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Perspectives on Nobel Laureate Barbara McClintock's Publications (1926-1984): A Companion Volume

Edited by Lee B. Kass

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Cover Photo

1. Dr. Charles R. Burnham (Ph.D. Wisconsin), National Research Council Fellow, Department of Plant Breeding; 2. Marcus M. Rhoades, graduate student, Department of Plant Breeding; 3. Dr. Rollins A. Emerson, Head, Department of Plant Breeding; 4. Dr. Barbara McClintock, Instructor, Department of Botany; 5. George W. Beadle, graduate student, Department of Plant Breeding. Photo taken at Cornell University, 1929, in the Plant Breeding Garden, outside the Plant Breeding field house (currently fondly called the "McClintock Shed"; see back cover). Beadle shared a Nobel Prize in 1958; McClintock was awarded an unshared Nobel Prize in 1983.

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Barbara McClintock and Harriet Creighton at Cornell University 1929
Stone Hall, Cornell University
(Image used with permission of H. B. Creighton)

Perspectives On Nobel Laureate Barbara McClintock's Publications (1926-1984) A COMPANION VOLUME Volume III

Edited by

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Publisher's Note

With this Internet-First publication we formalize a new publishing genre, an '**Incremental Book**' that becomes feasible due to the Internet. Unlike paper-first book publishing – in which each contribution is typically held in abeyance until all parts are complete and only then presented to the reading public as part of a single entity – with Internet publishing, book segments (*Increment*s) can be released when finalized. Book parts that have not been completed at the time of the initial release may be published as they become available. We anticipate releasing "increments" from time-to-time, i.e., this volume, that incorporates these increments. These digital collections may be freely downloaded for personal use.

The initial release of this Incremental Book of 782 pages, spread over two volumes includes a complete list of the publications of Nobel Laureate Barbara McClintock. In addition, with publisher's permissions, we have reprinted many of McClintock's research articles, provided online links for free access to many, and cited traditional sources for the remaining articles. Only six (6) of the citations listed in the Contents are not presently accessible online. With this incremental release we add one perspective to the initial fourteen essays ("perspectives"), which are paired with the original scholarship being discussed. Nine more perspectives are forthcoming, and will be offered as additional 'increments' in Volume III when they become available.

As these and other new perspectives become available online, the eCommons@Cornell digital repository will automatically provide a listing of the most recent additions to this collection. Furthermore, we will provide a chronologically ordered PDF file named '**Increment**' for content added to this eCommons collection. These 'increments' will be integrated into a Volume 3 in chronological order.

Note: To preserve the page numbering in Volumes I & II — despite the insertion of additional content — the page numbering in the increments uses a prefix to identify the location of the related content in the previously-released volume. For example, the page number "1.47.1", contains a prefix "1.47", to refer to Part 1, page 47 and the number following the prefix indicates the page number within the increment, e.g., "1", in this case. Volume I contains Part 1; Volume II contains Parts 2-5.

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Front and Back Covers:
Left to Right, Back Row: Charles Russell Burnham, Marcus M. Rhoades, Rolland A. Emerson, Barbara McClintock;
Front Row: and George Beadle (kneeling with dog).
(Courtesy William B. Provine and Department of Plant Breeding & Genetics, Cornell University)

Plant Breeding Garden, Cornell University, outside the Department Field Laboratory, now fondly called "The McClintock Shed."

To the memory of Royse P. Murphy,
Mentor, collaborator, and friend
&
To
William B. Provine,
For inspiring this project
&
My husband, Robert E. Hunt
For his constant encouragement of my efforts

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Volume I

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Part I: GOLDEN AGE OF CORN GENETICS (1926-1936)

1926 **Perspective**: forthcoming

Randolph, L. F. & B. McClintock. 1926. Polyploidy in *Zea mays* L. *American Naturalist LX*(666): 99–102.

1928 **Perspective**: Genetic analysis of meiosis using the *asynaptic* 1 mutant: A perspective on George W. Beadle and Barbara McClintock's 1928 contribution. by **Wojciech P. Pawlowski**, Cornell University

Beadle, G. W. and B. McClintock. 1928. A genic disturbance of meiosis in *Zea mays*. *Science* 68(1766): 433.

1929 **Perspective**: Commentary on Barbara McClintock's 1929 cytogenetic analysis of triploid maize: A cytological and genetical study of triploid maize. by **Mark E. Sorrells**, Cornell University

McClintock, B. 1929a. A cytological and genetical study of triploid maize. *Genetics* 14(2): 180–222.

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Perspective: Identifying the individual chromosomes of maize. by **Randy Wayne**, Cornell University

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1930 **Perspective**: McClintock's presence of mind and forward vision as illustrated in the analysis of an interchange in maize. by **Ron Phillips**, University of Minnesota

McClintock, B. 1930a. A cytological demonstration of the location of an interchange between two non-homologous chromosomes of *Zea mays*. *Proceedings of the National Academy of Sciences* 16(12: 791–796.

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Perspective: Proof of physical exchange of genes on the chromosomes. by **Edward Coe**, University of Missouri & **Lee B. Kass**, Cornell University

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Perspective: Increment #01 [in Vol. III, page 1.47.1] by Lee B. Kass and James A. Birchler

McClintock, B. 1931b. Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. *Missouri Agricultural Experiment Station Research Bulletin* 163: 1–30.

1932 **Creighton, H. B. and B. McClintock**. 1932 [EXHIBIT/ABSTRACT]. Cytological evidence for 4-strand crossing over in *Zea mays*. *Proceedings of the International Congress of Genetics II*: 392.

Perspective: An attempt at identifying the position of genes on the chromosomes of maize using X-ray induced chromosome deficiencies. by **Randy Wayne**, Cornell University

McClintock, B. 1932b. A correlation of ring-shaped chromosomes with variegation in *Zea mays*. *Proceedings of the National Academy of Sciences* 18(12): 677–681.

1933 (**McClintock, Barbara**. 1932a [ABSTRACT]. Cytological observations in *Zea* on the intimate association of non-homologous parts of chromosomes in the mid-prophase of meiosis and its relation to diakinesis configurations. *Proceedings of the International Congress of Genetics II*: 126–128.)

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1934 **Perspective**: McClintock's pioneering studies of the nucleolar organizer region in maize: exact chromosomal localization and its function. by S**tephen E. Bloom**, Cornell University

> **McClintock, B.** 1934a. The relation of a particular chromosomal element to the development of nucleoli in *Zea mays* with 21 figures in the text and plates *VIII–XIV*. *Zeitschrift für Zellforschung und mikroskopische Anatomie* 21(2): 294–328.

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Creighton, H. B. and B. McClintock. 1935. The correlation of cytological and genetical crossing-over in *Zea mays*. A corroboration. *Proceedings of the National Academy of Sciences* 21(3): 148–150.

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McClintock, B. and H. Creighton. 1936. Cornell University, Ithaca, N.Y. — 9. Several inversions … chromosome 9 … and chromosome 4,… detected and isolated by Creighton and [McClintock]. *Maize Genetics Cooperation News Letter* 10: 6.

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Volume II [The front matter is listed above and is reproduced in this volume.]

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1937 See 1938

1938 **McClintock, B.** 1938a. [ABSTRACT] A method for detecting potential mutations of a specific chromosomal region. *Genetics* 23(1): 159. [2.3][1]

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1 Page numbers in brackets take readers to sections in these volumes where perspectives, with links and/or relevant reprinted publications originate.

2 References preceeded by an asterisk (*) are not reprinted herein, but were collected and published previously in McClintock, B. 1987a. In most instances, we've provided an Internet link to the original article.

1941 **Perspective**: forthcoming [2.73]

*__McClintock, B.__ 1941a. The stability of broken ends of chromosomes in *Zea mays*. *Genetics* 26(2): 234–282.

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McClintock, B. 1944b. The relation of homozygous deficiencies to mutations and allelic series in maize. *Genetics* 29(5): 478–502. [2.135]

*__McClintock, B.__ 1944c. Maize genetics: Completion of the study of the allelic relations of deficiency mutants. The chromosome-breakage mechanism as a means of producing directed mutations. Continuation of the chromatid type of breakage-fusion-bridge cycle in the sporophytic tissues. Homozygous deficiency as a cause of mutation in maize. *Carnegie Institution of Washington Year Book No. 43*, 1943-1944: 127–135. [2.157]

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Part III: MOBILE GENETIC ELEMENTS: Corn & the Nobel Prize (1946-1987) [3.1]

1946 *__McClintock, B.__ 1946. Maize genetics: Continuation of the study of the induction of new mutants in chromosome 9. Modification of mutant expression following chromosomal translocation. The unexpected appearance of a number of unstable mutants. *Carnegie Institution of Washington Year Book No. 45*, 1945-1946: 176–186. [3.3]

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1948 *__McClintock, B.__ 1948. Mutable loci in maize: Nature of the *Ac* action. The mutable *c* loci. The mutable *wx* loci. Conclusions. *Carnegie Institution of Washington Year Book No. 47*, 1947-1948: 155–169. [3.7]

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1950 **Perspective**: Transposable controlling elements step out onto the broader scientific stage. by **Clifford Weil**, Purdue University [see also Fedoroff *PNAS*, 2012, 109(50): 20200-20203] [3.11]

*__McClintock, B.__ 1950a. The origin and behavior of mutable loci in maize. *Proceedings of the National Academy of Sciences*. 36(6): 344–355.

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Perspective: forthcoming [3.27]

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Increment #02 [Reprint in Vol. III, page 3.27.1]

1952 *__McClintock, B__. 1952. Mutable loci in maize: Origins of instability at the *A1* and *A2* loci. Instability of *Sh1* action induced by *Ds*. Summary. *Carnegie Institution of Washington Year Book No. 51*, 1951-1952: 212–219. [3.29]

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Perspective: McClintock and epigeneitcs: changes in phase of transposition activity. by **Nina V. Fedoroff**, King Abdullah University of Science and Technology & Penn State University [3.33]

*__McClintock, B.__ 1953b. Mutations in maize: Origin of the mutants. Change in action of genes located to the right of *Ds*. Comparison between *Sh1* mutants. Change in action of genes located to the left of *Ds*. Meiotic segregation and mutation. *Carnegie Institution of Washington Year Book No. 52*, 1952-1953: 227–237.

1954 *__McClintock, B.__ 1954. Mutations in maize and chromosomal aberrations in *Neurospora*: Mutations in maize. *Carnegie Institution of Washington Year Book No. 53*, 1953-1954: 254-260.]3.41]

1955 **McClintock, B.** 1955a. Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, Long Island, N.Y. 1. Spread of mutational change along the chromosome. 2. A case of *Ac*-induced instability at the *bronze* locus in chromosome 9. 3. Transposition sequences of *Ac*. 4. A *suppressor-mutator* system of control of gene action and mutational change. 5. System responsible for mutations at *a1m-2*. *Maize Genetics Cooperation News Letter* 29: 9–13. [3.43]

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Perspective: forthcoming [3.67]

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Increment #03 [Reprint in Vol. III, page 3.67.1]

1957 **McClintock, B.** 1957a. Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, Long Island, N.Y. 1. Continued study of stability of location of *Spm*. 2. Continued study of a structurally modified chromosome 9. *Maize Genetics Cooperation News Letter* 31: 31–39. [3.69]

*__McClintock, B.__ 1957b. Genetic and cytological studies of maize: Types of *Spm* elements. A modifier element within the *Spm* system. The relation between *a1m-1* and *a1m-2*. Aberrant behavior of a fragment chromosome. *Carnegie Institution of Washington Year Book 56*, 1956-1957: 393–401. [3.81]

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1961 **Perspective**: Comparative studies relevant to transposon function in plant development. by **Allan M. Campbell**, Stanford University [3.87]

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Part IV: ORIGIN AND DIVERSITY OF MAIZE IN THE AMERICAS (1957-1981) [4.1]

Moreno, U., A. Grobman, and B. McClintock. 1959. Escuela Nacional de Agricultura, La Molina, Lima, Peru: 5. Study of chromosome morphology of races of maize in Peru. *Maize Genetics Cooperation News Letter 33*: 27–28. [4.3]

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McClintock, B., T. Angel Kato Y. and Almiro Blumenschein. 1981. Chromosome Constitution of Races of Maize. Its Significance in the Interpretation of Relationships Between Races and Varieties in the Americas. *Colegio de Postgraduados, Escuela Nacional de Agricultura, Chapingo, Edo*. Montecillo, Mexico. xxxi, 517 pp.

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Part V: CYTOGENETIC STUDIES OF *Neurospora crassa* (1945-1954) [5.1]

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__McClintock, B__. 1945b. *Neurospora.* I. Preliminary observations of the chromosomes of *Neurospora crassa*. *American Journal of Botany* 32(10): 671–678.

*__McClintock, B.__ 1947 [1 July 1946–30 June 1947]. Cytogenetic studies of maize and *Neurospora*: Continuation of studies of the chromosomes of *Neurospora crassa*. *Carnegie Institution of Washington Year Book No. 46*, 1946-1947: 146–152. [5.13]

*__McClintock, B.__ 1954. Mutations in maize and chromosomal aberrations in *Neurospora*: Chromosome aberrations in *Neurospora*. *Carnegie Institution of Washington Year Book No. 53*, 1953-1954: 254-260. [5.15]

*Reprinted in McClintock 1987a. The articles in the issues of the *Carnegie Institution of Washington Year Book may be viewed online or downloaded:*
http://carnegiescience.edu/carnegie_institution_year_books_numbers_1_through_99_years_1903_through_2001
Note: The webpage title (Carnegie Institution Year Books Numbers 1 through 99, years 1902 through 2000) differs slightly.

APPENDICES (Volumes I and II)

Increments

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by Lee B. Kass, Cornell University and James A. Birchler, University of Missouri

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Note: To preserve the page numbering in Volumes I & II — despite the insertion of additional content — the page numbering in the increments uses a prefix to identify the location of the related content in the previously-released volume. For example, the page number "1.47.1", contains a prefix "1.47", to refer to Part 1, page 47 and the number following the prefix indicates the page number within the increment, e.g., "1", in this case.

[See Volume I, Part I, page 1.1, for introductory material.]

Reprint: 19. McClintock, Barbara. 1931b. Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. *Missouri Agricultural Experiment Station Research Bulletin* 163: 1–30. ①

Setting up a prepared mind: Summary and analysis of "Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*"

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Introduction

In this relatively unknown (and often misrepresented) assembly of investigations by McClintock (1931b), she analyzed chromosomal changes in several plants from material subjected to irradiation and genetically characterized by L. J. Stadler. Irradiation of course scrambles the chromosomes and her challenge was to decipher the changes by examination of the pachytene chromosomes and other stages of meiosis. Stadler had described X-irradiation of plant material just a few years earlier (Stadler 1928a, b) and McClintock had developed cytological analysis of maize chromosomes (*e.g.* McClintock 1929a, b, c, d, 1930; McClintock & Hill, 1929, 1931). By 1929 she had morphologically identified each haploid mitotic chromosome by size, arm length, centromere position, satellite position, and knobs (McClintock 1929d, see Wayne perspective, this volume). Her goal in 1929 was to use trisomic (extra chromosome, 2n+1) plants to correlate linkage groups with each chromosome. This marriage of these two lines of investigation ignited many fruitful subsequent studies by McClintock. Her later publication with Hill (1931) was the *first report* linking chromosome 10 with a gene, *R,* and linkage group, *R-g*, and is cited in this investigation. This paper (McClintock 1931b) correlates the locus for gene *R* with the long arm of chromosome 10, in addition to placing the "regional locations" of three other genes in the chromosomes of maize.

The paper considered here contains studies of several different types of complex chromosome rearrangements. The investigation and its publication is one of record, describing the changes that X-irradiation produce on chromosomes. Yet, McClintock brings to this study her distinctive and perceptive analytical processes. It is interesting that many of the observations are reported nonchalantly but set the stage for her later extensive groundbreaking studies on ring chromosomes, uncovering deficiencies, and the breakage-fusion-bridge cycle from anaphase bridges, among others. This experience with a wide range of chromosomal changes likely prepared her mind to grasp the mechanics of chromosomal aberrations that figured into her important discoveries that followed.

To make a study of the eight sections (I-VII A, B) of McClintock's paper easier to follow, we have included a table (Table I) of chromosomal alterations described and illustrated by McClintock, a glossary of cytogenetic terminology used by McClintock, and a *corrigenda* for three typographical errors. Table I uses gene symbols used by McClintock in this paper, and the glossary provides currently applied gene symbols.

Maize has 10 pairs of chromosomes, which were known only by their linkage group names before they were accurately correlated with their chromosomes (Emerson et al. 1935). Four of the 10 maize chromosomes reported by McClintock in this paper, and identified by their linkage group names (Table I), are correlated with the loss (deficiency) of four dominant gene loci (*Lg, A, Pl, R*) in chromosomes 2, 3, 6, and 10**,** respectively. A deletion McClintock found in chromosome 7 was responsible for the loss of genes Gl_1, V_5, (reported by Burnham 1962, p. 25). Evidence for a hypothesized deletion in the chromosome 6 section of a T6-4 interchange chromosome (Fig. 12) provided a "more definite location" (p.13) for gene *Pl* in chromosome 6 (Fig. 10). A deficiency in the chromosome 3 section of the T6-3 interchange chromosome (Fig. 29, p. 22) was "almost identical in extent" to the chromosome 3 deficiency in the end of the long arm, and correlated with loss of gene *A*, shown in Fig. 7 (p. 9). The deletion in interchange chromosome T6-3 resulted in a ring chromosome having a centromere. [X-rays causing breaks in centromere regions would be observed in a later study of ring chromosomes (McClintock 1932b, chr. 5, see Wayne perspective, this volume)]. Two inversions, one in the centric section of chromosome 2, and one in the

chromosome 4 centric section of the T6-4 interchange chromosome were neither correlated with gene loss, nor associated with bridge formations at anaphase I (Table I).

The Original Objective

Lewis J. Stadler invited McClintock to the University of Missouri in the summer of 1931 to "determine the location of specific genes in their respective chromosomes." The question at the time was to learn if mutations caused by X-rays were due to "chromosomal irregularities" or caused by a change in the "individual gene" (Stadler 1931). The focus of this study was to analyze cytologically the nature of chromosomal alterations and their relation to phenotypic genetic changes induced by X-ray treatment. By the time of her study, eight of the ten linkage groups had been associated with their respective chromosomes using trisomic plants (chrs. 2, 3, 5, 6, 7, 10), and interchange chromosomes (chrs. 8, 9) (Emerson et al. 1935). It was therefore possible to "correlate a genic loss with an alteration in the chromosome known to carry this gene" (p. 3).

