responsive types may also occur in many of the progenies of

+En colored (t7), but their presence is not detected because of their

similarity in phenotype to that of the t7 responding types (+En).

However, the putative t7 non-responders are identified by a progeny test

in which only t7 progeny appeared without any basic allele phenotype

(t2-3) or colorless non-responsive type (Table 4.25, example 1, 1121-4 X

1217). Four of these t7 non-responders occurred out of fifteen randomly

chosen t7 kernels (with and without colorless areas, Tables 4.24 and

4.25), indicating that approximately 25% of all colored (t7) kernels

are non-responding type.

Thus, the +En state of a-m-1 6078 (t7) yields many non-responsive

(tl and t7) as well as responsive (spotted and basic allele derivatives)

progeny kernel types. This indicates that this particular receptor

element (t7 with En) undergoes frequent changes in state under the influ-

ence of En and these changes are in contrast to the occurrence of only a

few changes in state of other mutable alleles in the present study.

Also unique with the a-m-1 6078 state is that it is colored (t7) in the

presence of En (Figure 4.10B), whereas the mutable alleles in general are

spotted in the presence of a regulatory element. Another peculiarity

with the +En colored state of a-m-1 6078 is that this phenotype often

contains colorless (tl) areas (Figure 4.11A). These three observations:
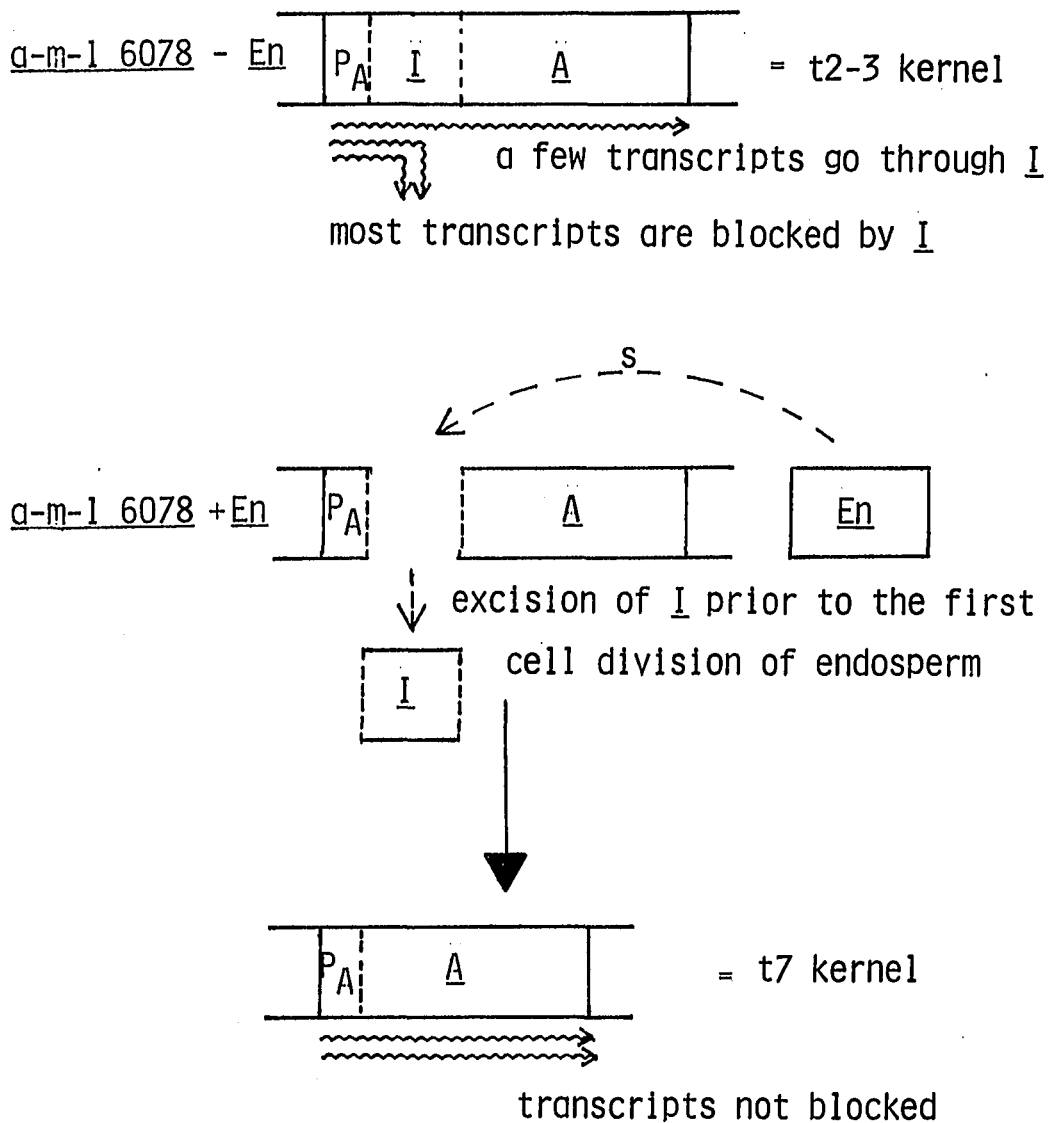
Figure 5.3. Model illustrating the change from t2-3 (−En) to t7 (+En) of a-m-1 6078. A = structural gene; I = receptor element; P_A = promoter of A; En = regulatory element; s = signal of En

i.e., (1) colored (t7) +En phenotype of a-m-1 6078, (2) presence of

colorless (t1) areas on the colored +En phenotype, and (3) frequent

changes in state from colored (t7) +En type to different responsive

and non-responsive types, call for molecular interpretations in terms

of changes in the receptor element (I) in the presence of En. The

following models are proposed for these molecular interpretations.

1) Model to explain the colored (t7) phenotype of a-m-1 6078

state in the presence of En   The original state of a-m-1 6078 is

light pale (t2-3) in the absence of En and it changes to colored (t7)

(Figure 4.10) with or without colorless areas (Figure 4.11) when En is

introduced. This t7 phenotype differs from the usual +En spotted pat-

terns of mutable alleles. It can arise by the excision of I prior to

the first cell division of the endosperm so that all the derivative

cells have the A locus relieved from suppression of I (Figure 5.3). But

the colored (t7) kernels are two types—those with and those without

colorless areas. To account for these two types of colored kernels, the

following models are proposed.