McClintock's study includes an analysis of a variety of chromosomal defects such as deficiencies, deletions, inversions, reciprocal translocations (interchanges), and small ring chromosomes. The behavior of these "alterations" (aberrations) during meiosis was documented, and crossing over (recombination) between chromatids from a normal chromosome and a translocation chromosome (T6-3), in which the centric part of 3 was deleted, resulted in the formation of anaphase I bridges. McClintock had already assigned some chromosomes to linkage groups at this writing (McClintock & Hill 1931) and had deciphered the nature of a reciprocal translocation together with Charles Burnham (Burnham 1930, McClintock 1930, Kass & Bonneuil 2004; Phillips perspective this volume). Interestingly, deficiencies, deletions, inversions, ring chromosomes and anaphase bridge breakage all figured into many of McClintock's later landmark investigations. McClintock (p. 5) was careful to define the difference between a deficiency, "any chromosomal loss," and a deletion, "deficiencies involving an internal region of the chromosome," – this distinction has not persisted in genetics literature.

Even though McClintock had clearly demonstrated genetically and cytologically the phenomenon of reciprocal translocation in 1930 (see Emerson et al. 1935, p. 6; Phillips perspective, this volume), her description of "reciprocal translocations" in this paper refers to more complicated and different arrangements of translocated chromosome segments. So as not to confuse the terminology, we use the term "interchange chromosomes" in place of McClintock's term "reciprocal translocation" (see Table I, Footnote 3 for references citing McClintock's older usage of this term).

Innovative Methods for Locating Genes on Chromosomes

McClintock studied adult maize plants that exhibited recessive phenotypes. These plants were the result of crosses made by Stadler using 1) irradiated pollen or 2) irradiated early embryos (developing kernels). In the first method, Stadler irradiated pollen grains (male parent) carrying dominant genes and applied these grains to the silks of female parent plants carrying recessive genes. The corn kernels (progeny, F_1) produced from this cross were germinated and grown to maturity. Most adult progeny showed the dominant phenotype. Occasionally, individual offspring appeared that expressed recessive phenotypes. When such plants were approaching reproductive maturity, McClintock examined the chromosomes cytologically (in anthers at microsporogenesis) at the mid-prophase (pachytene stage) of meiosis. In this study, McClintock exclusively applied the aceto-carmine smear technique, which she had modified and perfected years before to examine developing pollen grains (microsporocytes) in maize anthers, beginning in late prophase (Randolph & McClintock 1926, McClintock 1929b), and later at pachytene stage (McClintock 1930, and this study). Plants examined are identified by Stadler's culture numbers.

The second method employed adult plants grown from irradiated embryos in developing corn kernels**,** whose parents were heterozygous for known genes. The adult plants showing recessive traits were then examined at meiosis, using the same anther squash technique mentioned above.

In plants expressing the recessive phenotypes, McClintock observed, photographed, diagrammed and sketched, most of the chromosomes at meiotic pachytene stage (mid-prophase of meiosis). In one plant, she reported and photographed "a retarded synaptic complex" (a chromatid bridge) at anaphase I (Figs. 36, 37, 39) and late anaphase I/early telophase I (Fig. 38). The chromosome sketches were made with a *camera lucida* (see fig. 1, Sorrells' perspective, this volume).

In the irradiation experiments, pollen or early embryos had been irradiated such that the examined adult plants were heterozygous for dominant genetic markers. A missing dominant phenotype was used to select plants for analysis thus exposing the recessive/mutant character. These materials, examined cytologically, usually showed deficiencies or deletions for the chromosome carrying the marker gene in question and allowed a rough estimate of its cytological position in the chromosome and in one case provided confirmation of the genetic linkage group with chromosome 7 (*ra-gl*$_1$*-v*$_5$ chr., Table I; Rhoades & McClintock 1935, p. 296; Burnham 1962, p. 25). By 1933, McClintock used a similar method to establish the *j-ms*$_8$ linkage group with chromosome 8 (McClintock 1933, Rhoades & McClintock 1935, p. 296). Most of this publication

involves such correlations between a loss of genetic markers and chromosomal deficiencies or deletions recognized at the pachytene stage of meiosis.

Ground Breaking Results

Part I. The Regional Location of the *liguless* [*sic*] Gene (*lg*) in the *B-lg* Chromosome

Deficiency in chromosome 2

In Part I of this paper, McClintock demonstrated that a deficiency in the extreme short end of the short arm of the *B-lg* chromosome (chr. 2) was associated with the loss of the dominant *Lg* (*non-liguleless*) gene. She notes (p. 6) that genetic studies had placed the *lg* (*liguleless*) locus at one end of the genetic linkage map. She examined maize plants at meiosis that had exhibited the recessive phenotype *liguleless* leaf (*lg*). The leaves on these plants lack a ligule and auricles. The plants had been grown from irradiated embryos, whose pollen parent (male) was homozygous recessive (*lg, lg*) and had been crossed with a female parent that was homozygous dominant (*Lg, Lg*). All offspring (kernels, progeny) from this cross were expected to be heterozygous (*Lg, lg*) and to have leaves with ligules and auricles, the dominant phenotype. After irradiation, however, two plants grown from young embryos had instead expressed the recessive *liguleless* phenotype, and half of their pollen was sterile (p. 8). When these plants were examined cytologically at the pachytene stage of meiosis, McClintock found that one of the *B-lg* chromosomes (chr. 2) lacked a long portion of its shorter arm (Figs. 1, 2) and another plant lacked a shorter, four chromomere section, of its short arm (Figs. 3 to 5). She correlated the deficiencies with loss of gene *Lg* (p. 8). She compared these figures with synapsed chromosomes 2, observed at the pachytene stage from a plant expressing the normal dominant phenotype (*non-liguleless*), which showed no deficiency (Fig. 6).

Part II. The Regional Location of the Gene *A* in the *A-d_1-cr* Chromosome

Deficiency in Chromosome 3

In Part II, McClintock demonstrated that a deficiency at the end of the long arm of the *A-d_1-cr* chromosome (chr. 3) was associated with the loss of gene *A*. She knew from genetic studies that gene *A* was at one end of the genetic linkage map (p. 9).

She examined maize plants that showed the recessive green plant phenotype (*a,a*, loss of anthocyanin). The plants had been grown from kernels whose parent (male) pollen had been irradiated, carried dominant gene (*A*), and had been crossed with a female parent that was heterozygous dominant (*a,A*) (p. 9). Offspring (germinated kernels, F_1 progeny) from this cross were expected to be heterozygous (*A,a*) or homozygous (*A,A*) dominant and to exhibit anthocyanin color in the plant.

One adult plant, grown from the kernels derived from the cross resulting from irradiated pollen parents, had instead expressed the recessive green plant phenotype, and half of its pollen was sterile (p. 9). McClintock had sampled the plant cytologically at the pachytene stage of meiosis. She found that one of the *A-d_1-cr* chromosomes (chr. 3) lacked a portion of its longer arm (Fig. 7), and she correlated the deficiency with loss of gene *A* (p. 9). A similar deficiency involving a loss of the *A* gene from a section of chromosome 3 was seen in another plant (p. 9) and described in Part VII B for an alteration involving a T6-3 interchange chromosome (pp. 28-29).

Part III. The Regional Location of the Gene *Pl* in the Satellite Chromosome

Deficiency in Y-Pl Chromosome 6

In part III, McClintock's intention was to test the idea that a deficiency in the long arm of chromosome 6 was associated with the loss of the dominant *Pl* gene, which is expressed as a dominant *purple* color in the plant, in association with other non-allelic color genes (see Glossary for non-allelic color gene interactions). Previous trisomic inheritance studies had shown that gene *Pl* lies in the satellite chromosome. She examined maize plants that had exhibited the recessive phenotype *non-purple* (*pl*). The plants had been grown from irradiated embryos, whose pollen parent (male) was homozygous recessive (*pl, pl*) and had been crossed with a female parent that was homozygous dominant (*Pl, Pl*). All offspring from this cross were expected to be heterozygous (*Pl, pl*) and exhibit the dominant *purple* phenotype. After irradiation, however, some of the young embryos had instead developed into *non-purple* plants. When these plants were examined cytologically, McClintock found that one of the satellite chromosomes lacked a portion of its longer arm (Figs. 10, 11) and she correlated the deficiency with loss of gene *Pl* (p. 10), thus providing an explanation for the plant's recessive phenotype. Genetic crossover data also indicated that *Y* and *al* lie on the same side of chromosome 6 (p. 12) from the point of the deficiency toward the satellite. However, *al* (*albescent plant*) was later shown not to be linked with *Y* (Emerson et al. 1935, p. 53), and is now known to be on the short arm of chromosome 2 (Neuffer et al. 1997, p. 216).

McClintock explained that the satellite chromosome was readily distinguishable by its relation to the nucleolus and by a "deep staining knob," one third of the distance in from the end of the chromosome (p. 10). The beautiful photomicrograph of two synapsed satellite chromosomes (chromosome 6, Fig. 8) in mid-prophase (pachytene stage) of meiosis showing normal paired chromosomes, would be reprinted many times by McClintock (1933 photo 61; 1934, fig. 1, see Bloom perspective, this volume), and by others (Sharp 1934, p. 119; Swanson 1957, p. 68[1]; Phillips & Burnham 1977, p. 291). This photograph was taken from preparations which were only one to two weeks old, and did not show signs of deterioration (p. 5). In the later reports, the focus was on the locus and origin of the nucleolar organizer region (NOR) in the short arm of chromosome 6, described here as "a deep staining mass" (p. 10).

Interchange between non-homologous chromosomes 6 & 4 with a deletion

Another plant resulting from irradiated embryos of the same parents had a recessive green phenotype and was reported to have 98% pollen abortion. In this plant she sought evidence suggesting a more definite location of the gene *Pl*. She obtained confirmation from an interchange between chromosome 6 (satellite chromosome) and chromosome 4 (*su-Tu* chr.), a T6-4 interchange chromosome (pp. 13-15). One result of this exchange of non-homologous chromosomes was loss of an internal part of the satellite chromosome (Fig. 12a, b). At pachytene stage the interchange chromosomes paired with the normal chromosomes and formed a cross (Fig. 12c). The hypothesized deleted section of the interchange chromosome is diagrammed as a loop at the intersection of the cross (Fig. 12c). McClintock compared the position of the deletion in the interchange chromosome with the loss of the *Pl* locus in the long arm of the satellite chromosome (Fig. 10, arrow); the deletion and deficiency might provide additional evidence for the regional location of *Pl* in the satellite chromosome, she believed. She noted, also, that "No obvious evidence of deficiency was obtained from this plant … possibly because no figures showing close synapsis at the center of the cross were found" (Fig. 13). She could not exclude, therefore, the possibility that the loss of *Pl* was due to "recessive mutation" (p. 15).

Interchange T6-4 with a deletion & a pericentric inversion not correlated with gene loss

This interchange chromosome (T6-4) also included a pericentric inversion that was *not* shown in the diagram (Fig. 12c) and was not correlated with gene loss. The inversion of the centric portion of chromosome 4 could be observed, however, in the sketch of the normal chromosome 4 synapsed with the interchange chromosome T6-4 (Fig. 13); note the non-homologous pairing of the interchange 4 centromere represented by dotted lines. McClintock reprinted a modification of this sketch in a later report (1933, p. 220), emphasizing association of non-homologous parts of chromosomes; a study she first reported at the 6th International Congress of Genetics (McClintock 1932a).

Part IV. The regional location of the *R* gene in the *R-G* chromosome

Deficiency in chromosome 10

In Part IV, McClintock demonstrated that a deficiency in the entire long arm of the *R-g* chromosome (chr. 10) was associated with the loss of the r^r locus. McClintock examined the chromosomes in maize plants that had exhibited a recessive, totally green plant color. The plants had been grown from developing embryos (kernels) that had been irradiated, and whose pollen parent (male) also had been irradiated, carried color genes *A, b,* r^r, and which had been crossed with a female parent that carried color genes *a, b,* R^g (p. 15). Offspring (F_1 progeny, germinated kernels) from this cross were expected to be *dilute sun red* (*Aa, bb,* R^g, r^r; see Emerson et al. 1935, Table p. 27). Loss of genes r^r or *A* would result in a green plant.

She knew from her previous study of trisomic (2n+1) plants (McClintock & Hill 1931) that the *R-g* linkage group was associated with the smallest chromosome (chr. 10). From her current study (see Part II), she had shown that plant color gene *A* was on chromosome 3**;** therefore the loss of plant color must be associated with the chromosome carrying a deficiency—the *R-g* chromosome, 10. Note that McClintock's phenotypes fit with the male genotype carrying *b* instead of the stated *B* gene in her description, and we apply the recessive color gene *b* to our interpretation here**.**

One adult plant, grown from this cross, expressed the recessive green plant phenotype (p. 15). McClintock examined the plant cytologically at the pachytene stage of meiosis. She found that for one of the *R-g* chromosomes (chr. 10), the entire portion of its long arm (Fig. 14) was missing and had been broken at the centromere. She correlated the deficiency with loss of gene r^r (p. 15).

Part V. A Deficiency Involving the Short Arm of the Satellite Chromosome

A second chromosome 6 deficiency not correlated with gene loss

1 The reference for the photo in Swanson 1957, fig. 3-14 is misdated; correct references are McClintock 1931b, or 1933a (see reference 1933 in this perspective), photo 61, or 1934, fig. 1.

In another plant, McClintock found a second observation of a deficiency in chromosome 6. This plant resulted from a cross with irradiated pollen. McClintock does not provide the phenotype of this plant nor does she correlate a deficiency in the short arm of chromosome 6 with gene loss. McClintock provided a sketch (Fig. 15) and a photograph (Fig. 16) of the chromosome 6 deficiency, at the pachytene stage of meiosis. Its synapsed normal chromosome was also observed closely attached to the nucleolus (Fig. 16; see also Figs. 8, 9 for normal synapsed satellite chromosomes). In continued studies of satellite chromosomes, McClintock (1934) would show that this region of the short arm of chromosome 6 included the Nucleolar Organizer Region (see Bloom perspective, this volume).

Part VI. A Deletion within the Long Arm of Chromosome 7

A deletion within the ra-gl1-v5 chromosome

McClintock begins Part VI by explaining that the 4th smallest chromosome, chromosome 7, can be easily recognized by the relative size and relative length of its two arms, and by a short deep staining portion, immediately below the centromere ("clear insertion region;" p. 16). She observed pachytene chromosomes in an adult plant with a recessive phenotype. This plant had been derived from irradiated pollen placed onto the silks of an untreated plant. She described, and illustrated an internal deletion in chromosome 7 (Figs. 17 to 19), but she did not correlate gene loss with this deletion, or mention the phenotype of the recessive character. From her study of trisomic (2n+1) plants, McClintock had associated chromosome 7 with the *ra-gl$_1$-v5* linkage group (Emerson et al. 1935, "McClintock unpublished," p. 59). Burnham (1962, p. 25, fig. 5) reported that the chromosomal deletion described here was correlated with a loss of genes in chromosome 7 "deficient for an internal segment including the locus of *gl$_1$ v$_5$* in the long arm." Burnham had learned from McClintock ("personal comm.") that the seedling was *glossy* and *virescent*. Dominant genes *Gl$_1$* and *V$_5$*, therefore, were located in an internal section of the long arm of chromosome 7, and also provided confirmation of the linkage group.

Part VII. An Analysis of Several Chromosomal Alterations within an Individual plant

A. Cytological Evidence for Inversion of a Long Section of a Chromosome

Introduction to VII Parts A and B

McClintock begins this last section of her paper by introducing observations of alterations between two chromosomes (6 and 3), and a third alteration, an inversion, in chromosome 2 (second largest), for plant 397.1-5. This plant was the progeny (F_1) of a cross using irradiated pollen from a male parent and an untreated female parent (p.18). The male parent carried the dominant gene *A* and the female parent possessed the recessive allelemorph, *a* (p. 28). In Part VII A, she described the chromosome 2 alteration, "an inversion of a large section." She also mentioned that in the next section (VII B, pp. 21-29; see below), she would describe the alteration in which two chromosomes were involved in "a translocation … accompanied by deficiencies" and "in one of these chromosomes a deletion produced a functional fragment."

Pericentric Inversion in Chromosome 2 not correlated with gene loss

It is important to note that the pericentric inversion described here (Part VII A) for chromosome 2 (the *B-lg* chromosome and linkage group), and diagrammed and photographed in Figs. 20 to 28, is not involved with the "case of a reciprocal translocation (interchange), accompanied by deficiencies, and a deletion" described in section B. Additionally, crossovers within such pericentric inversions, as described by McClintock in section A, do *not* form bridges at anaphase I. We emphasize this point because in the literature[2] these images of pericentric inversion figures have been described and misrepresented as examples of heterozygous inversions producing bridges at anaphase I.

McClintock did not correlate this chromosome 2 pericentric inversion with gene loss. Instead, her emphasis was on the homologous association of the synapsed chromosomes in the inversion loop ("ring," Figs. 20c to 27), and the demonstration of a lack of pairing when close synapsis is lost between the inverted section and non-inverted chromosomes (Fig. 28). Also, Fig. 28 is missing any designation of the centromere; only the knobs are shown. Readers must be careful not to confuse the inversion loop, which she referred to as a "ring," with the "ring fragment" or "ring chromosome" described in Part VII B.

2 McClintock's (1931b) pericentric inversion (inversion loop includes centromere; Fig. 23, p. 19) was reprinted in Sturtevant & Beadle (1939, Plate III, B, flipped 90°) to show a paracentric inversion (centromere outside inversion loop), which they describe on p. 130. This misrepresentation was also published by Swanson et al. (1967, fig. 4.10, p. 107; 1981, fig. 613, p. 376); a preferable photo of a paracentric inversion can be found in McClintock, 1933 (Plate IX, photos 21-23). Comfort 2001, p. 57, inappropriately claimed that McClintock "predicted that a translocation *within the inversion* could produce one chromosome with no centromere and another with two centromeres." As explained here, Part VII B shows clearly that the translocation occurred between non-homologous chromosome 6 and 3 (at the arrows, Fig. 22) and *not* within the chromosome 2 pericentric inversion, described in Part VII A).

This was the *first cytological* account that clearly demonstrated an inversion loop at synaptic pairing of homologous chromosomes, when one member of the chromosome pair included an inversion [pachytene configuration in a maize plant heterozygous for an inversion in chromosome 2 (e.g. Fig. 23; Burnham 1962, p. 35)]. In this report, McClintock did *not* predict the fate of the chromosome 2 heterozygous inversion following synapsis, and made no mention of a cross-over occurring in the inversion loop – that would remain for later investigations involving paracentric inversions in chromosomes 8 and 4. The following summer, in studies not associated with ring chromosomes, McClintock and her student Harriet Creighton (1932) would describe a crossover in a paracentric inversion in chromosome 8 that produced bridges at anaphase I. Two years later, McClintock (1933, pp. 202-204) demonstrated how crossing over in inversion chromosome 8 (1933, photos 21-23) produced dicentric chromosomes and anaphase bridges (1933, photos 28-29) during meiosis, and in 1938, she followed the fate of a paracentric inversion in chromosome 4 that produced bridges at anaphase I and II (McClintock 1938c, see Burnham 1962, pp. 40, 51).