2) Models to illustrate the differences between colored

kernels with and without colorless areas

a) Precise and imprecise excisions of I   The colored

kernels without colorless areas can result from precise excision of I,

as depicted in Figure 5.3, whereas those with colorless areas may

involve two excision steps (Figure 5.4A). First, an imprecise excision

of I, yet relieving the A locus from suppression prior to the first cell

division of the endosperm would give rise to colored kernels. A second

A. TWO-STEP EXCISION OF I

a-m-1 6078 - En   [P_A | I | A]   = t2-3 kernel

a few transcripts go through I

most transcripts are blocked by I

a-m-1 6078 +En   [P_A | I]   [A]

En

imprecise excision of I
relieving A from suppression
of I; occurs prior to the first
cell division of endosperm

excision of residual I and
part of P_A during the endosperm
development

= t7 kernel with
colorless areas
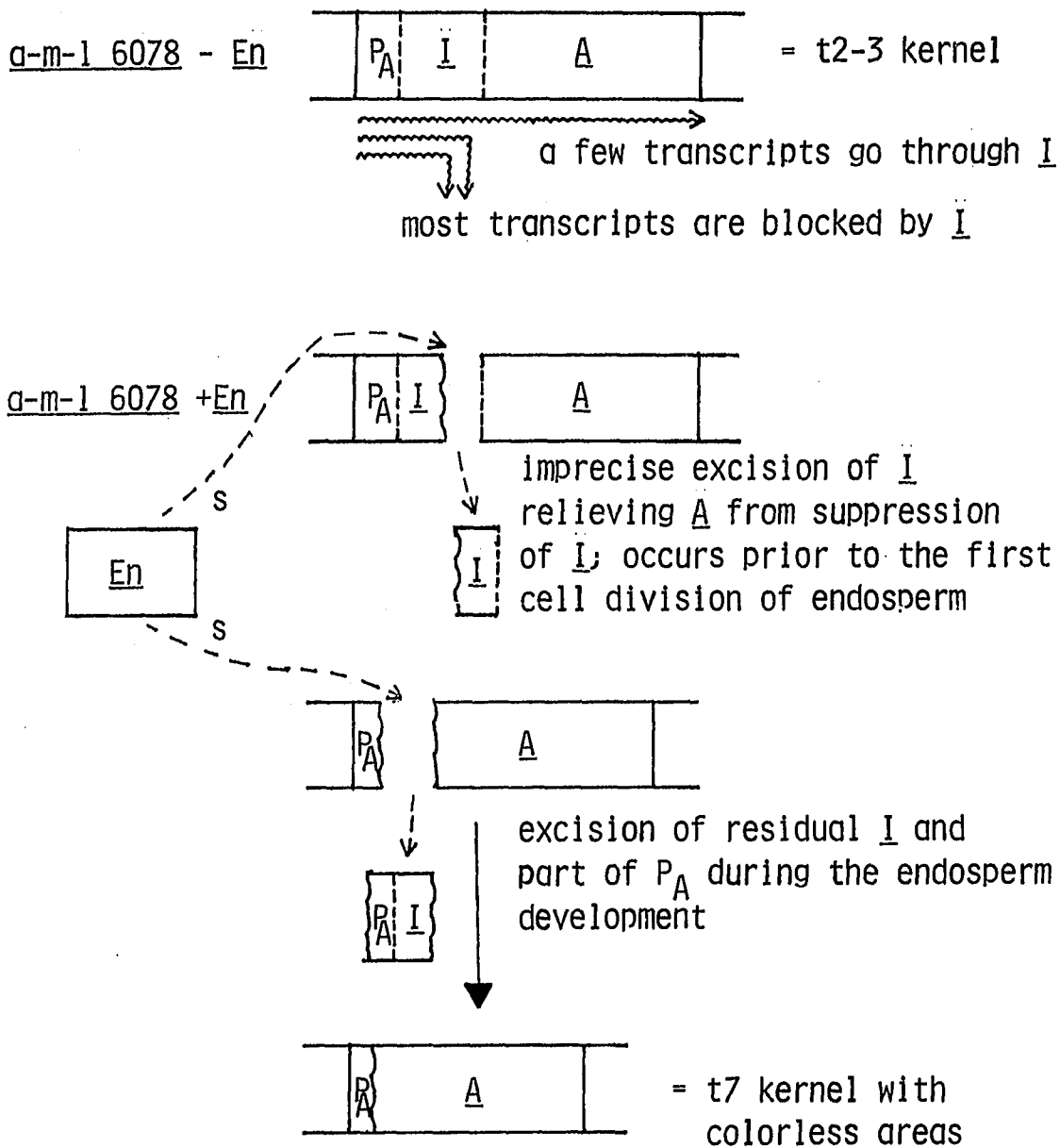
Figure 5.4. Model to explain colorless areas on colored (t7) kernels of
a-m-1 6078 + En; A = structural gene; I = receptor element;
P_A = promoter of A; En = regulatory element; s = signal of En

B. ALTERATION OF EN COMPONENTS

a-m-1 6078 - En P_A | I | A   = t2-3 kernel

a few transcripts go through I

most transcripts are blocked by I

a-m-1 6078
+En

En = S^+ | M^+

signal from S^+ elevates t2-3 to t7
signal from M^+ imprecisely excises I

A   = t7 kernel with colorless areas

excision of I
and part of P_A

germinal mutants are
expected in the progeny

change from M^+ M^-

En = S^+ | M^-

signal from S^+ elevates t2-3 to t7
no signal from M^-; ∴ no excisions of I

P_A | I | A   = t7 kernel without colorless areas

germinal mutants are not
expected in the progeny

Figure 5.4. Continued

excision of the residual $\underline{I}$ at $\underline{A}$ locus along with a part of the gene

material of the locus (causing a deletion of $\underline{A}$ locus) during the endo-

sperm development can result in colorless areas on colored kernels.

The differences between colored with and without colorless areas

can also be explained by another model.

   b) <u>Alteration</u> <u>in</u> <u>En</u> <u>components</u>   According to this

model, the original t2-3 state changes to t7 in the presence of $\underline{En}$ with

$\underline{S}^+\underline{M}^+$ components (Figure 5.4B). The $\underline{S}^+$ component elevates the t2-3 pale

to t7 colored type, whereas $\underline{M}^+$ component excises the receptor ($\underline{I}$) from

the locus. The excisions of $\underline{I}$ during the endosperm development result

in colorless areas. A change from $\underline{M}^+ \rightarrow \underline{M}^-$ would give colored kernels

without colorless areas. If $\underline{M}^+$ changed to $\underline{M}^-$, the progeny of colored

kernels without colorless areas ($\underline{S}^+\underline{M}^-$) would not be expected to have

germinal mutants because of the $\underline{M}^-$ component and should include the

original t2-3 basic allele phenotype due to the segregation of $\underline{S}^+\underline{M}^-$.