B. A Case of a Reciprocal Translocation [Interchange Chromosome] **Accompanied by Deficiencies, and a Deletion Producing a Functional Ring Fragment, Involving Two Chromosomes of the Haploid Complement.** [same plant as VII A]

Interchange between chromosomes 6 & 3, & a deletion producing a centric ring chromosome.

In one of the more interesting rearrangements examined, an interchange had been produced that would have joined together in one new chromosome two centromeres from nonhomologous chromosomes except that one centromere (centric fragment) was deleted, and formed an accompanying small ring chromosome.

Besides the inversion in chromosome 2 (mentioned in VII A) a translocation (interchange) occurred between the satellite (*Y-Pl*) chromosome (chr. 6) and the long chromosome associated with the *A-d_1-cr* linkage group (chr. 3; see Part II) in an order that ordinarily would contain two insertion regions. In this case, however, a deletion also took place in the translocation chromosome that removed the chromosome 3 centromere and flanking chromatin (internal centric region) to produce a separate ring chromosome. This resulted in the translocation chromosome having only one centromere (6) and the deleted fragment carrying a centromere (3) (Figs. 29a-c). The reciprocal pieces of the translocation not containing a centromere (acentric chromosome fragment) would be lost during cell division (p. 23). At synapsis, the centric interchange chromosome (T6-3) paired with normal homologous chromosomes 6 and 3, forming a cross configuration (Figs. 29c, 30 to 32). In the normal chromosome 3, at the position of the centromere, a bulge appeared because the homologous region in the translocation chromosome had been deleted (Figs. 29c, 30 to 32). Additionally, the centric fragment piece, which had been deleted from the translocated part of chromosome 3, carried the centromere and appeared as a ring chromosome. Figures 33 and 34 show synapsis of the deleted section with the normal chromosome; the bulge clearly shows the centromere and the portions of the chromosome on either side. The ring chromosome fragment was *not* synapsed with its homologous region (on chromosome 3). This was unexpected. Occasionally, the fragment was found "lying beside the bulge produced in the normal chromosome at the region of the deletion" (p. 23); this association is not illustrated.

Centric ring chromosome

The ring chromosome, which she observed at the pachytene stage (Figs. 29c, 30 to 32) and anaphase I (Fig. 36b), was found in a maize plant, whose parent pollen had been irradiated (p. 18). The ring chromosome was derived from a centric deletion of chromosome 3. The deleted and broken chromosome 3 joined with a broken chromosome 6 (at the arrows in Fig. 29a) to become the deleted T6-3 interchange chromosome (Fig. 29b; Table I, T6-3). The ring chromosome did not pair with its homologue (Figs. 29c, 30 to 32), and McClintock pointed this out without surprise. Navashin (1930) had first described a ring chromosome in *Crepis*, the year before, and Beadle had observed a ring chromosome in *Zea* (McClintock 1931b, footnote 1, p. 29). How the deletion that produced a ring chromosome might arise is hypothesized in her conclusions [section (3), p. 29].

Single crossover in interchange chromosome T6-3 producing a post synaptic bridge at anaphase I

Furthermore, based on genetic and cytological evidence (found by Creighton and by Rhoades, p. 24) that crossing over occurs "between two of the four chromatids of the two synapsed chromosomes in *Zea mays*," McClintock explained that a crossover between one translocation chromatid and one normal chromatid 3 would produce "one chromatid possessing two insertion regions (dicentric chromatid) and one lacking an insertion region (acentric chromatid) (p. 23; Fig. 35, crossover at a-a). At anaphase I, the two centromeres in the dicentric chromatid might move to opposite poles of the cell. This would retard the movement of the dicentric chromosome and leave the "synaptic complex (dicentric chromosome and synapsed homologues form a tie or bridge) … at the center of the spindle figure (Figs. 36a, b; 37)." The "split" (replicated) ring fragment was also delayed in its movement (Fig. 36b). McClintock describes without surprise that the ring sister chromatids actually separated at anaphase I, rather than proceeding to one pole or the other as the normal chromosomes typically do. In a later paper, McClintock (1938b), described this again without surprise. This of course is a failure of cohesion of sisters at meiosis I, suggesting that there is a threshold of chromosome length that is necessary for cohesion to be maintained. Such separation

does not happen for normal chromosomes, but has now been found repeatedly for small chromosomes in maize (Rhoades 1940, Maguire 1987, Brock & Prior 1996, Han et al. 2007, Birchler & Han 2013). The basis of this lack of cohesion of sister chromatids at meiosis I remains unknown even today. The chromatid that had no centromere was left at the equatorial plate region at anaphase I/early telophase I (Fig. 38). She observed, photographed (Figures 36-38), and interpreted this process (pp. 24-26).

These figures depict the *first* photographs of an anaphase bridge [in a dicentric chromosome formed by recombination in a heterozygote between the normal chromosome present and a translocation between non-homologous chromosomes 6 & 3, in which the centromere of 3 was deleted] in maize, or in any organism. Such bridge formations would subsequently play a prominent role in her studies of the bridge-breakage-fusion cycle (later called the breakage-fusion-bridge cycle); investigations of ring chromosomes at mitosis (McClintock 1937a, 1938a, 1938b), and of inversion heterozygotes at meiosis (McClintock 1938c, 1939) were continued at the University of Missouri and at Cornell (Kass 2005).

Double crossover in interchange chromosome T6-3 producing a post synaptic double bridge at anaphase I

McClintock also provided evidence that *two crossovers* probably occurred between one translocation chromatid and one normal chromatid 3 (Fig. 35, crossover at b-b). In this case two chromatids would be formed with no centromeres (acentric fragments) and two chromatids would be formed with two centromeres. Crossovers involving the four chromatids in this manner would result in exclusion (loss) of two chromatids from the anaphase group at the first meiotic division (meiosis I). This was visible in the anaphase I Figure (39) illustrated for the *first time* in maize sporocytes, or in any organism. Rhoades (1932) considered McClintock's report of a double dicentric bridge at anaphase I as "indirect cytological evidence," along with his genetic evidence (Rhoades 1932, trisomic 5), to support *Zea* as the *first* organism in which double strand crossing-over had been demonstrated. Direct cytological evidence was later presented by Creighton and McClintock (1932), Creighton (1933, pp. 43-46), and McClintock (1933, 203-204) using 1) heteromorphic synapsed chromosomes, 2) translocations, and 3) paracentric inversions showing bridges at anaphase I.

The experience of studying these materials no doubt prepared McClintock for subsequent studies of ring chromosomes (McClintock 1932b), especially the analysis of homozygous deficiencies using ring chromosomes (McClintock 1938b, chr. 5), and with studies of paracentric inversions, a type not reported in this paper nor associated with ring chromosomes (see above). All of these classic contributions led to her discovery of the breakage-fusion-bridge cycle initiated by anaphase bridge formation (McClintock 1939, chr. 9; see below).

In the following year, McClintock (1932b) published her classic on the correlation of ring chromosomes with variegation of plants (see Wayne, this volume) in which she recognized that ring chromosomes changed size and that dominant markers were lost from the chromosomes (chrs. 2, 5, 6) similarly to the findings in deficient chromosomes studied in much of this paper. The materials in which she found ring chromosomes in 1932(b) were also supplied by Stadler and were the result of both spontaneous changes and irradiation experiments.

Some years later, McClintock published an analysis of homozygous deficient tissues in plants that carried different sizes of small ring chromosomes (McClintock 1938b, chr. 5). The ring chromosomes carried the normal copy of genes together with chromosomes with deficiencies. When the ring chromosomes were lost during development, then there was no normal copy of these genes in plant sectors. Depending on the size of the ring chromosome involved, different types of phenotypes appeared in the mosaic plants. One can thus see the progression of thought from loss of dominant markers when a deficiency is formed and the behavior of ring chromosomes that were developed in her earlier work.

In McClintock's description of the breakage-fusion-bridge cycle (McClintock 1939, chr. 9), the first example noted in her paper involved an inversion chromosome that when recombination occurred with a normal homologue would produce an anaphase bridge that would break and then subsequent fusions would occur to form another dicentric that would again break in the following anaphase. In the paper considered here, McClintock had recognized chromosomal inversions (which were pericentric and not associated with bridge formation), and also that recombination between rearranged chromosomes (crossovers in an interchange chromosome, Part VII B), formed dicentric chromosomes that could produce anaphase I bridges. As mentioned above, she would also observe anaphase bridges due to crossovers in paracentric inversions in chromosomes 8 and 4 (Creighton & McClintock 1932, McClintock 1933, 1938c). Thus, the stage was set for McClintock to synthesize and extend these observations in the subsequent 1939 study in chromosome 9.

Science proceeds by building on the findings and concepts that precede each new advance. In this paper, one can recognize foundational findings that would have prepared McClintock's mind for the seminal discoveries that followed.

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Table I. Chromosomal alterations/derangements/structural aberrations (deficiencies, deletions, inversions, rings, translocations) described and illustrated by McClintock (1931b); applying the squash technique in maize anthers. Such alterations can be correlated with the location of genes (see text). McClintock examined the chromosomes of plants exhibiting a recessive (mutant) phenotype. Recessive plants that had apparently lost dominant genes were correlated with chromosomal alterations at the pachytene stage of meiosis. Alterations involved chromosomes 2, 3, 6, 7, 10, and chromosomal interchanges (T) between non-homologous chromosomes 6 & 3 (T6-3) and 6 & 4 (T6-4). One plant had crossovers during synapsis between chromosomes 3 & 6^3, and showed unusual anaphase I configurations. Chromosomes were known then by their linkage group names and are listed here with their **chromosome numbers** (column 1); **chromosome name** (column 2); type of **alteration/gene location** in the chromosome, and text Figures (column 3); and **recessive** (mutant) **phenotype** expression**/dominant gene**(s) **lost** (column 4). [Column 2 Roman numbers are sections in McClintock's text where descriptions/Figures appear; chr. = chromosome]

Chromosome(s)[1]	**Chromosome name**	**Alteration/ gene location**	**Recessive phenotype/ Dominant gene lost**
2	*B-lg* chr. [I]	**Deficiency**/short arm, extreme end, four chromomeres (I. Figs.1 to 5; compare with non-deficient Fig. 6)	*liguleless*/*Lg*
3	*A-d_1-cr* chr. [II]	**Deficiency**/long arm, end (II. Fig. 7, compare with T6-3, VII. B)	*green* plant (loss of *anthocyanin*)/*A*
6	Satellite (*Y-Pl*) chr. [III]	**Deficiency**/long arm, lower part [end] (III. Figs. 10, 11; compare with not-deficient Figs. 8, 9)	*non-purple* plant (54% pollen abortion)/*Pl*
T6-4, 6^4 + [**T4-6**, 4^6, acentric]	Satellite (*Y-Pl*) - *su-Tu* chr. [III; "interchange occurred," p. 13]	[*mechanism assumed*] **Interchange Chr.**[3] **Deletion**/chr. 6 of 6^4; (III. Fig. 12a-c, diagram; Fig. 13, no *obvious* deletion; probable short **deletion** at point of interchange; compare Fig. 12c & Fig. 10, arrow)	*non-purple* plant (98% pollen abortion)/*Pl* [plant with T6-4 *loss* provides "evidence" for "more definite location" of *Pl* in chr. 6 (see above)]
	"second alteration," [III. p. 14; Fig. 13]	**Inversion** [pericentric]/ $T6^4$, centric chr. 4 section; inversion loop/or rod at synapsis; centromeres "did not correspond in relative position" in rod chromosomes (Fig. 13). (III. p. 14:"inverted section not included in diagrams" [Fig. 12], but see Fig. 13. synaptic figure.)	Inversion is part of T6-4; Fig. 13 shows non-homologous synapsis of second insertion region (centromere), indicated by dotted lines [pericentric inversion; no bridge would form at anaphase I^2]; no correlated gene loss for inversion
10	*R-g* chr. [IV]	**Deficiency**/entire long arm (IV. Fig 14)	totally *green* plant (*not dilute sun red)*/r^{-r} (cannot be gene *A*)[*A* is on chr. 3, see above]

6	Satellite (*Y-Pl*) chr. [another observation, another plant] [V]	**Deficiency**/short arm (V. Figs. 15, 16)	phenotype not given/ no correlated gene loss
7	*ra-gl*$_1$*-v*$_5$ chr. [VI]	**Deletion**/long arm, 1/5th (VI. Figs. 17a, b, c; 18, 19) synapsis of deleted (missing internal) acentric chr. section with normal chr. shows looping-out of normal chr. (Fig. 17c, diagram)	phenotype not given/no correlated gene loss [but see Burnham (1962, p. 25), phenotype *glossy, virescent/* loss of *Gl*$_1$*,V*$_5$]
		Several within <u>one plant</u>:	
2	*B-lg* chr. [VII. A.]	**Inversion** [pericentric]/ centric chr. section (VII. A. Figs. 20a, b, c; 21; 22, 23; 24, 25; 26, 27; 28)	no correlated gene loss [pericentric inversion *not part* of alteration-T6-3; see below]
&			
T6-3, 6^3 + [**T3-6**, 3^6, acentric]	Satellite (*Y-Pl*) - *A-d*$_1$*-cr* chr. [VII. B.]	**<u>Interchange Chr.</u>**[3] **deficiencies** (acentric-fragment(s), chrs.3 & 6). **deletion** (chr. 6^3, centric-fragment of chr. 3) = **ring fragment** [ring chr.] (VII. B. Figs. 29a, b, c; 30, 31, 32; 33, 34)	*green* plant, sterile/*A* [compare II. chr. 3, Fig. 7][3]
		Post synaptic **<u>Interchange Chr.</u>** *<u>assumed</u> single crossover at synapsis*; anaphase I **bridge**; **dicentric chr.** (6^3 + 3), **acentric fragment** (3^6); **deletion** (chr. 6^3, centric-fragment of chr. 3) = **ring fragment** [ring chr.] (VII. B. Figs. 35 [crossover at a-a]; 36a, b; 37; 38 [late anaphase])	same as above [anaphase I, bridge]
		Post synaptic **<u>Interchange Chr.</u>** *<u>assumed</u> double crossover at synapsis*; anaphase I **bridges; two-dicentric chrs.** (6^3 + 3), **two-acentric fragments**(3^6); **two-deletions** (chr. 6^3, centric fragments of chr. 3) = **two-centric fragments** [ring chrs.] (VII. B. Figs. 35 [crossing over at a-a & b-b]; 39)	same as above [anaphase I, double bridge]

[1] After McClintock & Hill 1931; Emerson et al. 1935; Anderson 1935, p.73; chr. 7: Rhoades & McClintock 1935, p. 269 & Burnham 1962, p. 25.

[2] See McClintock 1933, photo. 48, chr. 4, a pericentric inversion not associated with a translocation.

[3] "reciprocal translocation with loss of one acentric fragment," citations to work of McClintock 1931b (Anderson 1935, p.77; Anderson 1936, p. 1304; Jones 1941, p. 298).

Corrigenda (for McClintock 1931b)
p. 29, line 12 (3), Change "Section XII, B" to "Section VII, B."
p. 13, line three, Change "50 hours after pollination" to "20 hours after pollination."
p. 15, 9 lines from bottom "genes ***A, B, r^{-r}***, …" change ***B*** to ***b***

GLOSSARY (for McClintock 1931b)
[Note: Current nomenclatural usage for gene symbols replaces subscript or superscript numerals to a single line format – examples: *A1*, *r-r* or *r1-r*.]

***A*:** A_1 (phenotype-anthocyanin color expression in plant, aleurone and pericarp)

acromatic spindle fiber insertion region; insertion region; **primary constriction:** centromere

chromosomal alterations, derangements: structural aberrations

allelomorphic: allelic

allelemorph: allele

maize chromosome names: maize chromosomes are currently named by their numbers and not by their linkage group names. There are 10 maize chromosomes, numbered from 1 (the largest) to 10 (the smallest).

mid-prophase of meiosis: pachytene stage

reciprocal translocation: chromosomal interchange; interchange; segmental interchange; forms a cross shaped complex when chromosomes synapse at pachytene stage of meiosis (Figs. 12, 29 (diagrams); 31 (photo); 13, 30, 32 (sketches). McClintock 1931b found two instances of chromosomal interchanges between chromosomes six & four [T6-4, T4-6 (acentric fragment)], and chromosomes six & three [T6-3, T3-6 (acentric fragment)]. A crossover in interchange chromosomes T6-3 produced a "retarded synaptic complex" (p. 26; a chromatid bridge) at anaphase I of meiosis (see text).

alteration about the insertion region: break on either side of the centromere; centric chromosome section

derangement of the chromatin: rearrangement or alteration of chromosome material

deficiency: any chromosomal loss (p. 5)

deletion: deficiency involving an internal region of the chromosome (p. 5); currently also applied to any chromosomal loss

inversion: An inversion in a chromosome occurs when a segment of the chromosome breaks off and becomes reinserted in the same place, but in the reverse direction relative to the rest of the chromosome. A loop arrangement occurs, when homologous chromosomes pair, due to the inversion in one of the homologues. Inversion loops are of two types, named with respect to the position of the centromere about the inversion. A **pericentric inversion** includes the centromere in the inversion loop. A **paracentric inversion** has the centromere outside of the inversion loop. The inversion reported in McClintock's paper (1931b, p. 19, Figs. 21 to 26) is now known as a **pericentric inversion,** which does not produce bridges at anaphase I of meiosis.

***Lg*:** Lg_1 (dominant phenotype-*ligules and auricles* present in leaves)

Pl: Pl_1 (dominant phenotype-*purple* plant color, in association with other non-allelic color genes); see *R*.

***R*:** *R* (dominant phenotype-colored aleurone and plant) gives *purple* and *red* aleurone and *red* or *purple* plants, in genotypes with other plant color genes; see Emerson et al. (1935) for descriptions of alleles (p. 19) and phenotypes (Table p. 27) with interactions with plant color genes a_1, a_2, *B*, *Pl* and *R*.

retarded synaptic complex: chromatid bridge (Fig. 36); tie (Sturtevant & Beadle 1939)

Y: Y_1 (dominant phenotype-*yellow endosperm* color)

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Increment #02 [in Vol. III, page 3.27.1]

Note: To preserve the page numbering in Volumes I & II — despite the insertion of additional content — the page numbering in the increments uses a prefix to identify the location of the related content in the previously-released volume. For example, the page number "3.27.1", contains a prefix "3.27", to refer to Part 3, page 27 and the number following the prefix indicates the page number within the increment, e.g., "1", in this case. Part 3 is in Volume II.

[See Volume II, Part III, page 3.1, for introductory material.]