But in tests to determine the basic allele phenotype of t7 types without

colorless areas, 6 out of 10 have colorless (t1) germinal mutants and

the other 4 yielded only the parental type t7 (without colorless areas).

These latter 4 were without any colorless germinal mutants and t2-3

basic allele phenotype (Table 4.24). These tests confirmed that the

colored kernels without colorless areas are not a consequence of a

change from $\underline{S}^+\underline{M}^+$ to $\underline{S}^+\underline{M}^-$ but they represent either responsive (with

germinal mutants) or non-responsive (without germinal mutants) type to

$\underline{En}$ ($\underline{S}^+\underline{M}^+$). Therefore, the explanation of $\underline{S}^+\underline{M}^+$ to $\underline{S}^+\underline{M}^-$ change of $\underline{En}$ in

Figure 5.5. Model illustrating the derivation of responsive (spotted) and non-responsive (colorless and colored) germinal mutants from the original colored (t7) a-m-1 6078 + En state. A = structural gene; I = receptor element; P_A = promoter of A; En = regulatory element

a-m-1 6078 + En   [P_A | I | A]   = t7 kernel
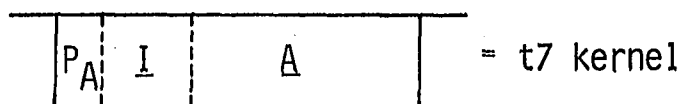
germinal mutants

spotted
(responsive to En)

1. Altered I
   (deletion with in I)

I is smaller due
to deletion

2. Change in
   position of I
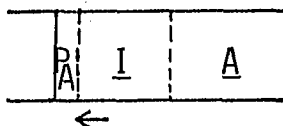
I is repositioned
into P_A region;
still responds to En

3. Alteration and
   change in
   position of I

colorless (t1)
(non-responsive to En)

1. Excision of I
   and part of A
   gene
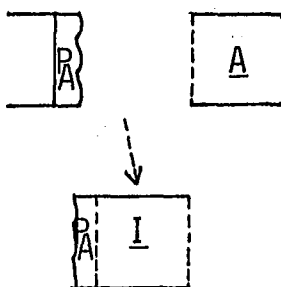
2. Change in
   position of I
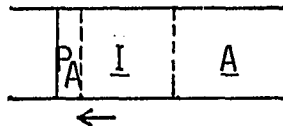
I is repositioned
into P_A region and
is not responsive
to En in this new
position

colored (t7)
(non-responsive to En)

Precise excision
of I
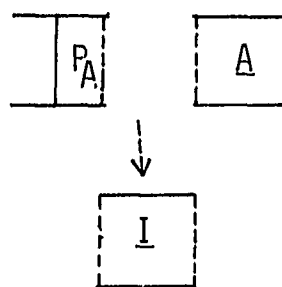
colored kernels is not valid.

3) <u>Model for the derivation of En-responsive spotted and non-responsive colorless and colored exceptions</u>   Among the progenies of

t7 colored state (+<u>En</u>) of <u>a-m-1 6078</u>, several <u>En</u>-responsive spotted and

non-responsive colorless and colored exceptions are derived (Figure 5.2).

From the spotted types, derivatives with a basic allele phenotype (-<u>En</u>)

varying from colorless (t1) to colored (t7) are obtained. The diversity

of expression of these derivatives indicates that the receptor element

(<u>I</u>) of <u>a-m-1 6078</u> state frequently undergoes changes in state. As

illustrated by the model in Figure 5.5, these changes in state of <u>I</u> can

be interpreted to arise by either of the following molecular changes

in <u>I</u>:

(1) Alteration in <u>I</u>;

(2) Change in the position of <u>I</u> within the <u>A</u> locus; or

(3) Both alteration and change in position of <u>I</u>.

These molecular changes in <u>I</u> are reflected in a change in response

of <u>I</u> to <u>En</u> and also in the degree of suppression of <u>A</u> gene by <u>I</u> in the

absence of <u>En</u> (basic allele phenotype). The change in response of <u>I</u> to

<u>En</u> is recognized either by an altered response (spotted derivatives vs.

colored (t7) phenotype of the original <u>a-m-1 6078</u>) or by a complete

lack of response (non-responsive colorless and colored derivatives).

In the model for the original colored (t7) phenotype (Figure 5.3), it

has been interpreted that <u>I</u> is excised immediately after fertilization

but prior to the first cell division of the endosperm. In the spotted

derivatives, however, the changed <u>I</u> responds to <u>En</u> (excised) later during

the development of the endosperm so that the relief of the A locus is

expressed only in some cells of the endosperm. In addition to these

changes differing in response to En, the new states of I also differ

in control of the gene activity at A locus ranging from full suppression

(colorless) to full expression (colored). Such diverse gene activity is

also evident in non-responsive colorless (t1) and colored (t7) deriva-

tives. However, these non-responsive types may either represent a

changed state of I (I is present at the locus) or lack I at the locus.

If they lack I, the colored non-responsive type can be interpreted to

arise by precise excisions of I in the germ line cells, whereas the

colorless non-responders result from excision of I plus a portion of A

gene (imprecise excision) and thus equivalent to a deletion at the locus.

The same explanation is given to the presence of colorless areas on

colored kernels (Figure 5.3), but in this case the excisions are somatic.

This explanation is valid because the presence of colorless non-

responders in the progeny is coincident with that of colored with color-

less areas (Table 4.24).

The precise and imprecise excisions of the receptor element (I) in

these models are comparable to those demonstrated at the molecular level

for transposable element Tn 10 in His⁻ insertion mutants. A precise

excision of Tn 10 is genetically expressed as reversions of an insertion

mutation and it involved a deletion between the short direct repeats of

target DNA that flank an inserted Tn 10 (Kleckner et al., 1981). Such

deletion was shown to restore the wild-type target sequence (Foster

et al., 1981). In nearly precise excision, deletion of all but 50 bp
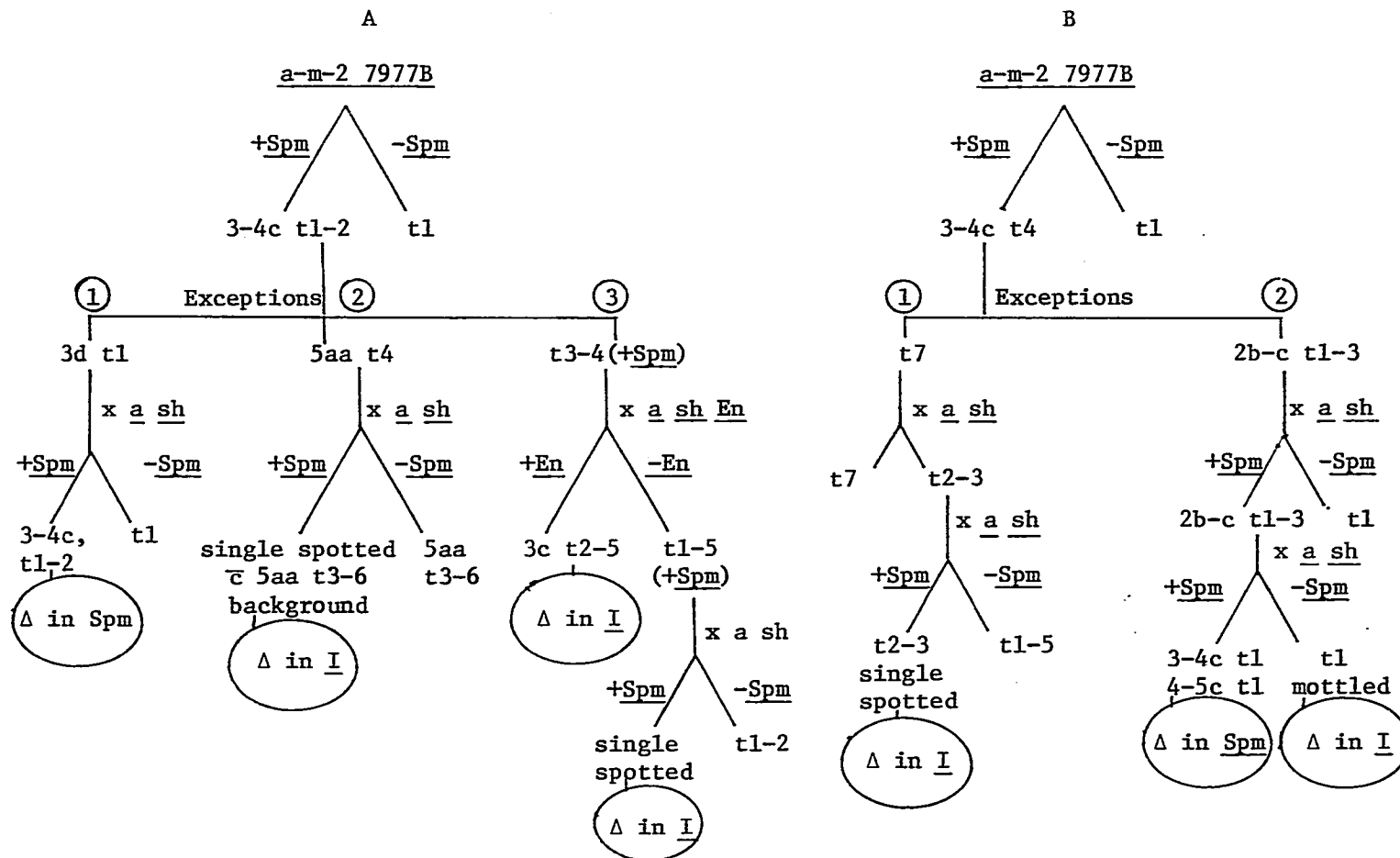
Figure 5.6. Exceptional phenotypes in a-m-2 7977 cultures representing changes in I or Spm (Δ = change)

of Tn 10 occurred.

Like the a-m-1 6078 state, a-m-2 7977B also shows changes in both
spotting pattern and basic allele phenotype. However, with a-m-2 7977B,
only two such exceptions are derived. They are 5aa t4 and t2-3 (Figure
5.6A②, B①). The 5aa t4 appeared among test cross progenies of 3-4c
t1-2 phenotype of a-m-2 7977 cultures (Figure 5.6A②). The t2-3 excep-
tions had a more circuitous origin. They were extracted from t7 excep-
tional kernel which in turn arose among selfed progeny of 3-4c t4
cultures of a-m-2 7977B (Figure 5.6B①).

The 5aa t4 appears as pale (t4) but fine specks of 5aa type are
seen under a microscope. In the presence of Spm, the 5aa t4 exhibits
single spots. Similarly, the t2-3 exception shows single spots in the
presence of Spm, and its basic allele phenotype (-En) ranges from t1 to
t5. Thus, these exceptional phenotypes represent new states of the
receptor element (I) that exhibit low response to Spm and also variable
level of gene activity (basic allele phenotype) in the absence of Spm.
However, it is not clear what causes the fine specks on 5aa t4 kernels.