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CHROMOSOME ORGANIZATION AND GENIC EXPRESSION

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During the past six years, a study of the behavior of a number of newly arisen mutable loci in maize has been undertaken. This study has provided a unique opportunity to examine the mutation process at a number of different loci in the chromosomes. For some of these loci, several independent inceptions of instability have occurred during the progress of this study. The types of mutation that appear, and the types of instability expression, need not be the same at any one locus. In fact, comparisons of the behavior of these different mutable conditions at a particular locus have shown striking diversity, not only with regard to the changes in phenotypic expression that result from mutations at the locus, but also with regard to the manner in which mutability is controlled. Knowledge of the genetic constitutions, with respect to mutable loci already present in the plants in which new mutable loci have arisen, and the subsequent behavior of the newly arisen mutable loci, have provided evidence that allows an interpretation of their mode of origin and also their mode of operation. As a consequence of this study, some rather unorthodox conclusions have been drawn regarding the mechanisms responsible for mutations arising at these loci. The same mechanisms may well be responsible for the origins of many of the observed mutations in plants and animals.

Instability of various loci—whether referred to by the terms mutable loci, mutable genes, or variegation, position effect, etc.—has been known for many years, and many such cases have received considerable study. The conditions associated with the more obvious position-effect phenomena in *Drosophila* are well known. Those associated with instability of phenotypic expressions in other organisms have been less well understood. It is because of the distinctive advantages that the maize plant offers for such a study that it has been possible to obtain precise evidence concerning some of the events associated with the origin and behavior of mutable loci. The first of these advantages relates to the ease of observing the chromosomes, and thus determining the nature of some of the changes that occur in them. The presence of a triploid endosperm in the kernel provides a second advantage. This endosperm, with its outer aleurone layer that can develop pigments, and the underlying tissues that may develop starches of several types, or sugars, or carotenoid pigments, permits the detection of differences in phenotypic expression of various types. Some of these may be quantitatively measured. Thirdly, there are a number of different loci known in which heritable alterations have given rise to changes in the expression of these several endosperm components. The mutations at some of these loci affect characters of both the endosperm and the plant tissues. This applies particularly to those mutations that affect the development of the anthocyanin pigments. In the studies to be described, the presence in the short arm of chromosome 9 of four marked loci that affect endosperm characters has been of particular importance for analyzing the events occurring at mutable loci. The necessity of having such markers will become evident in the discussion. For this study, the accumulated knowledge of the behavior of newly broken ends of chromosomes in maize has been of particular importance. Its significance for interpreting the origin of mutable loci will be indicated in the sections that follow.

The Chromatid and Chromosome Types of Breakage-Fusion-Bridge Cycle

The diagrams of Figure 1 illustrate the mode of origin of newly broken ends of chromosomes at a meiotic mitosis and the subsequent behavior of these ends in successive mitotic cycles. A chromosome with a newly broken end entering a telophase nucleus in the gametophytic or endosperm tissues will give rise in the next anaphase to a chromatid bridge configuration (McClintock, 1941). The bridge is produced because fusion occurs between sister chromatids at the position of previous anaphase breakage. This sequence of anaphase breaks and sister-chromatid fusions will continue in successive mitoses. It has therefore been designated the chromatid type of breakage-fusion-bridge cycle. This cycle is illustrated in A of Figure 1. In the sporophytic tissues, however, this cycle usually does not occur. The broken end entering a telophase

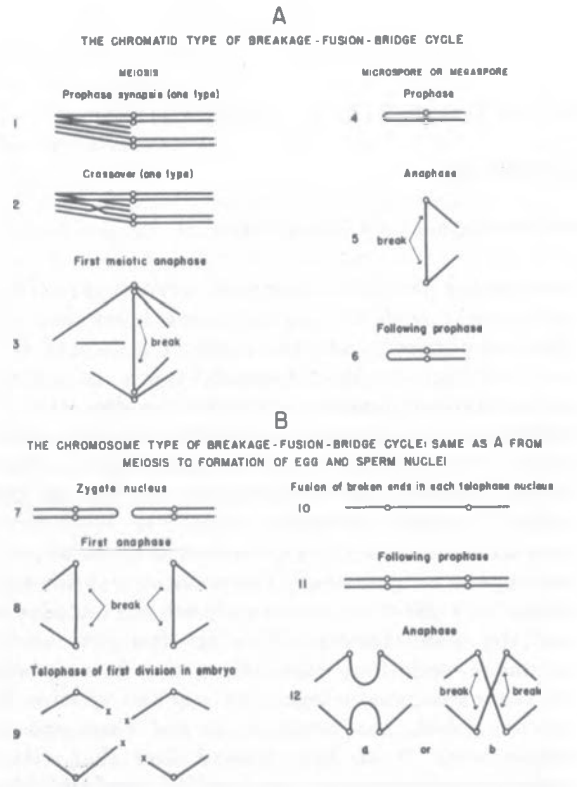


FIG. 1. Diagrams illustrating the origin of a newly broken end of a chromosome at the meiotic anaphase and its subsequent behavior. A. The chromatid type of breakage-fusion-bridge cycle. 1. One type of synaptic configuration at the first meiotic prophase between homologous arms of a pair of chromosomes, one member of which carries a duplication of this arm in the inverted order. 2. The production of a dicentric chromatid as the consequence of a crossover. It is composed of two complete chromatids of this chromosome. 3. Anaphase *I*. Bridge configuration produced by separation of centromeres of the dicentric chromatid. A break in the bridge occurs at some position between the two centromeres. 4. Fusion of sister chromatids at the position of the previous anaphase break is exhibited in prophase of the microspore or megaspore nucleus. 5. Separation of sister centromeres at anaphase in the microspore or megaspore produces a bridge configuration. This bridge is broken at some position between the two centromeres. 6. Fusion of sister chromatids occurs at the position of the preceding anaphase break. Separation of sister centromeres at the next anaphase again produces a bridge which is broken at some position between the two centromeres. This cycle continues in successive mitoses during the development of the gametophyte and the endosperm.

B. The chromosome type of breakage-fusion-bridge cycle. It may be initiated in the sporophyte if each gamete contributes a chromosome which has been broken in the anaphase of the division preceding gamete formation. The zygote nucleus will then contain two such chromosomes. In the prophase of the first division of the zygote, 7, each of these is composed of two sister chromatids fused at the position of the previous anaphase break. In the first anaphase of the zygotic nucleus heals, and its subsequent behavior resembles that of a normal, nonbroken end of a chromosome. (Note: The chromatid type of breakage-fusion-bridge cycle can continue throughout the development of the sporophytic tissues under certain conditions. These conditions are usually not present in the genetic stocks of maize.) If, however, a chromosome with a newly broken end is introduced into the zygote by each gamete nucleus, the broken ends of the two chromosomes are capable of fusion (McClintock, 1942). This establishes a dicentric chromosome. A different type of breakage-fusion-bridge cycle is thereby initiated. In the telophase nuclei, the fusions now occur between the broken ends of chromosomes rather than between the broken ends of sister chromatids, as described above. This sequence of events has been called the chromosome type of breakage-fusion-bridge cycle, and is illustrated in B of Figure 1. A study of the consequences of these cycles has revealed that they may initiate breakage events in chromosomes of the complement other than those undergoing the cycle. This complication has been of significance, for it appears that these unanticipated alterations of the chromosomes may be responsible primarily for the origin of mutable loci and of other types of heritable change.

Unexpected Chromosomal Aberrations Induced by the Breakage-Fusion-Bridge Cycles

In the course of an experiment designed to induce small internal deficiencies within the short arm of chromosome 9, a number of plants were obtained that had undergone the chromosome type of breakage-fusion-bridge cycle in their early developmental period. The short arm of each chromosome 9 was involved in this cycle. It is

division, 8, these two chromosomes give rise to bridge configurations as the centromeres of the sister chromatids pass to opposite poles. Breaks occur in each bridge at some position between the centromeres. In the telophase nuclei, two chromosomes, each with a newly broken end, are present as diagrammed in 9. The crosses mark the broken ends of each chromosome. Fusion of broken ends of *chromosomes* occurs in each telophase nucleus, 10, establishing a dicentric chromosome. In the next prophase, 11, each sister chromatid is dicentric. At the subsequent anaphase, several types of configurations may result from separation of the sister centromeres, two of which are shown in 12. Separations as shown in *b* of 12 give rise to anaphase bridge configurations. Breaks occur in each bridge at some position between the centromeres. The subsequent behavior of the broken ends, from telophase to telophase, is the same as that given in diagrams 9 to 12.

known that the cycle will often cease suddenly in certain cells and that these cells are then capable of developing sexually functional branches of the plant. In order to determine the nature of the chromosome changes produced by this cycle, the sporocytes of many of these plants were examined at the pachytene of meiosis. The expected types of altered constitution of the short arm of chromosomes 9 were found. In addition, other quite unexpected types of chromosome aberration appeared in a number of the plants. These alterations had been produced in the early developmental periods when the breakage-fusion-bridge cycles were occurring. With a few exceptions, the chromosome parts in which alterations had been initiated were the knobs and the centromeres, or the nucleolus organizer of chromosome 6. In the majority of cases, either the knob or the centromere of one of the chromosomes 9 that had been undergoing the breakage-fusion-bridge cycle was involved in the structural rearrangement. Nonrandomness was apparent with regard to the other chromosome involved in the aberration. For example, four cases were found in which the centromere of chromosome 9 had fused with the centromere of another chromosome—chromosome 2 in three of the four cases. Chromosome 8 was also very frequently involved in these structural changes.

The breakage-fusion-bridge cycle was obviously responsible for the induction of these alterations in the knobs, centromeres and the nucleolus organizer. That alterations in such elements were occurring without obvious direct participation of the knob or the centromere of the chromosome 9 undergoing the breakage-fusion-bridge cycle has also been indicated. This was made evident by the presence in one plant of an inversion involving the nucleolus organizer and the centromere region in chromosome 6, by an inversion in chromosome 5 in another plant involving the centromere and the knob regions, and by an inversion in chromosome 7 in a third plant involving the centromere region and the knob region in the long arm of this chromosome. In addition, some of the plants examined showed the presence of a ring chromosome that was not composed of segments of chromosome 9, so far as could be determined. It now must be emphasized that it was in the self-pollinated progeny of plants that had undergone the chromosome type of breakage-fusion-bridge cycle in their early developmental period that the initial burst of newly arisen mutable loci appeared. It might be suspected that this burst was a reflection of the mechanism that had produced the alterations mentioned above. If so, the origin of mutable loci would be associated with change in these particular elements of the chromosome complement. It was some time, however, before sufficient evidence had accumulated to allow deductions to be drawn regarding this presumptive relationship. A description of the origin and behavior of some of the representative types of mutable loci should be given before this topic is again considered.

Recognition of the Relation of Mutation to the Mitotic Cycle

Interest in these mutable loci, appearing unexpectedly and in large numbers in the self-pollinated progeny of plants that had undergone the chromosome type of breakage-fusion-bridge cycle in their early developmental periods, was aroused when it was realized that in each case some factor was present which controlled the time or the frequency of mutations. This factor could be altered as a consequence of some event associated with the mitotic process. This was made evident by the appearance of sectors of tissue, derived from sister cells, that exhibited obvious differences in time of mutations, mutation frequency, or both. In many cases, it was also apparent that the mutations themselves arose as a consequence of some event associated with the mitotic cycle. This basic behavior pattern was exhibited by all the various newly arisen mutable loci. It directed attention to the mitotic mechanism as the responsible agent. It was concluded, therefore, that further investigation of these mutable loci might produce some evidence leading to an appreciation of the nature of the responsible mitotic events.

During six years of study of a number of newly arisen mutable loci, some well-established facts have accumulated concerning the processes associated with the origin of mutable loci and their subsequent behavior. Observation of consistent behavior in many mutable loci, where the cytological events associated with a change in phenotype could be determined, and comparison of the behavior of these loci with others in which cytological determinations could not readily be made, have provided an assemblage of interrelated facts upon which the conclusions to be stated later are based.

The Origin of *Ds* and its Behavior

The first evidence of the type of chromosomal event that is associated with the expression of mutability came with the discovery of a locus in

the short arm of chromosome 9 at which chromosome breaks were occurring. This was observed in the self-pollinated progeny of one of the plants that had undergone the chromosome type of breakage-fusion-bridge cycle in early development. When first seen, the "mutability" was expressed by the time and frequency of the breaks that occurred at this locus in some cells during the development of a tissue. Also, some change could occur in somatic cells that affected the time and frequency; and this latter event likewise was associated with the mitotic process. The behavior pattern resembled in considerable detail the patterns exhibited by the mutable loci. In this case, however, a mechanism associated with chromosome fusion and subsequent breakage was responsible for the behavior observed. The mutations from recessive to dominant exhibited by the mutable loci would not alone have suggested a chromosome-breakage mechanism as being responsible. Because of this similarity of the patterns of behavior, it was suspected that the basic mechanism responsible for mutations at mutable loci could be one associated with some form of structural alteration at the locus showing the mutation phenomenon. This conclusion was consistent with the very first observations of the behavior of mutable loci. These observations had indicated that the events at mutable loci leading to mutations and also other events controlling their time and frequency of occurrence were associated with alterations that were in some manner produced during the course of a mitotic cycle.

Intensive study of this locus in chromosome 9 at which structural alterations occur at regulated rates and at regulated times in development has been rewarding. A "break" in the chromosome at this locus was the event first recognized. The factor responsible was therefore given the symbol *Ds*, for "Dissociation." The nature of the breakage event was later determined. It arises from dicentric and acentric chromatid formations. The acentric fragment is composed of the two sister chromatids, from the *Ds* locus to the end of the short arm. The complementary dicentric component includes the sister segments from the locus to the centromere plus the long arms of the two sister chromatids. This is the type of recognizable event found to occur most frequently at *Ds*. Other recognizable aberrations, however, may sometimes arise. One of them is the formation of an internal deficiency in the short arm of chromosome 9. Such deficiencies include the regions adjacent to *Ds*, and vary in extent from minute to quite large. Translocations between this chromosome and another chromosome of the complement may arise, with one of the points of breakage at the *Ds* locus. Duplications, or inversions, of segments within chromosome 9 may also be produced, one of the breakage points being at *Ds*.

It was realized early in this study of *Ds* that changes could occur at the locus leading to marked alterations in frequency of the detectable breakage events. The original isolate was showing high frequencies of formation of dicentric chromatids and the associated acentric fragments. Changes arose at the locus, however, as a consequence of some event occurring in a somatic cell. These changes resulted in the appearance, in subsequent cell and plant generations, of lowered frequencies of these events. Such changes in the behavior pattern of *Ds* were called "changes in state"; and the *Ds* with the altered state behaved in inheritance as an allele of the original isolate of *Ds*. A subsequent change could occur, which again was recognized by an altered frequency of detectable breakage events, and which behaved in inheritance as an allele of the initial state, of the derived state, or of other unrelated derived states. By selecting altered states of *Ds*, a series of alleles of the original *Ds* has been isolated. The changes in state of *Ds*, and those occurring at other mutable loci, are of considerable significance in understanding the nature of the events responsible for the patterns of behavior of all mutable loci. A discussion of this significance will be postponed until the behavior of some other mutable loci have been considered. The meaning of the term will then be readily apparent.

Transposition of *Ds*

An important aspect of this study, with regard to the origin of mutable loci and nature of their mutation process, is related to transposition of *Ds* from one location in the chromosome complement to another. The discovery of such transpositions occurred in the course of studies aimed at determining the exact location of *Ds* in chromosome 9. These tests involved linkage relationships. A sequence of six marked loci along the chromosome arm were used, and the linkage studies clearly established the location of *Ds* as shown in Figure 2. This genetically determined location fitted the position of breaks in the chromosome observed in some of the sporo-

cytes of plants having *Ds* in either one or both chromosomes 9. Such chromosome breaks are illustrated in the photographs of microsporocytes at pachytene given in Figures 4 to 8. This was the location of *Ds* when it was first discovered, and has been called the standard location.

In the course of studies of the inheritance behavior of *Ds*, an occasional kernel appeared which showed that *Ds*-type activity—that is, chromosome breakage—was occurring at a new position in the short arm of chromosome 9. Attempts were made to germinate such kernels when they were found. If a plant arose from one, a study was then commenced to determine the new location of the *Ds*-type activity. Over 20 cases of the sudden appearance of *Ds*-type activity in new locations in the short arm of chromosome 9, and several cases of its sudden appearance in other chromosomes of the complement, have been investigated. Within the short arm of chromosome 9, such activity has appeared at various positions. All the isolates studied have shown sharply defined locations of the *Ds*-type activity. In these cases, the cytological determination of breakage position and the genetic determination of location were in agreement. New positions of *Ds*-type activity have appeared between all of the marked loci shown in Figure 2. For example, in four independently arisen cases, the new position of *Ds* has been located between *I* and *Sh*. In two of these, it is to the right of *I*, at or close to the same position in each case—approximately one-fifth the crossover distance between *I* and *Sh*. In the other two it is to the left of *Sh*, with a very low percentage of crossing over between *Ds* and *Sh* in each case.

The mode of detecting new locations of *Ds*-type activity has been selective, in that those arising in the short arm of chromosome 9 are immediately revealed on many of the ears coming from test crosses. *Ds*-type activity has suddenly appeared, however, in other chromosomes of the complement. Only when appropriate genetic markers are present can it be detected readily; and in most tests, such markers have not been present.