The changes in both spotting pattern and basic allele phenotype
are also reported in other studies (Peterson, 1970a; Fowler and Peterson,
1974). Germinal mutants exhibiting varied patterns of mutability and
levels of pigmentation were derived from a-2-m(r-pa-pu) allele. In
these studies, the differences among mutable patterns were attributed
to the transposition of an unaltered I to a new location within the A2
locus. Non-responsive types showing different levels of pigmentation
were interpreted to result from transposition of an altered I within
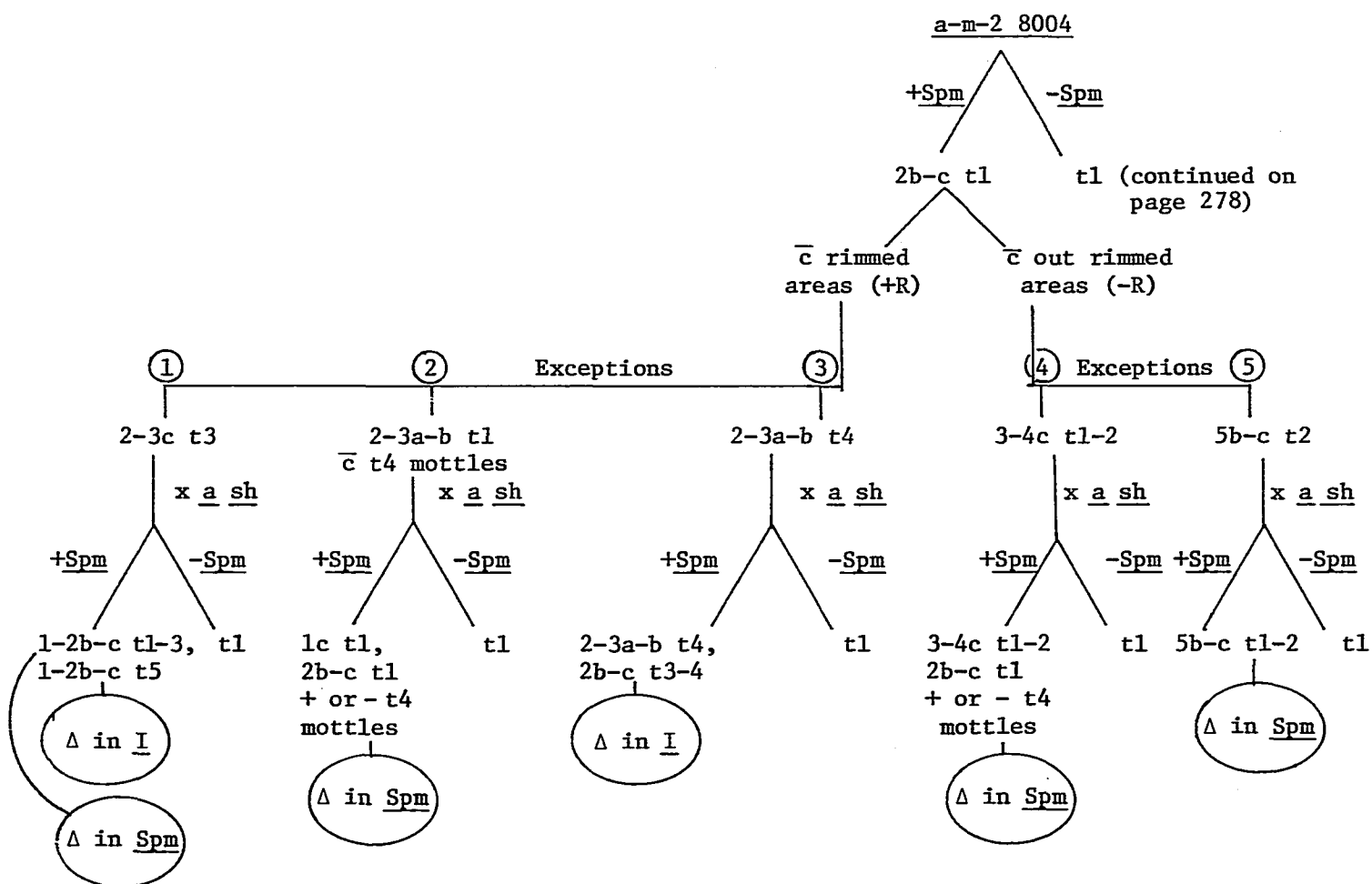
Figure 5.7. Exceptional phenotypes in a–m–2 8004 cultures representing changes in I or Spm (En) (Δ = change)
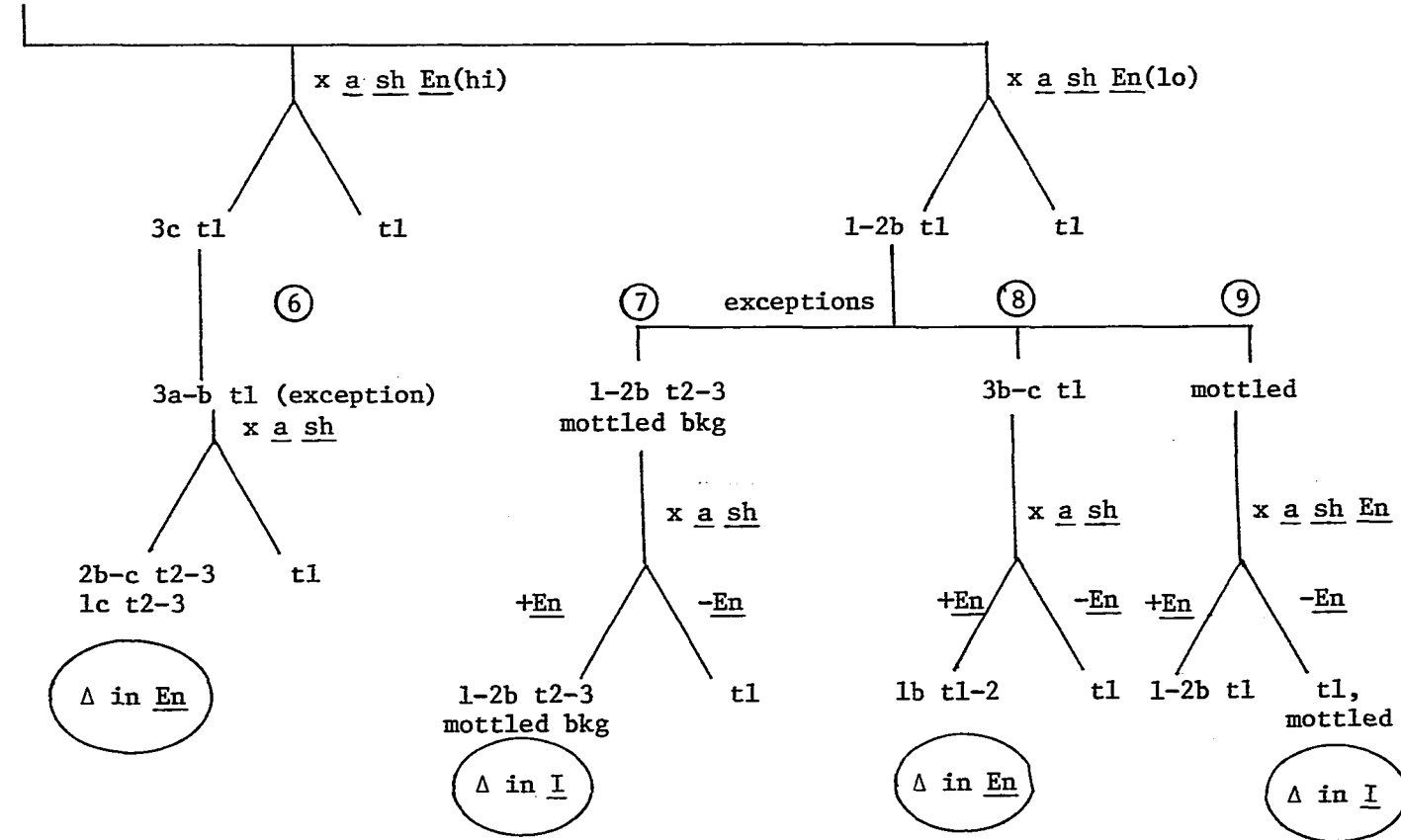
Figure 5.7.   (Continued)

the A2 locus or away from the locus.

   c.   Change in only the background coloration of the spotted exceptions   Among a-m-2 8004 cultures, three confirmed spotted exceptions showed no change in spotting pattern but did have darker background pigmentation than the parental kernels (Figure 5.71 , ③ and⑦). With a constancy in the spotting pattern but with an alteration in the background coloration, this combination must indicate that the change in I is confined to the site that receives the signal from the S component of En. This change in the I element must be different among the spotted exceptions for each exhibits a different level of background pigmentation. However, since all the spotted exceptions are background coloration changes and thereby involve a change of the same site of I, it is likely that this site is more prone to change than the kinds of events that would lead to spotting pattern changes, that is, those receiving M signals from En.

   d.   Change of only the basic allele phenotype without a change in response to En or Spm   From a-m-2 7977B and a-m-2 8004, several non-En(Spm) types are derived that are mottled (Figure 5.6B under ②, 5.7 ⑨). When these mottled are tested with En or Spm, the response is similar to the parental type. What is different is that without En or Spm, the parental type is colorless while these exceptions are mottled. Thus, the mottled does represent a change in the basic allele phenotype and expresses a change in the effect of the I element on the activity of the locus. However, since the mottled phenotype is not fully heritable (Figure 5.7⑨ , Table 4.47a, 4.57c), it represents only a transitory change of the I

element. This inheritance pattern is comparable to that of a-m-2 7977B basic allele phenotype of colorless with faint pale areas (Table 4.41).

The mottled phenotype resembles the preset pattern of a-m-2 7977B state reported by McClintock (1967b) in that both exhibit pale areas in a colorless background in the absence of the regulatory element Spm or En. Also, the response of mottled and presets to Spm or En is the same as that of the basic allele. However, the mottled and presets differ in one respect. That is the progeny of mottled include several kernels of the same type, whereas the preset pattern is either not heritable or occasionally inherited into one or two progeny kernels.

There is one difference with the preset pattern that is not confirmed with the mottled phenotype. The kernels with preset pattern always arise in the progeny of spotted phenotype. In the present study, although the mottled appeared in the progeny of spotted (+Spm or En), they do not occur in all the progenies of spotted (Table 4.45).

e. A change that differentiates En from Spm mode of action

Among a-m-2 7977B progenies, 9 pale (t3-4) exceptions are isolated. They have no spots. In tests of these exceptional derivatives with a-m(r)/ a-m-1, a highly active Spm (high frequency spotted) is recognized (Table 4.38b). Further, when tested with En (a sh En), the progeny kernels are spotted (3c t2-5) (Table 4.38a). These tests are summarized as follows, where + stands for spotted and - for no spotted.

	Spm	En
a-m-2 7977B t3-4	-	+
a-m(r)/a-m-1	+	+

These findings would indicate that the receptor I responds to En but not to Spm. This suggests that the receptor sites for Spm and En are different or modes of action of these two regulatory elements differ from each other. This is not unlike the Fcu-Spf differential cited by Gonella and Peterson (1978).

2. Changes in state or phase of the regulatory element En or Spm

For the purpose of present study, changes from an active to another active or to an inactive En or Spm are considered as changes in state. If the changed state reverts to the original state, then the whole series of changes (original active state → another active or an inactive state → original active state) are regarded as changes in phase of activity. With En or Spm, these changes in state or phase may involve either one or both of suppressor (S) and mutator (M) components. A change in S is recognized by a change in the background pigmentation of a spotted pheno-type, whereas a change in M alters the spotted pattern (spot size and frequency) itself.

In the present study, changes in state of En and Spm occurred both to lower and higher levels of activity. With En, a change from 2-3b tl to 1-2a-b tl in a-m-1 5719A-1 cultures confirmed that the change involved only the M component (Figure 5.10①,②). The changed En in 1-2a-b tl is seen to undergo further changes in S component from S → s in a few of the progeny kernels (1a t6) (Figure 5.10①).

In a-m-2 7977B cultures, Spm changed in state to several levels (both low and high) of activity. The original state of 3-4c t4 changed

to 2b-c t1-3 (Figure 5.6B ②), indicating that the M component is weakened. A change in S component (S → s) is also suggestive (t4 → t1-3), but it is not as clear cut as the M change (S component elevates pigmentation of a-m-2 in contrast to suppression of the a-m-1 allele). The M component in 2b-c t1-3 exceptions further changed to higher levels of activity that included 3-4c and 4-5c spotted. The background pigmentation of these spotted, however, was colorless (t1), indicating that the S component is inactive (s) (Figure 5.6B under ②).

These changes in a-m-2 7977B cultures confirm that the Spm under-goes changes in state to different levels of activity. The change from 3-4c t4 → 2b-c t1-3 → 3-4c t1 can be considered as a change in phase of activity (Figure 5.6B ②).

Like the changes in a-m-2 7977B cultures, Spm and En in a-m-2 8004 also showed several changes in state or phase of activity (Figure 5.7 ①, ②, ④, ⑤, ⑥ and ⑧). Of particular significance is the change of Spm from 2b-c t1 with rimmed areas (+R areas) to 2-3a-b t1 with t4 mottles (Figure 5.7 ②). The R areas in 2b-c t1 spotted kernels are due to a change in Spm from an active to an inactive phase of activity early during the endosperm development (McClintock, 1967a). The absence of these R areas in exceptional kernels with changed 2-3a-b t1 spotting pattern and coincidental presence of t4 mottles in these kernels indi-cate that the t4 mottles represent changed R areas. Also, the t4 pale pigmentation of these mottles does correspond to the dark rims of the R areas (Figure 4.17). However, since the t4 mottles are smaller than R areas and do not enclose non-spotted colorless areas in contrast to R

areas, the mottles can be considered to represent late changes in $\underline{Spm}$ from an active to an inactive phase of activity during the endosperm development. Thus, a change from 2b-c t1+R areas to 2-3a-b t1 with t4 mottles represents two concomitant changes in $\underline{Spm}$, one a change in state (2b-c t1 → 2-3a-b t1) and the other a change in the time at which $\underline{Spm}$ undergoes changes in phase of activity during the endosperm development.

Changes in state and phase of activity of $\underline{Spm}$ and $\underline{En}$ have been reported in other studies. McClintock (1962) isolated several changed states of $\underline{Spm}$ from the original state ($\underline{Spm}^S$) that was recognized by many deeply pigmented spots in a colorless background with the $\underline{a-m-1}$ mutable allele. The changed states of $\underline{Spm}$ produced several tiny spots against a colorless background, indicating that the mutator ($\underline{M}$) component of these states was very much weakened and were appropriately designated as $\underline{Spm}^W$. These $\underline{Spm}^W$s also differed in the frequency of reversion to $\underline{Spm}^S$.