Several questions must now be asked. How do new positions of *Ds* activity arise? And what conditions are responsible for their occurrence? The methods used in seeking answers to these questions may be described. In some cases, it could be established that the appearance of *Ds* activity at a new location was associated with its disappearance at the known former location. It has been emphasized that the mechanism underlying *Ds* events is one that can give rise to translocations, deficiencies, inversions, ring-chromosomes, etc., as well as the more frequently occurring dicentric chromatid formations with reciprocal formation of acentric fragments. It has also been stated that in each such case one breakage point is at the known location of *Ds*. The appearance of *Ds* at new locations is probably associated with such a break-inducing mechanism. This was indicated by extensive analysis of the constitutions of two independent duplications of segments of the short arm of chromosome 9 when a new location of *Ds* activity was also present in this arm. In both cases, only one of the many tested gametes of one of the parent plants carried the particular chromosome aberration with the new location of *Ds*. It was detected in two single aberrant kernels on separate ears coming from similar types of crosses made in two different years. The female parent carried two morphologically normal chromosomes 9, each with the markers *C*, *sh*, *bz*, and *wx*. No *Ds* (or *Ac*, see below) was present in these plants. The male parent (one *Ac* present) carried two morphologically normal chromosomes 9. The markers *I*, *Sh*, *Bz*, *Wx*, and *Ds* (at its standard location) were present in one chromosome 9. The homologous chromosome carried *C*, *sh*, *bz*, *wx*, but no

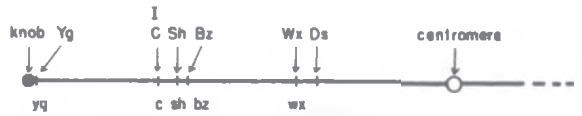
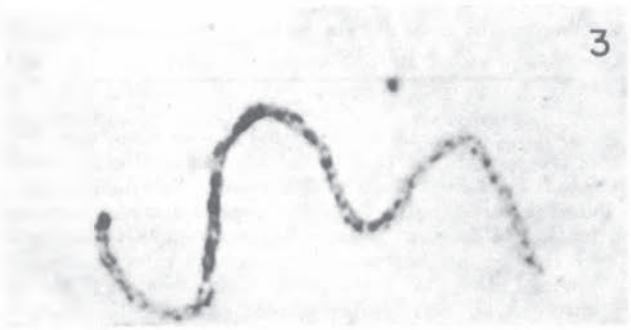


FIG. 2. Diagram showing the approximate locations of the genetic markers in the short arm of chromosome 9 that have been used in this study. In symbolization, dominance is indicated by a capital letter or capitalization of the first letter. Recessiveness is indicated by lower-case letters. The symbols refer to the following plant or endosperm characters: *Yg*, normal chlorophyll; *yg*, yellow-green chlorophyll color in early period of development of the plant. *Sh*, normal endosperm; *sh*, shrunken endosperm. *I*, *C*, and *c* form an allelic series associated with pigment development in the aleurone layer of the endosperm. *I*, inhibitor of aleurone color formation, dominant to *C*. *C*, aleurone color, dominant to *c*, colorless aleurone. The *Bz* factor is associated with development of aleurone and plant color. When homozygous, the recessive, *bz*, (bronze), gives rise to an altered anthocyanin color in the aleurone and plant tissues, from a dark red or purple to a bronze shade. When *Wx* is present, the starch in the pollen and endosperm stains blue with iodine solutions, due to the presence of amylose starch; when only the recessive *wx* (waxy) is present, no amylose starch is formed and with iodine solutions, the starch stains a reddish-brown color. The position of *Ds*, indicated in the diagram, is the standard location (see text).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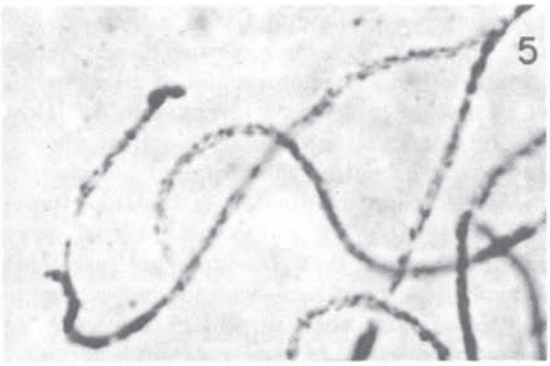
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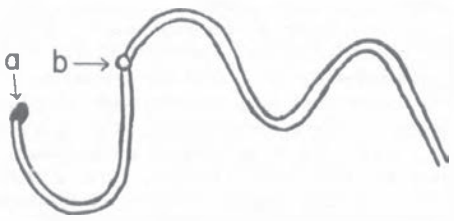
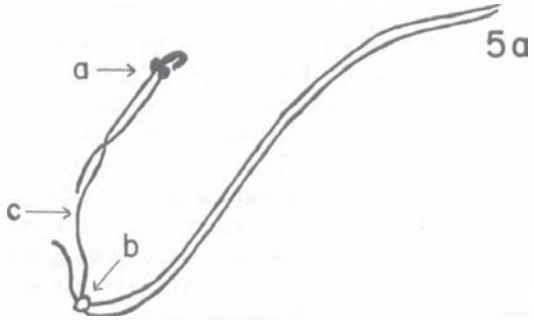

3a
a
b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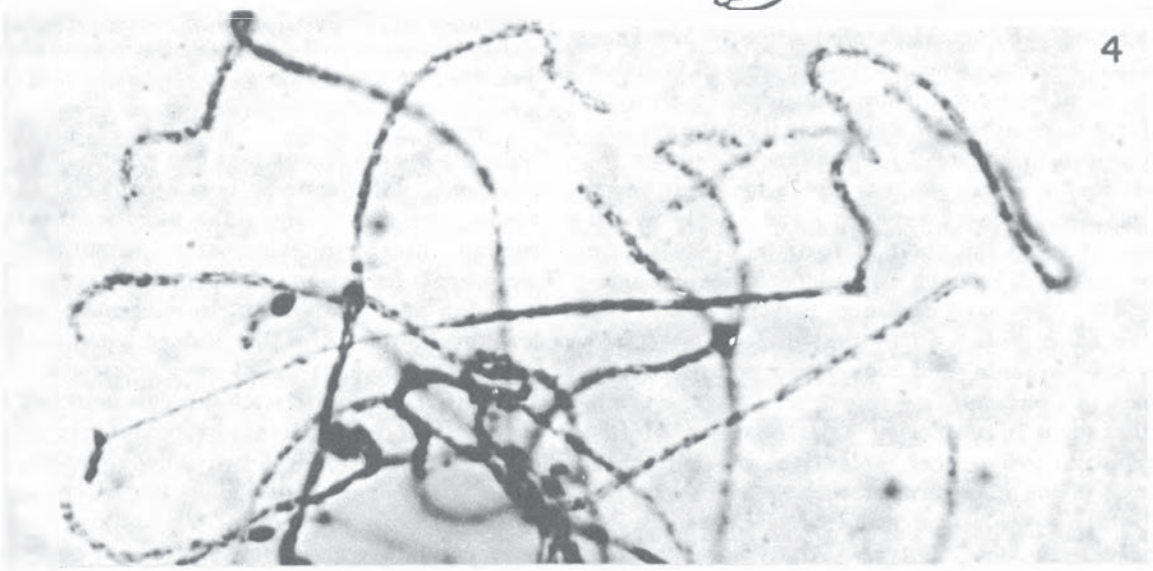
a
c
b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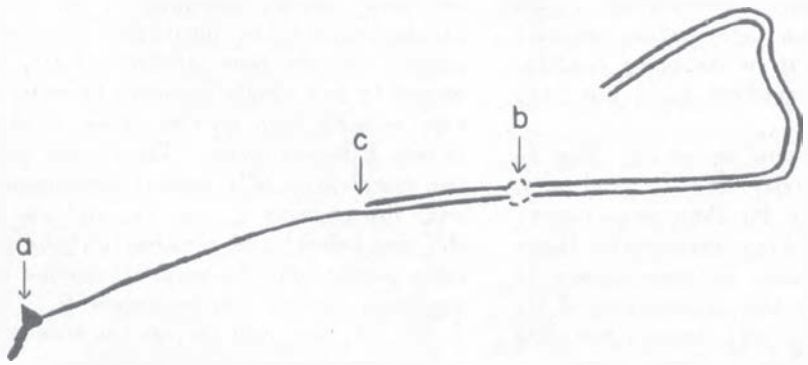
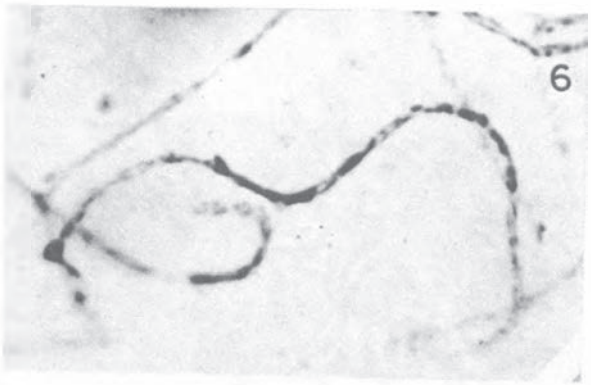

4a
c
b
a



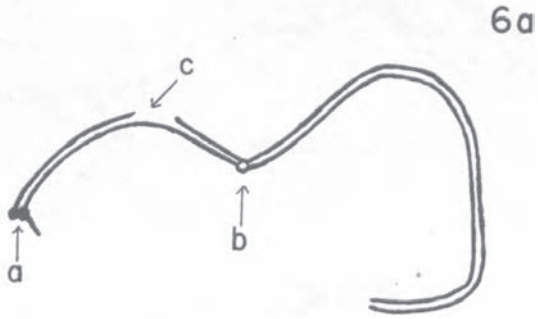
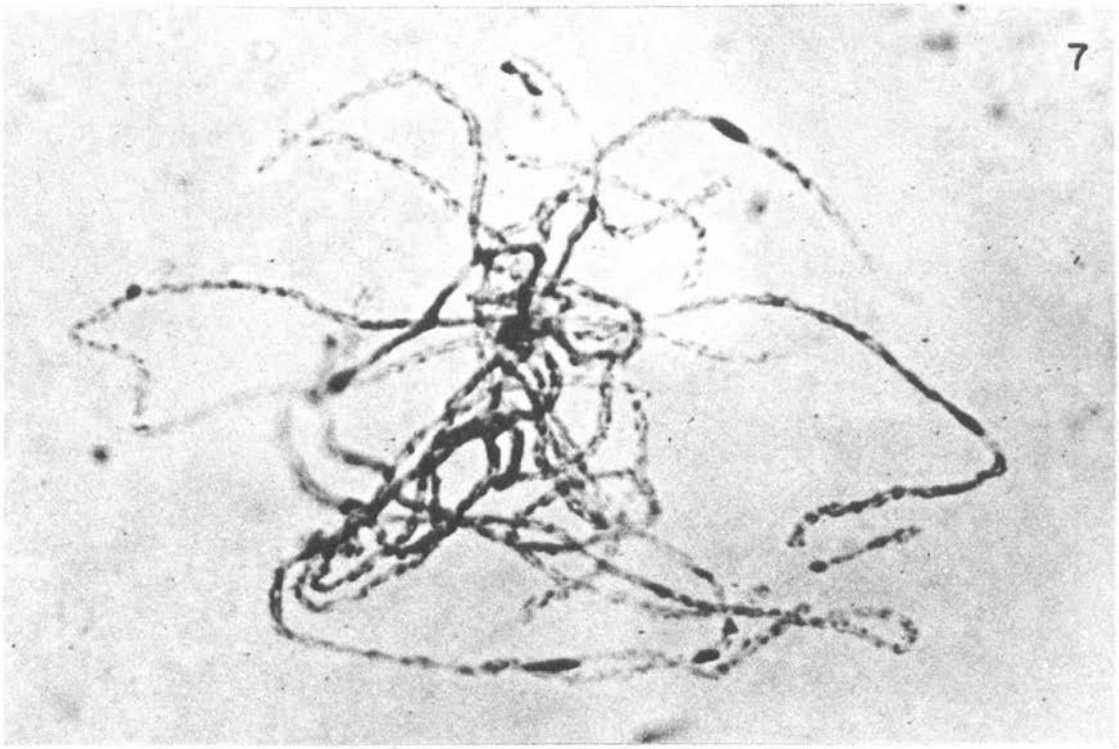
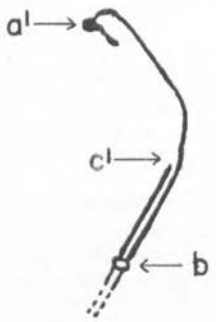


FIG. 3. Photograph of a normal bivalent chromosome 9 at pachytene of meiosis. In the accompanying diagram, 3a, the knob terminating the short arm is indicated by the arrow, *a*. The centromere is indicated by the arrow *b*. Mag. approximately 1800x. Fusion of homologous centromeres appears to occur at pachytene. Consequently, in the diagrams accompanying Figures 3 to 8, this region is indicated as single rather than double.

FIGS. 4 to 7 and accompanying diagrams, 4*a* to 7*a*. Illustrations of the position of breaks at the *Ds* locus as seen at pachytene of meiosis in plants having *Ds* at its standard location in one chromosome 9 and no *Ds* in the homologue. The two homologues are distinguishable. At the end of the short arm of the chromosome 9 having no *Ds*, a segment of deep-staining chromatin extends beyond the knob. The short arm of the chromosome 9 carrying *Ds* terminates in a knob. Magnifications approximately 1800x. In Figure 4, a break at *Ds* occurred in a premeiotic mitosis. The acentric fragment, from *Ds* to the end of the arm, was lost to the nucleus. Consequently, this segment is missing in the bivalent. The homologous segment in the chromosome 9 having no *Ds* is therefore univalent. In making the preparation, this segment was considerably stretched. In the accompanying diagram, arrow *a* points to the knob and the small deep staining segment extending beyond the knob. Arrow *b* points to the centromere region, not clearly shown in the photograph. Arrow *c* points to the position of the break in the chromosome 9 that carried *Ds*. Figures 5 and 6 show the appearance of the bivalent chromosome 9 when a break in the *Ds* carrying chromosome occurred at the meiotic prophase and when the free segment, from *Ds* to the end of the arm, paired with its homologous segment in the chromosome 9 having no *Ds*. In the accompanying diagrams, arrow *a* points to the knobs, arrow *b* points to the centromeres and arrow *c* to the position of the *Ds* break in one of the homologues. Figure 7 is similar to Figures 5 and 6 except that the free fragment, from the position of *Ds* to the end of the arm, did not pair with its homologous segment in the chromosome 9 having no *Ds*. In the accompanying diagram, arrow a^1 points to the knob and the deep-staining chromatin extending beyond the knob in the chromosome having no *Ds*. Arrow a^2 points to the knob of the unpaired acentric fragment. Arrow *b* points to the position of the centromeres, not observable in the photograph. Arrow c^1 points to the broken end of the centric segment, and arrow c^2 points to the broken end of the acentric segment. (For Fig. 7, see next page.)





7a

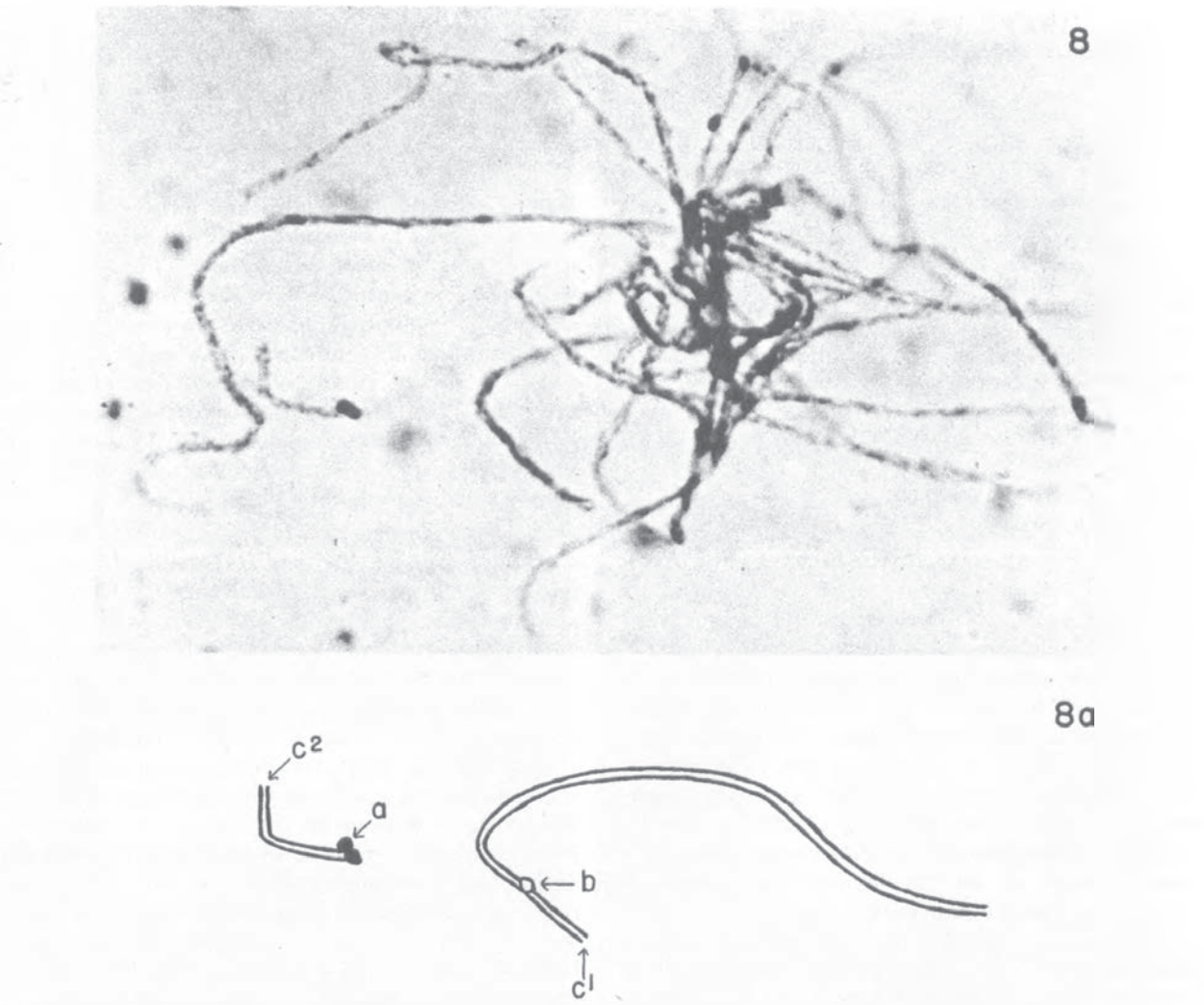


FIG. 8. The chromosome 9 bivalent at pachytene in a plant having *Ds* at its standard location in each chromosome 9. A *Ds* break occurred at the meiotic prophase in each chromosome. Consequently, the bivalent is composed of two free segments: one short acentric segment, from *Ds* to the end of the arm, and a long centric segment, from the position of *Ds* to the end of the long arm. In the accompanying diagram, arrow *a* points to the large knob terminating the short arm of each member of the acentric bivalent segment, and arrow *b* points to the centromeres of the centric bivalent segment. Arrow c^1 points to the broken ends of the centric bivalent segment and arrow c^2 points to the broken ends of the acentric bivalent segment.

Ds. The constitutions of the aberrant chromosomes 9, which were present in an individual pollen grain in each case, are shown in Figure 9. Each has a duplication of a segment of the short arm of chromosome 9. A study of these constitutions reveals that, in each case, the new position of *Ds* activity coincided with the position of one of the breaks that produced the duplication. Also, the position of the second break coincided with the previously known location of *Ds* in the morphologically normal *Ds*-carrying chromosome 9 of the male parent plant. It seems clear from the analysis of both these cases that the breaks were *Ds* initiated, and also that both breaks involved sister chromatids at the same location. Two other cases of newly arisen duplications associated with new positions of *Ds* activity have received study. These have proved to be similar in their modes of origin to the two cases diagrammed. Although these cases suggest that the new positions of *Ds* activity may arise from contacts with the *Ds* that is present in the chromosome complement, they do not constitute evidence that *Ds* is composed of a material substance and that the new positions arise from insertion of this material into new locations.

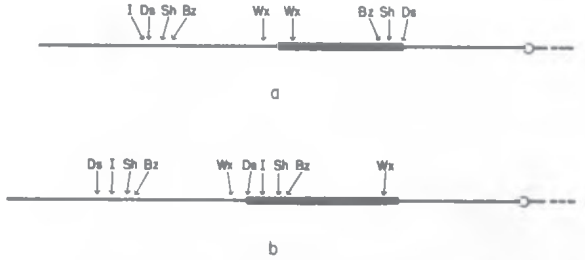


FIG. 9. Diagrams illustrating the constitution of two chromosomes 9, each of which carries a duplicated segment (heavy line). A transposition of *Ds* accompanied the formation of the duplication in each case. For details, see text.

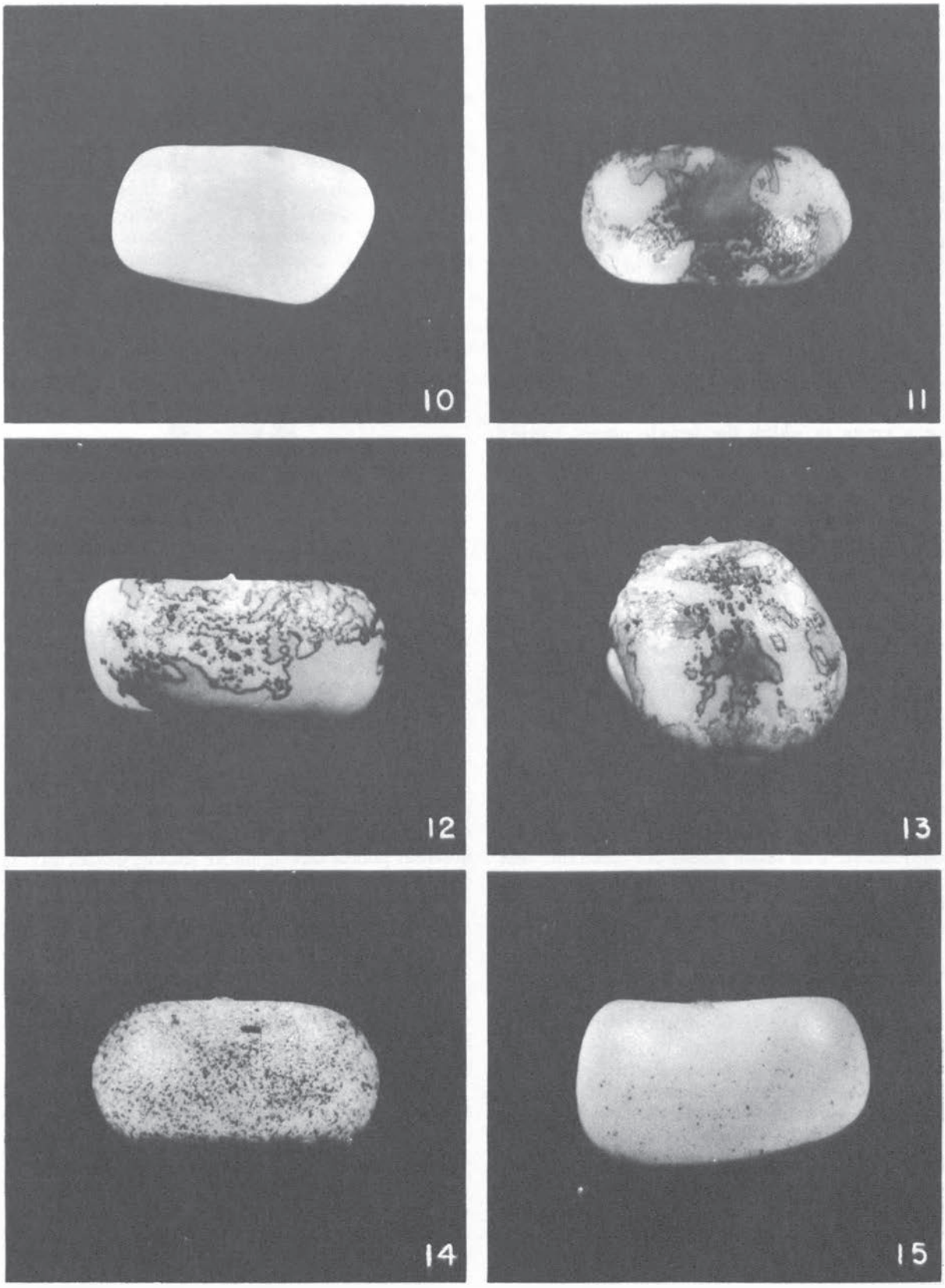
Ac Behavior and Inheritance: General Considerations

Before continuing discussion of the sudden appearance of *Ds* at new locations, it is necessary to consider another heritable factor called Activator (*Ac*). *Ac* is a dominant factor that must be present if any breakage events are to occur at *Ds*. If *Ac* is absent from the nucleus, no detectable events whatsoever occur at *Ds*, wherever it may be located; and no new positions of *Ds* activity appear. Because of this, and because new positions of *Ds* arise only in a few cells, during the period of development of a tissue when breakage events are occurring at *Ds*, it may be concluded that these new positions are one of the consequences of the mechanism that produces chromosome breakage events at *Ds*.

The location of *Ds* in chromosome 9 was first suggested by the altered phenotype of sectors of tissue derived from cells in which a break had occurred at *Ds*. Such sectors will appear if the factorial constitutions of the homologues differ. The chromosome arm carrying *Ds* must also carry dominant markers, and the homologue must carry the recessive alleles. If one chromosome 9, delivered by the male parent, carries *I*, *Sh*, *Bz*, *Wx*, and *Ds* at its standard location, and if the homologue delivered by the female parent carries the recessive alleles *C*, *sh*, *bz*, and *wx*, and has no *Ds*, the events at *Ds* that form a dicentric chromatid and an acentric fragment will lead to elimination of the acentric fragment during a mitotic division. This fragment will form a pycnotic body in the cytoplasm, which subsequently disappears. All the dominant markers carried in this acentric fragment will be removed from the nuclei after such an event has occurred. The sector of tissue derived from these cells will exhibit the collective phenotype of the recessive alleles carried by the homologous chromosome 9 having no *Ds*.

If pollen of plants having one *Ac* factor and carrying *I*, *Sh*, *Bz*, *Wx*, and *Ds* (standard location) is placed upon silks of plants carrying *C*, *sh*, *bz*, and *wx* in their chromosomes 9, but having no *Ds* or *Ac*, two types of kernels will appear, those that received an *Ac* factor and those without *Ac*. The latter kernels will be colorless and nonshrunken, and will show the *Wx* phenotype. No variegation for the characters exhibited by the recessive alleles will appear, as shown in Figure 10. If *Ac* is present in the endosperm, however, the described *Ds* events will occur in some cells during the development of the kernel. This will result in elimination of the segment in the short arm of chromosome 9 carrying the dominant factors. All the cells arising from one in which such an event has occurred will exhibit the *C*, *sh*, *bz*, and *wx* phenotypes. Consequently, these kernels will be variegated. The photographs of kernels in Figures 11 to 13 will illustrate the nature of this variegation.

FIGS. 10 to 15. Photographs of kernels illustrating the effects produced by breaks at *Ds*, and the relation of the presence or absence of *Ac* and the doses of *Ac* on the time of occurrence of *Ds* breaks. The kernels arose from the cross of a plant (♀) carrying *C* and *bz* and having no *Ds* in each chromosome 9, by plants (♂) having *I*, *Bz*, and *Ds* at its standard location in chromosome 9. In Figure 10, no *Ac* is present; the kernel is completely colorless due to the inhibition of aleurone color when *I* is present. In Figures 11 to 13, one *Ac* is present. Breaks at *Ds* occur early in development with consequent loss of *I* and *Bz* to the nuclei. The cells without *I* and *Bz* give rise to sectors showing the *C bz* phenotype. It should be noted that *Bz* substance diffuses through several cell layers, from the *I Bz* areas into the *C bz* areas, producing a *C Bz* phenotypic expression in these genotypically *C bz* cells. Thus, all large areas of the *C bz* genotype are rimmed with the dark aleurone color of the *C Bz* phenotype. Small *C bz* areas may be mostly *C Bz* in phenotype because of this diffusion, and very small *C bz* areas are totally *C Bz* in phenotype. These relationships are clearly expressed in the photographs. In Figures 11 to 13, both large and small *C bz* sectors are present as well as areas in which no *C bz* sectors appear. This irregularity arises from alterations that occur to *Ac* during the development of the kernels. These give rise to sectors with no *Ac* or with altered doses of *Ac* (see text). Figure 14 shows the pattern that may be produced by *Ds* breaks when two *Ac* factors are introduced into the primary endosperm nucleus. There are many small sectors of the *C bz* genotype, all produced by relatively late occurring *Ds* breaks. This results in a heavily speckled variegation pattern. Figure 15 shows the pattern that may appear when three *Ac* factors are present. Only relatively few *C bz* specks, produced by late occurring *Ds* breaks, are present in this kernel.


10
11
12
13
14
15

In the early studies, it soon became apparent that the time of occurrence of *Ds* breakage events in the development of a tissue depends upon the dose of *Ac* present. The higher the dose of *Ac*, the later in development will such events at *Ds* occur. Because the endosperm is triploid—the female parent contributing two haploid nuclei derived from the female gametophyte, and the male contributing one haploid nucleus—single to triple doses of *Ac* may readily be obtained after appropriate crosses. The time of response of *Ds* to double and triple doses of *Ac* is shown in Figures 14 and 15.

Initial studies of the inheritance behavior of *Ac* showed that it follows the mendelian laws known to apply to single genetic units. An illustration of this inheritance behavior is given in Figure 16. The ear in this figure was derived from a cross in which the female parent carried the recessive factor *c* in each chromosome 9, but had no *Ds* or *Ac* factors, and the male parent carried in each chromosome 9 the factors *C* and *Ds* (standard location). It also carried a single *Ac* factor. The expected 1-to-1 ratio of colored, nonvariegated kernels (no *Ac* present) to kernels showing sectors of the *c* phenotype (*Ac* present) was apparent on this ear. Efforts to determine the location of *Ac* in the chromosome complement were commenced, but were soon abandoned when it was realized that *Ac* need not remain at any one location in the complement. It can appear at new locations and in different chromosomes. Because of this highly unexpected behavior of a genetic factor, extensive studies were made to determine the mode of inheritance of *Ac* and to learn how these new positions arise. Much has been learned from them about *Ac* behavior and inheritance. The modes of investigating alterations of *Ac* will be considered later. Some of the facts concerning its behavior, however, may be stated here in summary form, by describing the results obtained from several types of experiments.

If plants having one *Ac* factor are self-pollinated, the expected mendelian ratios may appear in the F_2 populations. These are: one with two *Ac*, to two with one *Ac*, to one with no *Ac*. The ratios obtained in one such test were 61 : 145 : 68, which is close to the statistical expectancy. When, however, these F_2 plants having two *Ac* factors are crossed by plants having no *Ac*, the expectation would be that all the progeny would have one *Ac* factor. Usually, most of the plants do have one *Ac* factor; but sometimes there are plants displaying other conditions with respect to *Ac*. The following unexpected types have appeared: plants having no *Ac* factor; others having two nonlinked *Ac* factors; others having two *Ac* factors that appear to be linked; still others having an *Ac* factor that acts as a single unit in inheritance but gives the same dosage action as would two doses of the *Ac* factor in the parent plant that contributed *Ac*. The dosage action of *Ac* may be altered in other ways, moreover, so that a single *Ac* factor, as determined genetically, may exert an action either less than that of the *Ac* factor contributed by the *Ac*-carrying parent plant or falling between one and two doses of this factor.

FIG. 16. Photograph of an ear produced when pollen from plants having *C* and *Ds* (standard location) in each chromosome 9 and carrying one *Ac* factor is placed on silks of plants carrying *c* in each chromosome 9 and having no *Ds* or *Ac* factors. Approximately half of the male gametes have no *Ac* factor. The kernels arising from the functioning of such gametes are fully colored; no variegation appears. The other half of the male gametes carry *Ac*. The kernels arising from the functioning of these gametes are variegated. They show a number of sectors with the *c* phenotype. Note the approximate 1 to 1 ratio of fully colored kernels to variegated kernels.

In the early studies of *Ac* inheritance, the *Ac* factor was found not to be linked with the genetic markers in the short arm of chromosome 9. In one series of tests of *Ac* inheritance, where a number of *Ac*-carrying plants were derived from a cross between a plant having no *Ac* and a plant

having one *Ac*, a single aberrant plant was present in the F_1. Tests of this plant showed that it possessed a single *Ac* factor, which was obviously linked to the markers in the chromosome 9 delivered by the *Ac*-carrying parent plant. In the sister plants, and in the parent plant that contributed *Ac*, no such linkage was evident. Studies were then conducted to examine the linkage relationships of this *Ac* with the marked loci. The very same types of tests for linkage of *Ac* with the genetic markers in chromosome 9 were also conducted with the sister plants. A summary of one set of these comparative tests of *Ac* inheritance is presented in Table 1. In part I of this table, no linkage of *Ac* with *I* or *Wx* is evident; in part II, however, linkage is obvious. The data place *Ac* to the right of *Wx*. Approximately 20 per cent crossing-over appears to have occurred between *Wx* and *Ac*. Actually, this figure is only approximate, for a few kernels in the *Ac-wx* class do not carry *Ac* in chromosome 9. They do not belong in the crossover class. The *Ac* in these kernels had been transposed from chromosome 9 to another chromosome. Also, a few kernels in the *Wx*-no *Ac* class have not lost *Ac* because of crossing over but rather because it was removed from its location in chromosome 9.

The described case of sudden appearance of *Ac* in chromosome 9 is not the only one that has been found and similarly studied. Seven independent cases have so far been identified. In two of these, *Ac* appeared in the short arm of this chromosome: in the first case, a short distance to the left of *Wx*; and in the second case, a short distance to the left of *I*. Studies were then undertaken to investigate not only the linkage behavior of *Ac* but also the types of events that alter *Ac* in these two sharply delimited locations. It was determined that in the majority of the *Ac*-carrying gametes produced by plants having an *Ac* at either of these two stated positions, no change in location or action of *Ac* occurred. Exchanges of *Ac* from one homologue to another took place as a consequence of crossing over, with consistent frequencies in each case. In a few of these gametes, however, the above-described types of aberrant *Ac* conditions were present, as follows: (1) *Ac* was no longer present in chromosome 9, but was carried instead by another chromosome. (2) Two *Ac* factors were present, one at the given location in chromosome 9, and one carried by another chromosome of the complement. (3) The *Ac* factor was unchanged in its location but showed an altered action; in a single dose it could be equivalent to the double dose before the alteration occurred, or could show an increased but not doubled dosage action, or a decreased dosage action.

The behavior of *Ac* was also studied in plants in which the *Ac* factor was present at an allelic position in each chromosome 9. Again, it could be determined that in a few gametes produced by such plants the above-described aberrant conditions with respect to *Ac* position and action were present. In addition, a few gametes were formed that had no *Ac* factor at all.

From what has been said about both *Ds* and *Ac*, it is apparent that with respect to inheritance

TABLE 1

Comparisons of *Ac* inheritance: (1) when *Ac* was not linked to markers in chromosome 9, and (II) when *Ac* was linked to markers in chromosome 9, in crosses of

(I) $\male \frac{I\ Sh\ Bz\ Wx\ Ds}{C\ Sh\ Bz\ wx\ Ds}$ 1 *Ac*, nonlinked

♀ *C sh bz wx/C sh bz wx*, no *Ds*, no *Ac* by (I) and

(II) $\male \frac{I\ Sh\ Bz\ Wx\ Ds\ Ac}{C\ Sh\ Bz\ wx\ Ds\ ac}$

Chromosome-9 Constitution of ♂ Gamete	I		II	
	Variegated* *Ac* present	Nonvariegated* No *Ac*	Variegated* *Ac* present	Nonvariegated* No *Ac*
I Wx	268	255	928	246
C wx	248	242	164	893
I wx	88	91	62	387
C Wx	84	83	344	100

*Presence of *Ac* detected by sectors of the *C sh bz wx* phenotype in kernels on ears obtained from cross I or cross II. In the absence of *Ac*, no *Ds* events occur; the kernels are therefore nonvariegated.

behavior they are much alike. The same questions concerning mode of appearance in new locations in the chromosome complement apply to *Ac* as to *Ds*. With this relationship in mind, we may now return to further considerations of *Ds*. It should be emphasized again, however, that the described events occurring at *Ds*, wherever it may be located in the chromosome complement, depend upon the presence of an *Ac* factor in the nucleus, regardless of where this latter factor is located in the chromosome complement. New positions of *Ds* activity arise only when *Ac* is also present in the nucleus, and, again, regardless of where *Ac* may be located. In addition, any one altered state of *Ac*—for example, an altered dosage action—affects *Ds* wherever it may be located, and in exactly the same manner for every *Ds*, regardless of its state. In other words, it is the state and the dose of *Ac* that control just when and where *Ds* events will occur, and it is the state of a particular *Ds* that controls the relative frequency of any one type of event that occurs at *Ds*.

The Origin and Behavior of c^{m-1}

In the discussion of the appearance of *Ds* at new locations, the question was raised whether or not this involves the transposition of a material substance from one location to another. The question applies equally to *Ac*. If no material substance is transposed, a serious problem is presented regarding the basic action of any known genetic unit or factor that has been assigned to a particular locus in a particular chromosome. *Ac* clearly produces an obvious, measurable, phenotypic response, wherever it may be located. It shows dosage action, mendelian inheritance, and linkage behavior of the expected type, in any location—with the exception, already mentioned, of a few transpositions, changes in state, and losses of *Ac*. It might be considered that *Ds* and *Ac* represent forms of altered chromosome organization producing somewhat similar effects in each case, much like the Minutes in *Drosophila*. The evidence now to be presented, however, makes that assumption unlikely. This evidence considers the origin and the behavior of many different mutable loci. To begin this part of the discussion, we may consider the origin of mutable c^{m-1}, the first-detected mutable *c* locus that arose in a chromosome carrying a normal-behaving *C* locus (*C*, aleurone color; *c*, recessive allele, colorless aleurone).