McClintock (1957) also derived from the original $\underline{Spm}$ ($\underline{Spm}^S$), an $\underline{Spm}$ that showed cyclical changes in phase of activity. It was first detected with $\underline{a2-m-1}$ mutable allele (McClintock, 1957) and later (McClintock, 1964, 1967a) in $\underline{a-m-2}$ cultures. Since the $\underline{a2-m-1}$ basic allele phenotype is pale and because it does not respond to $\underline{M}$ component of $\underline{Spm}$, frequent changes in $\underline{S}$ component of $\underline{Spm}$ from active → inactive → active are recognized by changes in kernel pigmentation from colorless → pale → colorless areas, respectively. In $\underline{a-m-2}$ cultures, changes in phase of both $\underline{S}$ and $\underline{M}$ components are detected. A change from $\underline{S}$ → $\underline{s}$ is recognized by colorless sectors ($\underline{S}$ component elevates pigmentation of

the a-m-2 allele in contrast to the suppression of the a-m-1 allele)

and an M → m change by a sector without spots. Changes in phase of

activity of S component (S → s → S) are also demonstrated with En in

kernels with the a-m-1 allele (Peterson, 1981).

3. Molecular interpretations of changes in state
   of controlling elements

There are two alternatives proposed to explain the origin of new

states of the receptor and the regulatory elements. McClintock (1958)

interpreted that the changes in state arise by an alteration or change

in the composition of the elements. Peterson (1976a) proposed that a

change in the position of the elements in the genome could account for

these changes.

According to McClintock, the strong and weak component differences

of Spm are due to differences in composition of the regulatory element.

But experimental data are not available to support the qualitative dif-

ferences among different states.

That a change in the position of a controlling element is responsi-

ble for the origin of a new state gains support from several experimental

findings. The most illustrative experiment is that conducted by

Peterson (1976b) in which a single source of En was used to induce

mutability at A2 and C loci. The spotting patterns of the mutable

alleles thus obtained were compared to that of the same En on a standard

a-m(r) allele. Lack of correlations in these comparisons indicated that

En changes its state when transposed to a new location in the genome.

However, it is not known whether the transposition process itself changes

the composition of the controlling element. If the transposition

involves both excision and insertion events, there is a greater possi-

bility of compositional change of the element.

Support for the compositional change can be seen in the transposi-

tion of bacterial transposable element Tn 10. Kleckner et al. (1981)

reported that both precise and imprecise excisions of Tn 10 occur.

But Berg (1977) showed that there is a lack of association between

excision and transposition of transposable elements in bacteria. In the

Uq-ruq controlling element system in maize, the derivation of a second

Uq element from parents containing only one Uq element indicated that

transposition of Uq is not associated with the excision of the original

Uq (Friedemann, 1981; Friedemann and Peterson, 1982).

In the absence of an association between excision and transposition

of controlling elements, the other possibility for a change in composi-

tion of the elements is by insertion. It is known that insertions of

transposable elements in bacteria cause chromosomal aberrations

(Starlinger, 1980b). The most common aberration is the deletion of

adjacent DNA extending from one terminus of a transposable element to a

variable site in the bacterial chromosome. Such adjacent deletions are

reported with ISI (Reif and Saedler, 1975), IS2 (Peterson et al., 1979),

Tn 3 (Nisen et al., 1978), Tn 10 (Kleckner et al., 1979), and bacterio-

phage Mu (Toussiant et al., 1977). If similar adjacent deletions occur

with the insertion of controlling elements in maize, the changes in

state can be attributed to deletions of DNA in or adjacent to the target

locus but not to deletion (and thus no change in composition) of the

controlling element itself.

However, in a specific case of Tn 10 in bacteria, internal dele-
tions and inversions have been reported (Kleckner et al., 1979). Tn 10
carrying a gene for tetracycline resistance (2500 bp) is flanked by
inverted repeats (IS 10 = 1400 bp) at both ends (Kleckner et al., 1975).
Mutants with deletions of internal termini of IS 10, unique DNA of Tn 10
were obtained. In some mutants, an inversion of one IS 10 and some DNA
adjacent to it was observed (Ross et al., 1979a). The possibility of
similar internal deletions and inversions of controlling elements
during transposition would account for a change in composition and thus
for a change in state.

With these analogies of bacterial transposable elements, it can be
surmised that a change in state of controlling elements might depend on
both their composition and position in the genome. But conclusive data
will come forth only when molecular techniques will enable the cloning
of different states of controlling elements and thereby permit the DNA
sequencing of these elements.

B. Special Cases of Unexpected Phenotypes
and Their Heritability

1. Unexpected segregations of + and - En phenotypes
   of a-m-1 5996-4

The -En phenotype of the original state of a-m-1 5996-4 is color-
less (tl). When En is introduced through crosses (Figure 4.6, 1979
3215), this state exhibited 4a, c-d tl spotted pattern (Figure 4.4).
In test crosses, it is expected that the 4a, c-d tl spotted kernels

will yield progeny kernels of the same phenotype and the colorless (t1)

basic allele phenotype (-En) in 1:1 ratio (one independent En). Instead,

two unexpected and distinct features appeared among the progenies

(Table 4.15). First, progenies are found with less than the expected

number of spotted progeny (the <50% group) and others with more than

the expected number of spotted (the >50% group). In addition, among

the non-spotted progeny of each of the two groups, only a small per-

centage of kernels show the original colorless (t1) basic allele pheno-

type. Most have variable pale pigmentation with the largest class being

t2-3 and a few are t4-5 and t6-7.

When the spotted kernels of each of these groups (<50% and >50%)

were test crossed, the resulting progenies showed the heritability con-

sistent with the parental type (Tables 4.19, 4.20). The progenies with

>50% spotted can be explained by the presence of more than one En and/or

a linked En.

But this explanation is not applicable for those cases with <50%

spotted. For these cases, one could consider a change in phase of

activity of En. Though such an explanation can be entertained for the

progenies of <50% group, this cannot explain why this En originating

from a common source as the <50% spotted group does not exhibit the same

phenomenon in progenies of >50% group.