The presence of an alteration at the known locus of *C*, which produced c^{m-1}, was detected probably within a few nuclear generations after it occurred. It was present only in one kernel among approximately 4,000 examined that had come from a cross of a single plant, used as a male, to 12 genetically similar female plants. All the other kernels on these ears gave the expected types of phenotypic expression. The male parent carried in both chromosomes 9 the genetic markers *Yg*, *C*, *Sh*, *wx*, and *Ds* (standard location). It also had one *Ac* factor, not linked to these markers. The female parents carried the stable recessive *yg*, *c*, *sh*, and *wx*. No *Ds* was present in their chromosomes 9, and also no *Ac* factor was present in these plants. The types of kernels to be expected from such a cross, and their relative frequencies, are the same as those shown in Figure 16. Approximately half the kernels should show the *C Sh wx* phenotype, with no variegation for the recessive characters since no *Ac* factor is present. The other half should carry *Ac* and thus be variegated. Sectors showing the *c sh* phenotype should be present. In these crosses, the expected classes of kernels appeared with the exception of one kernel. Instead of showing colorless areas in a colored background (resulting from losses of *C* following breakage events at *Ds*), this exceptional kernel showed a colorless background in which colored areas were present. The plant derived from this kernel was tested in various ways in order to determine the reason for this unexpected type of variegation. The early tests indicated, and subsequent tests proved, that mutations were occurring from the recessive *c*, to the dominant *C*, and that the mutable condition had arisen in one of the chromosomes 9 contributed by the *Ac*-carrying male parent plant.

Ds-type activity was also present in the chromosome carrying the new mutable *c* locus. The location of this activity was no longer to the right of *wx*, as would be expected since this was its location in both chromosomes 9 of the male parent plant. The new location was inseparable from that of the mutable *c*. All the recognizable breakage events associated with *Ds*-type activity now happened at this new location. In addition, mutations to *C* occurred. It was soon discovered that the mutations to *C* would appear only if *Ac* were also present in the nucleus, and that the time of occurrence of these mutations was controlled by the state and dose of *Ac* in precisely the same way that the state and dose of *Ac* controls *Ds* breakage events wherever *Ds* may be located. If *Ac* were absent, neither mutations to *C* nor *Ds*-type breakage events would occur. Thus when *Ac* is absent, the behavior of c^{m-1}

is equivalent to the previously known recessive *c*. If, however, *Ac* is again introduced into the nucleus by appropriate crosses, the potential mutability of this recessive is realized, for then mutations to *C* occur. The previously known recessive *c*, used for many years in genetic studies, is unaffected by the presence of *Ac*; and it remains stable, nonmutable, when present in nuclei in which c^{m-1} is also present and undergoing mutations.

In considering the mode of origin of c^{m-1} and its behavior, the following points may be reviewed: (1) the appearance of a new recessive that is mutable; (2) its derivation from a normal dominant *C*, which is nonmutable; (3) its appearance in a single gamete of a plant carrying *Ds* and *Ac*; (4) *Ac* control of the new mutable condition in exactly the same manner as *Ds* is *Ac*-controlled; (5) *Ds*-type chromosome breakage events also occurring at this mutable locus; and (6) disappearance of *Ds* from its former location in the same chromosome that carries the new mutable locus. This series of coincidences is striking enough in itself to command consideration of the possibility that this mutable recessive originated by transposition of *Ds* to the locus of the normal *C* factor. It is immediately apparent that, if this is true, the transposition of *Ds* from its former location to the new location created a condition that affects the formation of pigment in the aleurone layer; for no pigment is formed until some event occurs at this locus, and only when *Ac* is present. Previous tests have shown that the same *c* phenotypic expression can also arise if the tissues of the endosperm are homozygous deficient for the segment of chromatin carrying *C* (McClintock, unpublished). This might suggest that the presence of *Ds* has inhibited the normal action of the chromatin materials at the *C* locus. A final and most significant argument for the origin of c^{m-1} by a transposition of *Ds* to the *C* locus is derived from the fact that a mutation to *C* is associated with the loss of any further recognizable *Ds* events at the immediate location of *C*. It is apparent, therefore, that the mutation-producing event is associated with one involving *Ds* at this locus. All the evidence is consistent with the assumption that c^{m-1} arose by transposition of *Ds* to the locus of *C*, thereby inhibiting its action, and that removal of *Ds* is associated with removal of this inhibitory effect. The restored activity at the *C* locus is permanent, and its subsequent behavior resembles that present before *Ds* activity appeared at the locus. *Ac* no longer has any effect on its action and behavior, just as it had no effect before *Ds* appeared at the *C* locus to give rise to c^{m-1}.

Additional *Ds*-Initiated Mutable Loci

Simple coincidence rather than a relationship with *Ds* might still be claimed for the origin and behavior of c^{m-1} if it were the only case of such origin and behavior. Two other cases, similar to c^{m-1} and involving another marked locus, have appeared independently in the *Ds*- and *Ac*-carrying plants. Both involve the locus in chromosome 9 associated with the bronze phenotype (see Fig. 2). These two independent cases have been designated bz^{m-1} and bz^{m-2}. The description of the types of events occurring at c^{m-1} may be applied also to bz^{m-1} and bz^{m-2}. *Ds*-type breakage events occur at the mutable locus, as well as mutations from *bz* to an apparently full *Bz* expression. Both the *Ds*-type breakage events and the mutations to *Bz* are *Ac*-controlled; for if *Ac* is absent, neither will occur. In these cases also, the time when mutations to *Bz* or chromosome breaks occur is under the control of the state and the dose of *Ac*. Again, as with *C*, previous investigations had shown that a homozygous deficiency of the segment of chromosome including the *Bz* locus will reproduce the known recessive phenotype, *bz* (McClintock, unpublished).

That the presence of *Ds* close to a marked locus in a chromosome may result in frequent changes in the phenotypic expression of the marker has been indicated. Two independent cases of transposition of *Ds* from its standard location to a position near and to the left of *Sh* have been studied (see Fig. 2). In these two similar cases, less than one-half of one per cent crossing over occurs between *Ds* and *Sh*. In both cases, however, many gametes are produced that carry a "spontaneous" mutation from *Sh* to *sh*. These mutations occur only in those chromosomes carrying *Ds* immediately to the left of *Sh*, and only in plants that also have *Ac*. If *Ac* is absent, no such mutations appear. *Ac*-controlled events, therefore, in each of these two cases where *Ds* is near and to the left of *Sh*, are responsible for this high frequency of mutation to *sh*. When such a mutation occurs, *Ds* is not always lost to the chromatid; it is sometimes still present between *C* and *Bz*. Some of these "mutations" may prove to be newly arisen mutable *sh* loci, but the tests for this mutability have not been concluded.

Knowledge gained from the cases reviewed has led to the conclusion that the appearance of *Ds* at or close to the locus of a known genetic

factor can give rise to frequent changes in the action of the factor. The initial change is to an action resembling that of the known recessive allele. In the cases of c^{m-1}, bz^{m-1}, and bz^{m-2}, a subsequent alteration produces a return to apparently full dominant expression of the factor. This common type of mutational expression in these cases is of considerable significance. Its importance will become evident in the discussion of the behavior of other newly arisen mutable loci.

Origin and Behavior of One Class of Autonomous Mutable Loci

The behavior of some other types of mutable loci may now be considered for the additional and important knowledge they have contributed to an understanding of the basic processes involved. One of them is called mutable luteus. The luteus character is distinguished by a yellowish chlorophyll expression. This mutable luteus first appeared in the progeny derived from self-pollination of one of the original plants that had undergone the breakage-fusion-bridge cycle in early development. It resulted from some alteration at a normal locus, but the position of the locus in the chromosome complement was not known. This mutable locus is characterized, first, by its autonomous behavior. It required no recognizable, separate activator factor in order to undergo the mutation phenomenon. The mutations are registered in sectors of a plant as changes in the amount of chlorophyll that is produced. Alleles arise from germinal mutations. They are characterized by various quantitative grades of chlorophyll expression. These alleles, in turn, need not be stable; some of them may mutate to give higher or lower levels of chlorophyll expression. Even an allele apparently producing the full dominant expression may be unstable for it may mutate to or towards the lowest expression, which is luteus.

In studying one aspect of the behavior of this mutable luteus locus, a number of sister plants in one culture were all self-pollinated. On a resulting ear of one, and only one, of these plants, the presence of a new mutable locus was revealed. The mutability, registered in some of the kernels on this ear, involved a factor associated with the formation of pigment in the aleurone layer. Colorless kernels were present in which mutations to color occurred. None of the ears produced by the sister plants showed the presence of such a mutable factor; all kernels on these ears had the full aleurone color. Further study of the plants derived from the variegated kernels on the aberrant ear showed that a mutable condition had arisen at a previously known locus, the A_2 locus in chromosome 5 (A_2, aleurone and plant color; a_2, recessive allele colorless aleurone, altered plant color). Before the appearance of the mutable condition in this one plant, the A_2 locus had given the normal dominant expression in both parent plants. It had shown no indication whatsoever of any instability. One parent had contributed mutable luteus to this culture. The mutable luteus locus, however, was not linked with the A_2 locus. It should be emphasized that this newly arisen mutable a_2 behaved in many respects like mutable luteus. It was autonomous; and quantitative alleles were produced, some of which, in turn, were mutable.

Another mutable locus arose in a culture derived from one having mutable luteus. It first appeared as a single aberrant kernel on one ear. This ear was produced from a cross in which a plant carrying mutable luteus was used as a female parent. The male parent was homozygous for the stable recessive a_1. (A_1, aleurone and plant color, located in chromosome 3; a_1, recessive allele, colorless aleurone and altered anthocyanin pigments in the plant.) This single aberrant kernel exhibited variegation. Sectors of colored aleurone appeared in an otherwise colorless kernel. Tests were initiated with the plant derived from this kernel, and continued with the subsequent progeny. From these tests it was learned that the aberrant kernel carried a newly arisen mutable a_1 locus, designated a_1^{m-1}, whose general behavior resembled that shown by mutable luteus. It was autonomous; it produced a series of alleles showing various grades of quantitative expression of anthocyanin, in both the aleurone and the plant; and of these alleles, in turn, some were unstable, mutating to give higher or lower levels of quantitative expression of the anthocyanin pigments in both aleurone and plant. Other important aspects of the mutation phenomenon at this locus will be considered later.

In the discussion of the *Ac*-controlled mutable loci, c^{m-1}, bz^{m-1} and bz^{m-2} that arose in *Ds-Ac* carrying plants, it was emphasized that the types of mutational response were similar. Here also, the mutational expressions of the two mutable loci that have arisen in the mutable luteus stocks are much alike, and they resemble that shown by mutable luteus itself. One further example of related mutable loci will be given. It also shows the similarity of behavior of the newly

arisen mutable locus to the one already present in the plant. The direction this discussion is taking may now be apparent. It is towards the conclusion that the type of mutation occurring at a locus is a function of the type of chromatin material that is present at the locus or is transposed to it, and does not involve changes in the components of the genes themselves. Rather, it is this chromatin that functions to control how the genic material may operate in the nuclear system. With this in mind, a third example of related origins and behaviors of mutable loci may now be considered.

Origin and Behavior of c^{m-2} and wx^{m-1}: Two Related Mutable Loci

The progeny derived from self-pollination of another one of the original plants that had undergone the breakage-fusion-bridge cycle in early development was grown, and a number of these plants again self-pollinated. On a resulting ear of one of these plants, a new mutable locus was recognized. The factor involved was again associated with the production of pigment in the aleurone layer. Some of the kernels on this ear showed colored areas in a colorless background. Beginning with the plants derived from these kernels, a study was made of the condition responsible for the variegation. This proved to be due to another new mutable locus, and involved the previously discussed *C* locus in chromosome 9. This locus in the parent plant and in the sister plants of the culture, gave the normal dominant *C* expression. The new mutable condition was designated c^{m-2}, because it was the second case that appeared in this study. The types of mutation that arise from events at c^{m-2} are strikingly different from those shown by c^{m-1}. A series of alleles, as expressed by quantitative grades of pigment formation associated with the production of at least two different precursor-type diffusible substances, is produced by mutations at c^{m-2}. The intermediate alleles are not always stable, for some of them, in turn, can mutate to alleles showing higher or lower grades of color expression.

In the course of the study of c^{m-2}, a number of crosses were made, using pollen of plants homozygous for c^{m-2} and carrying a normal dominant *Wx* factor in each chromosome 9, on silks of plants carrying a stable recessive *c* and a stable recessive *wx* in both chromosomes 9 (see Fig. 2). A single aberrant kernel appeared on one of the ears resulting from this type of cross. It showed mutations to *C* and of the c^{m-2} type, and, in addition, mutations from the *wx* to and towards the *Wx* phenotype. A plant was obtained from the kernel and a study commenced to determine the nature of this instability expression. It proved to be a new mutable *wx*, and was designated wx^{m-1}. The tests showed that it had arisen in the male parent plant, which carried c^{m-2} and normal dominant *Wx* in each chromosome 9. It was present, however, in only one of the many tested male gametes of this plant. The pattern of mutational behavior of wx^{m-1} strikingly resembled that shown by c^{m-2}. A series of quantitative alleles was produced by mutations of wx^{m-1} as registered by the amount of amylose starch produced. These alleles, in turn, could mutate to give greater or lesser amounts of amylose starch. Another endosperm character also was affected by some of the mutations of wx^{m-1}. This was expressed by an altered growth of the endosperm tissue, and accompanied some but not all of the mutations to the intermediate alleles, appearing particularly often in association with a mutation to one of the lower alleles. This accompanying mutation behaved as a dominant or a semidominant.

It is of particular significance, in comparison of the behavior of the series of mutable loci c^{m-1}, bz^{m-1}, and bz^{m-2} with that of the series c^{m-2} and wx^{m-1}, that the members of both series are controlled by the very same *Ac* factor—wherever it may be located—and in precisely the same manner, with respect to time and place of occurrence of mutations. When *Ac* is absent, no mutations occur in either series. In the latter series, mutations of the intermediate alleles also occur, but only when *Ac* is present. Because of this, it has been possible to isolate a series of quantitative alleles of *C*, and also a series of quantitative alleles of *Wx*, that are stable. Stability is maintained when *Ac* is removed. The percentages of amylose starch in the endosperm, produced by a number of the alleles arising from mutations at wx^{m-1} and freed of *Ac*, have been determined in terms of single, double, and triple doses of the particular allele. (Note: The writer is grateful to Dr. G. F. Sprague and to Dr. B. Brimhall, of Iowa State College, and also to Dr. C. O. Beckmann, of Columbia University, and Dr. R. Sager, of the Rockefeller Institute for Medical Research, for their chemical analyses of the amylose content produced by some of these mutants.) The preliminary tests suggest that alleles, falling into an almost continuous series with respect to the quantity of amylose starch they produce, may be obtained. It should

be mentioned here that, as with *C* and *Bz*, previous investigation had shown that a tissue homozygous-deficient for the *Wx* locus will give the known recessive *wx* expression (McClintock, unpublished).

In order to compare the action of *Ac* on the members of these two series of *Ac* controlled mutable loci, crosses were made combining several of them in a single plant so that they might be present together in the nuclei of a tissue. By this means, it was possible to determine that the mutations at these various mutable loci arise as a function of the state and dose of *Ac*, irrespective of which mutable locus is involved or how many such loci from the same series or from the two different series are present in the nuclei of an individual plant.

In further comparison of the behavior of the different *Ac*-controlled mutable loci, one very significant correlation may now be given. It is known that all these loci show one other common characteristic. At all of them, some chromosome-break-inducing events occur, but only when *Ac* is also present in the nucleus and only at those times in the development of a tissue where mutations leading to changes in expression of the respective phenotypic character are also occurring. Again, it has been determined that such breaks may occur at the locus of *Ac* itself. The conclusion that the mutation-producing events in these two series of related mutable loci, and also at *Ac* itself, are associated with such a chromosome-break-inducing mechanism is difficult to avoid. This relationship will be explored after consideration has been given to a comparison of the types of mutability that may arise at any one known locus in a chromosome.

The descriptions of mutable loci given so far in this discussion have shown that the type of mutability and the mode of its control are not alike for all. Nevertheless, there appear to be classes of mutable loci, the members of which show similar types of changes in phenotypic expression—that is, of mutations—and similar types of control of these mutations. It is now necessary to indicate the extent to which various types of mutability expression may arise at any one particular locus. For this purpose the *Wx*, the *C*, and the A_1 loci will be chosen as examples.

Comparisons of Types of Mutability Arising at any One Locus

a. The Wx locus

Six independent mutable conditions are known for the *Wx* locus. Five of them have arisen during the present study. One, $wx^{m\text{-}1}$, has been considered above. It is *Ac*-controlled, and produces quantitative alleles that may be unstable when *Ac* is present but are stable when *Ac* is absent from the nucleus. Mutations at this locus also give rise to an endosperm-growth-altering factor that is dominant in expression. The second mutable *wx*, $wx^{m\text{-}2}$, arose from a previously stable recessive *wx* carried in genetic stocks for many years. This mutable condition first appeared in a chromosome 9 in which a complex chromosomal rearrangement was present. Its mutations are expressed by different quantitative grades of the *Wx* phenotype. In the endosperm tissues, the sector produced by a cell in which a mutation has occurred is always markedly distorted in growth-type. The third mutable condition at this locus, $wx^{m\text{-}3}$, originated in a plant carrying a normal dominant *Wx* and also several mutable loci. It is autonomous, in that no separate activator factor is required for mutations to be expressed. It almost always mutates to give the full *Wx* phenotypic expression. The derived mutant giving the dominant expression is also mutable, for it produces mutants giving the full recessive expression, that is, *wx*. This recessive, in turn, may mutate again to give the full dominant expression. No altered growth conditions in the endosperm tissue accompany any of these mutations. The fourth case was recognized by a sudden change in the behavior of a previously normal *Wx* locus. It shows mutations producing various grades of quantitative expression between the full dominant and the full recessive. It is autonomous, and no alterations in growth conditions accompany the mutations. Another case, somewhat similar to the last, has recently arisen. It produces alleles giving various lowered expressions of the *Wx* phenotype, and appears to be autonomous although the information on its behavior is too incomplete to allow a full description. The sixth case is one that has been investigated by Sager (1951). It is autonomous, and gives quantitative grades of expression in the endosperm; but the germinal mutations that have been studied all give full or nearly full *Wx* expression, and the mutants are stable. No altered growth conditions appear to accompany these mutations.

Genetic analyses have indicated that all these various mutational changes occur at this one locus in chromosome 9, and yet all show a different kind of mutational behavior. It is evident that each arose in association with a particular type of alteration at the locus, and that different mutation-controlling mechanisms can be involved.