In addition to this aberrant segregation ratio of spotted to non-

spotted, two other anomalies were obvious with respect to the phenotype

of the spotted and non-spotted progenies. One, there is a discrepancy

between progeny kernels and the parentals in that the frequency and the

size of spots were variable among progenies. Secondly, the non-spotted progeny were consistently different between the two groups. Among the group with <50% spotted progeny, the non-spotted varied from t1 to t7 (Table 4.19) and differing from this, the non-spotted among the group with >50% spotted ranged from t1 to t3 (Table 4.20). From these comparative analyses, the following deductions can be made:

(1) There are independent factors that induce the change in basic allele phenotype (-En) from colorless (t1) to variable pale types.

(2) Since a correlation exists between the variable pale pigmentation of the non-spotted and the frequency of spotted in individual crosses (Tables 4.19, 4.20), the same factors must be responsible for the aberrant segregation ratios of spotted to non-spotted.

An alternative interpretation for the fewer than expected (<50%) spotted and for the non-heritability of 4a, c-d t1 spotted phenotype is a change in state of either the mutable allele (receptor) or the regulatory element En. Since the spotted progeny are very few and are different from the parental spotted (Table 4.19), these changes must involve the following:

(1) The changes in state must occur in germ line cells and at a high frequency.

(2) A change in state of the mutable allele should be from a responsive to a non-responsive state, whereas a change in En must be from an active to an inactive state.

A few spotted progeny kernels that are different from the parental type can result from changes to another responsive (mutable allele) or another active (En) state.

The major feature of this aberrant segregation of spotted to non-spotted that can be tested is whether the change is in the receptor or in the regulatory element. To test this, the non-spotted pale types (t2-3, t4-5 and t6-7) from <50% spotted group (Table 4.19) were crossed with En from the a sh En segregates selected from >50% spotted group (Table 4.20). If the resulting progeny of this cross contain <50% spotted, it is the property of the receptor allele and not of En that is responsible for fewer spotted than expected. On the other hand, if there are >50% spotted progeny kernels, then it is the En in <50% spotted group of cultures (Table 4.19) that changes in its phase of activity.

Among t2-3 pale X a sh En crosses, two out of four contained <50% spotted and the rest with 50% spotted (Table 4.16). The other two classes of pales, t4-5 and t6-7 in crosses with a sh En yielded all the progenies with <50% spotted (Tables 4.17 and 4.18). These results confirm that it is the receptor at the mutable allele and not the regulatory element En that is responsible for the unexpected <50% spotted progenies. However, this mutable allele associated property can be attributed either to changes within the allele or to changes influenced by some factors in the genome affecting the allele.

2. <u>Variable pale phenotypes of a-m-1 5719A-1 state</u>

The original <u>a-m-1 5719A-1</u> state is colored (t7) without En and 3-4c-d t1 spotted with En. The spotted phenotype contains large pale

areas (approximately t4) in addition to the t7 type spots (Figure 4.1).

Among test cross progenies of the original colored (t7) state (-En), unexpected variable pale type kernels (t1 to t6) and a few low spotted kernels appeared (Figures 4.1A, B and C). In test crosses, the low spotted did not yield any of the same type, but included variable pale types. The variable pale types were suspected to represent changes in state of a-m-1 5719A-1 allele. Therefore, they were classified into t1, t4-5 and t7 and were studied for their heritability and response to En. In heritability studies, the progeny types of these three variable pales were as follows (Tables 4.3, 4.4, 4.5):

Parental type	Progeny types
t1	t1, t2-3, t4-5
t4-5	t2-3, t4-5
t6-7	t2-3, t4-5, t6-7

Though other pale types are represented along with the parental type among the progenies, these other pale types fall closer to the parental class. For example, the progeny of t1 do not contain t6-7 and similarly the progeny of t6-7 are without t1 type kernels.

When all the variable pale types (t1, t4-5, t7) were tested in En-response studies, all responded in the same way in that the spotting was 3-4c-d t1. This result indicates that despite the variable pale types, they all represent the same state of a-m-1 5719A-1 in terms of response to En. Then, how do these variable pale types arise? It is generally considered that a change in state of a mutable allele (receptor element) is not expected to occur in the absence of a regulatory element.

But in the case of a-m-1 5719A-1, the potential for generating variable

pigmented types could be an intrinsic property of the allele itself.

There is some precedence for such a property of allelic activity.

Gonella and Peterson (1978) reported variable dilute pigmentation of

r-cu mutable allele in the absence of the regulatory element Fcu. The

variable pigmentation of r-cu has been interpreted to result from setting

of gene activity at some point in the endosperm development. Similar

setting of gene activity could be involved in a-m-1 5719A-1 variable

pigmentation. But it is not known what conditions or genetic components

set a particular level of gene activity in individual kernels in the

absence of a regulatory element.

McClintock (1967b) reported a setting of gene activity of a-m-2 7995

and a-m-2 7977B mutable alleles. But this setting, in contrast to that

of a-m-1 5719A-1 allele in the present study and of rcu allele (Gonella

and Peterson, 1978), requires the presence of Spm regulatory element in

association with the mutable alleles during the early stages of plant

growth. According to McClintock, the alleles are "preset" by Spm at an

early stage of plant development. But the preset alleles are expressed

as mottled phenotype only in progeny kernels without Spm. However,

not all the kernels without Spm exhibit the characteristic mottled pre-

set pattern. To explain this expression of preset pattern only in a

few kernels, McClintock proposed another step called "setting" occurring

during meiosis.

Since the preset and setting of gene activity require the presence

of an active Spm, such gene control mechanisms are not expected to occur

in cultures of a-m-1 5719A-1 lacking an Spm or En. However, there could be some genetic mechanisms or factors responsible for variable pale phenotypes of a-m-1 5719A-1 state.

Another possibility for the origin of variable pale types of a-m-1 5719A-1 allele is the presence of a regulatory element such as Spm with weak mutator (M) and an unstable suppressor (S) components. The S component changes in phase to different levels of activity in germ line cells so that the individual kernels express different levels of pale pigmentation. However, for the individual kernels to be uniformly pigmented, as is the case with the variable pales of a-m-1 5719A-1, the S component should remain stable during the endosperm development.

This possibility of the presence of an Spm (with weak M and unstable S components) in a-m-1 5719A-1 cultures is supported by the occurrence of a few low spotted kernels among the progenies of the original colored (t7) kernels (Flow diagram 4.1A, B and C, 1976 progeny) and their disappearance in subsequent test crosses (1977 progenies). Also, similar low spotted (1b t1) reappeared as exceptions in test cross progenies of 4c t1 spotted (Table 4.7) and they were neither heritable nor could induce mutations when tested on a-m(r)/a-m-1 tester (Table 4.9).

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