This is especially well illustrated by a comparison of the types of mutations produced by wx^{m-1} and wx^{m-3} and of their controlling mechanisms.

b. The C locus

The contrasts in the kinds of phenotypic expression produced by mutations at c^{m-1} and c^{m-2} have been discussed above. Although several other independent expressions of mutability at this *C* locus have also arisen, the study of them is too incomplete to allow detailed comparisons to be made. With respect to this locus in chromosome 9 it is necessary to mention, however, that in the cultures having mutable loci a heritable factor carried by a chromosome other than 9 has appeared on several occasions. The presence of such a factor results in the production of pigment in the aleurone when the endosperm is homozygous for the well-investigated stable recessive *c*, used for many years in genetic investigations. The pattern of pigment formation differs markedly from that produced by mutations at c^{m-1} or c^{m-2}. It resembles that associated with the factor *Bh* (Blotch), previously studied by R. A. Emerson (1921) and Rhoades (1945b). To complete the discussion of the series at this locus, it may be mentioned that a mutable condition has also arisen involving the expression of *I*, an allele of *C*. Changes in the degree of inhibitory action of *I* occur as a consequence of such mutations.

c. The A_1 locus

A study of changes at the locus of A_1 has contributed some very important information regarding the origin and behavior of mutable loci. For a number of years, a type of control of mutability of the recessive, a_1, has been known. A dominant factor, called Dotted (*Dt*), provokes mutability at the a_1 locus (Rhoades, 1936, 1938, 1941, 1945*a*). In many respects, *Dt* is comparable to *Ac*. It is an activator, for it produces mutations at a_1, just as *Ac* produces mutations at c^{m-1}, c^{m-2}, bz^{m-1}, bz^{m-2}, and wx^{m-1}. Moreover, when *Dt* is absent no mutations occur at a_1; the a_1 locus then gives a stable recessive phenotype. In the presence of *Dt*, mutations occur at a_1 to give mainly the higher alleles of the A_1 phenotypic expression. The time of occurrence of visible mutations at a_1 is usually late in the development of the plant or the endosperm tissues; and they occur in only some of the cells. This results in the presence of dots of the A_1 phenotype. The *Dt* factor has been located by Rhoades in the knob region terminating the short arm of chromosome 9. *Dt* and *Ac* appear not to be the same activator, as plants and endosperm tissues that are homozygous for the recessive a_1 have not shown the dotted-type mutations to A_1 in the presence of *Ac*.

In the early period of this investigation of newly arisen mutable loci, the unexpected appearance of modifications in the knobs, and in the other chromosome elements previously mentioned, of plants that had undergone the breakage-fusion-bridge cycle in their early development, suggested that disturbances in these elements might have been responsible for the initial burst of mutable loci, including the origin of *Ac* itself. It was suspected, therefore, that this cycle might induce alterations in the heterochromatic elements that could initiate a *Dt* factor as they may have originated the *Ac* factor. Once initiated, this factor would activate a_1 to undergo alterations in somatic cells leading to A_1-type expression. The most direct way to induce changes in the heterochromatic elements was considered to be the breakage-fusion-bridge cycle itself. By subjecting tissues to this cycle during their developmental periods, and then examining the matured tissue, this hypothesis could be tested. A preliminary experiment, designed to test for production of mutations of a_1 to A_1 by the breakage-fusion-bridge cycle as an inductor, was performed in 1946. The experiment was repeated in 1950 on a much larger scale. Because this experiment was of particular significance in revealing the mode of origin of mutable conditions, and because it provided evidence about the relation of chromosome organization to genic expression and its control, the details will be given.

The silks of plants homozygous for a_1 and carrying no *Dt* factor received pollen from plants of similar constitution with respect to a_1 and *Dt*. The pollen parents carried one chromosome 9 with a duplicated segment of the short arm. The homologous chromosome 9 was deficient for a terminal segment of the short arm. Newly broken ends of chromosome 9 were produced in some meiotic cells, as diagrammed in Figure 1. Pollen grains of these plants carried either: (1) a deficient chromosome 9, which did not function in pollen-tube growth, (2) a chromosome 9 with a full duplication of the short arm—that is, the homologous chromosome 9, or (3) a chromosome 9 with a newly broken end. Among this last type, duplications or deficiencies of the short arm were present. Those carrying an extensive deficiency were nonfunctional but those carrying a relatively short duplication were better able to compete in functioning than those carrying the full duplication of the short arm. Thus the

majority of the functioning pollen grains of these plants carried a newly broken end of the short arm of chromosome 9 in their nuclei. These chromosomes had undergone the breakage-fusion-bridge cycle since the meiotic anaphase and continued to do so after being incorporated into the primary endosperm nucleus. Either before fertilization or during the development of the kernel, the breakage-fusion-bridge cycle might produce alterations in heterochromatic elements and some of them might include an alteration that would recreate the condition associated with *Dt* action. Mutations at the a_1 locus to give the A_1 phenotype could subsequently appear in the descendant cells. If this should occur early in development of the endosperm, a sector with dots of the A_1 phenotype would be produced. Examination of 95 ears resulting from the preliminary test conducted in 1946 revealed A_1 dots on 15 kernels from 14 different ears. In five of these kernels, more than one A_1 dot was present, and in one restricted region of the kernel in each case. The number of kernels with mutations to A_1 in this trial experiment was lower than anticipated, and the experiment was not expanded the following year. Later, as the probable relation between the origins of mutability and the alterations induced in the knobs or other chromosome elements by the breakage-fusion-bridge cycle became more clearly apparent, the same experiment was conducted in 1950 on a much larger scale. The results were rewarding, for now many kernels were obtained that had one or more A_1 dots. One hundred and twenty such kernels appeared in this second trial, and 24 of them had more than one A_1 spot. One of these kernels had 84 A_1 spots, distributed rather evenly over the kernel. In the other 23, the spots were not distributed at random over the aleurone layer but were restricted to well-defined sectors. In none of these kernels did any large areas of the A_1 phenotype appear. In all cases, the time of mutation, the pattern of mutation, and the type of mutation were much like those produced when the known *Dt* factor is present in endosperms homozygous for a_1. It was obvious that in each case the initial alteration had occurred in the ancestor cell that produced the dotted sector. This initial event was responsible for mutations that occurred at a_1 in some cells during the subsequent development of the endosperm. The observed mutations at a_1, therefore, were not produced directly by the breakage-fusion-bridge cycle but arose secondarily, as a consequence of an event that altered some particular component in the nucleus. It was the alteration of this component that was responsible for the subsequent mutations at the a_1 locus. And this initial alteration was one that imitated the effect produced when the known *Dt* factor is present. It is difficult to avoid the conclusion that a new *Dt*-like factor has been produced in each such case, and that it was created by some event associated with the breakage-fusion-bridge cycle. Unfortunately, the plant grown from the one kernel having 84 dots distributed over the whole aleurone layer did not show any mutations to A_1, nor did mutations appear in the kernels when this plant was crossed to plants homozygous for a_1. The effective alteration probably was present in only one of the two sperms carried in the pollen grain. Because the break in chromosome 9 that initiated the breakage-fusion-bridge cycle was produced at the meiotic anaphase, the event giving rise to the *Dt*-like factor would have had to occur in the subsequent microspore division in order to be incorporated in the two sperm nuclei. An even larger experiment of this same type must be conducted if such a case is to be obtained. It should be mentioned in this connection that the size of the sectors within which A_1 spots appeared graded from large to small, the smaller sectors being most frequent. Also, about three-fifths of the kernels showing mutations to A_1 had only one A_1 spot. These frequencies are to be expected if the creation of a *Dt*-like factor is a consequence of an event, associated with the breakage-fusion-bridge cycle, that has a probability of occurrence in a limited number of mitotic cycles. In order to indicate why the dotted pattern of mutations to A_1 is to be anticipated, rather than any other, it is necessary to review the origin of the previously discovered $Dt - a_1$ mutable condition.

The $Dt - a_1$ mutable condition first appeared on one ear after self-pollination of a plant belonging to the commercial variety known as Black Mexican Sweet Corn. This variety is homozygous for A_1. The recessive a_1 in this case represented a new mutation from A_1, and was associated with the appearance of *Dt*. The original a_1 mutant, known for many years and used in genetic studies, had originally been found to be present in a commercial variety of maize. Both a_1 mutants responded in much the same manner when *Dt* was present in the nucleus. In both cases, the dotted mutation pattern was produced in the presence of *Dt*. The states of the two a_1 mutants thus appeared to be alike. This suggests that the older a_1 mutant may

have been produced by a mechanism similar to that responsible for the origin of the newer a_1 mutant. A *Dt* factor may have arisen at the same time but subsequently been lost from the commercial variety during its propagation, leaving an apparently stable a_1 mutant. The change at *C* that produced c^{m-1} would have behaved quite comparably had *Ac* been absent from the nuclei in the initial gamete carrying c^{m-1}, or had it been removed by crossing before the change at this locus had been detected. If the mutation had been discovered several generations after its origin, and if *Ac* had been removed by a previous cross, it would have appeared to be a newly arisen, stable, recessive *c*. Only after an incidental cross to a plant carrying *Ac* would its potential mutability have been revealed. It is possible, therefore, that many known recessives may prove to be potentially mutable.

The essential similarity of the $Dt - a_1$ system to the $Ac - c^{m-1}$, etc. system is also expressed in the changes in state of a_1 that may occur in the presence of *Dt*. Such changes in state of a_1 have recently been described by Nuffer (1951). They are recognized individually by marked departures in frequency of visible mutations, in types of mutation and in time of occurrence of these mutations. The types of different phenotypic expression produced by mutations at altered states of a_1 are much the same as those produced by a mutable a_1 locus that has appeared in the Cold Spring Harbor cultures. This new mutable a_1 locus, called a_1^{m-1}, differs from the mutable a_1 studied by Nuffer in that it is autonomous and does not require *Dt* for mutability to be expressed. In this respect, mutability at the A_1 locus behaves like that at the *C* and the *Wx* loci, for both autonomous and activator-controlled mutable conditions may arise.

The origin of a_1^{m-1}, in a culture carrying mutable luteus, was described previously. It is autonomous, and produces a series of quantitative alleles, many of which are unstable in that they may mutate to give higher or lower levels of quantitative expression. Difference in degree of quantitative expression is only one of the consequences of mutations occurring at a_1^{m-1}, however. The diversity of phenotypic changes arising from these mutations is so great that an adequate analysis of all the observed types is a large task. They are distinguished not only by quantitative but also by qualitative differences in the anthocyanin pigments formed. Diversity is shown in other respects. For example, some of the mutations giving pale aleurone color are related to changes involving the rate of a particular reaction responsible for pigment formation. Others appear to be related to the absolute amount of pigment that may be produced, regardless of a time factor. This becomes evident when comparisons are made of pigment-forming capacities in plants arising from kernels carrying different mutants of a_1^{m-1}, each producing a pale color in the aleurone of the kernel. In some cases, such plants are pale in their expression of anthocyanin color throughout their lives. Others are pale in anthocyanin color up to the time of anthesis, when growth of the plant terminates; but in the six or seven following weeks, as the plants mature their ears, the anthocyanin color gradually deepens, becoming intensely dark by the time the ears are mature. The kernels derived from both these two types of plants, however, may be equally pale. The fact that pigment forms late in the development of the kernel, and dehydration of the tissues occurs shortly thereafter, may explain this similarity in color of the kernels.

Other types of mutation occur at a_1^{m-1}. Some produce sectors of deep color that are rimmed by areas in which the color gradually fades off to colorless, as if a diffusible substance associated with pigment formation had been produced in excess in the mutant sector. The area of diffusion may be very extensive for some mutations, and only slight for others, whereas still other mutations give rise to no such diffusion areas at all. Some of the mutations that result in strong A_1 pigmentation are associated with failure of development or degeneration of some of the aleurone cells within the mutated sector.

Besides the mutational changes at a_1^{m-1} that affect the type and amount of pigment formation, a number of other changes occur which affect the subsequent behavior of a_1^{m-1}. These alterations are termed changes in state, since they affect not only the times when pigment-forming mutations will occur at the locus in future plant and endosperm cells but also the kinds and frequencies of such mutations, and their distributions and their sequences in the development of the tissues.

Any interpretations that attempt to explain the primary action of a specific locus in a chromosome, and how this action may be changed, must take into consideration the facts just enumerated concerning the behavior of this a_1^{m-1} locus. It is not reasonable to regard such changes in expression and action as being produced by changes in a single gene—that is, according to the usually accepted concepts of the gene that have

been developed. The evidence suggests, rather, that the observed changes result either from alterations at a locus that has many individual components or from alterations at the locus affecting its relationship to other loci in the chromosome complement. If the latter is true, a combination of loci functions as an organized unit in the production of pigmentation. If such functional organizations exist within the nucleus —and it is reasonable to assume they do—then the large numbers of alleles known to arise at certain loci need not express altered genic action at the identified locus. Rather, any one alteration may affect the action of the organized nuclear unit as a whole. The mode of functioning of various other loci concerned may thereby be modified. In other words, the numerous different phenotypic expressions attributable to changes at one locus need not be related, in each case, to changes in the genic components at the locus, but rather to changes in the mechanism of association and interaction of a number of individual chromosome components with which the factor or factors at the locus are associated. According to this view, it is organized nuclear systems that function as units at any one time in development. In this connection it may be repeated that at a_1^{m-1}, and also at other mutable loci, many of the alterations observed represent changes in the potential for patterns of genic action during development (changes in state). Thus a pattern-controlling mechanism is being altered. If particular nuclear components are formed into organized functional nuclear units, the evidence would suggest that this may happen only at prescribed times in the development of an organism. In this event it may readily be seen that changes in pattern-controlling mechanisms would serve as a primary source of potential variability of genic expression without requiring any changes in the genes themselves.

A few more pertinent facts about the A_1 locus may now be mentioned. Mutability has arisen at this locus independently on a number of different occasions. Several cases have recently appeared in the stocks having *Ds* and *Ac*. Analysis of these cases has not proceeded to a stage where a complete description of their behavior may be given. Both Laughnan (1950) and Rhoades (1950) have found new cases of instability at this locus. Several have appeared in plants derived from kernels that had been aged for some time (Rhoades, 1950). Such aging is known to give rise to chromosomal aberrations as well as mutations; the observed instability may be an expression of one such structural change. Laughnan (1949, 1951) has shown that mutations at the A_1 locus may be associated with the mechanism of crossing over, suggesting again that mutations arise from structural changes at the locus. The crossover studies may elucidate the nature of some of these changes. Not only the cases described in this report but also others have produced evidence converging in support of a hypothesis that mutations originate from structural alterations in chromosome elements. The evidence derived from a study of progressive changes in state of c^{m-1} has shown the close relation between a structural change at a locus and one that so often has been called a "gene mutation." This study will now be described.

Significance of Changes in State of a Mutable Locus: Selected Examples

The foregoing review of the very different types of phenotypic expression that may be produced as a consequence of mutations arising at any one locus, and of the relation between the origin of a mutable condition and the type of mutations expressed, clearly indicates the necessity for caution in attempting to interpret the mutation process as one associated with a "change in a gene." With respect to events occurring at *Ac*, c^{m-1}, c^{m-2}, bz^{m-1}, bz^{m-2}, and wx^{m-1}, it has been established that a mechanism capable of producing chromosomal breaks at the locus is associated with the mutation-producing process. It could be argued from the evidence so far presented that these cases fall into a special category, and that what they may indicate regarding the mechanism of mutation at these mutable loci may not be used to interpret the mutation process in general. Knowledge gained from a study of the changes in state of c^{m-1} has shown, however, that no line may be drawn between those events at a locus that produce detectable chromosomal alterations and those that give rise to mutations but produce no readily detectable chromosomal alterations.

The origin of c^{m-1} by a transposition of *Ds* to the normal *C* locus has been discussed above. The state of *Ds*, when first transposed to the *C* locus, was one that produced many detectable chromosome breaks at this locus and few mutations to *C*. When plants having this state of c^{m-1} were crossed to plants that were homozygous for the stable recessive *c*, the majority of the resulting kernels showed this relationship. Some of these kernels, however, had sectors with higher rates of mutation to *C* and lower rates of detectable

chromosome breaks. In some sectors, no chromosome breaks were evident; but often a high frequency of mutation to *C* had occurred. There were also a few kernels on these ears that had this pattern throughout the kernel. When found, such kernels were selected from the ears (as well as others that showed changed mutational patterns). The plants grown from them were again crossed to plants homozygous for the stable recessive *c*. The kernels on the resulting ears now showed the types and frequencies of the different detectable events at c^{m-1} that had been observed in the kernel from which the plant had arisen. It was possible to determine in this manner that a heritable change had occurred at c^{m-1}. It was this change which was responsible for the altered frequencies of expression of the detectable events that subsequently occurred at this locus. That the altered response, in each isolated case, arose from a change at c^{m-1} and was not produced by a change at *Ac*, was determined by testing the responses of these c^{m-1} isolates with different isolates of *Ac*, each having a known type of action. *Ac* controlled, in each test, the time and place of the event occurring at c^{m-1}, but not its type. Because, in each case, the change in behavior of c^{m-1} was heritable, it must have arisen by an event that produced an alteration at this locus. It is this heritable altered condition that has been termed an altered state of the locus.

The various altered states of c^{m-1}, as previously mentioned, arise only when *Ac* is also present in the nuclei, and only at the times in development when mutations or chromosome breaks may occur at c^{m-1}. For our purposes, the most instructive of the changed states are those giving reciprocal frequencies of chromosome breaks and mutations to *C*. A series of isolates, each showing a particular relation between these two events at c^{m-1}, has been studied. The isolates ranged from those showing no mutations to *C* or only a very occasional one, but having a very high frequency of detectable chromosome breaks, to those showing a high frequency of mutations to *C* and no detectable breakage events or only an occasional one. The states of c^{m-1} that give high frequencies of chromosome breaks are unstable, for other altered states may be produced as a consequence of events at the locus, but only, as emphasized above, when *Ac* is also present in the nucleus. A particular state of c^{m-1} remains constant if maintained in plants having no *Ac*. The state of c^{m-1} giving no detectable chromosome breaks, and a correspondingly high frequency of mutations to *C*, is very stable with respect to the absence of breakage events. Had the state of c^{m-1} been of this type when it first arose, there would have been no opportunity to discover that the chromosome-break-producing mechanism and the mutation-producing mechanism were related. If chromosome breaks are not exhibited by a mutable locus, therefore, it cannot be argued that because of this the basic mechanism producing the mutations must be different from that known to operate at c^{m-1} and at the other *Ac*-controlled mutable loci. The evidence obtained from this study of the origin and subsequent behavior of altered states of c^{m-1} argues, rather, for similarities if not identities in the basic mechanism.

Another type of change in state of c^{m-1} should be mentioned, although it occurs infrequently. It is detected by a much altered expression of the mutation at this locus that affects aleurone color. The frequency of origin of such states of c^{m-1} is so very low as to suggest that they represent entirely new modifications at this locus, comparable to the original inception of a mutable condition at any locus. They may well represent just such new inceptions, for these are to be expected in view of the fact that very different types of mutational behavior are exhibited at this same locus by c^{m-1} and c^{m-2}, both of which are *Ac*-controlled.

All the *Ac*-controlled mutable loci exhibit changes in state. These are characterized by changed relative frequencies of the different recognizable types of mutations that occur. In other words, as we have already seen, the types of mutation produced in *Ac*-controlled mutable loci are related to the state of the mutable locus itself. The time and place during development of occurrence of mutations, on the other hand, are controlled by the state and dose of *Ac;* and therefore alterations in them are related to changes in state of *Ac* rather than to changes in state of the mutable locus. The changes in state of *Ac* have been described. The autonomous mutable loci also undergo changes in state, as described previously for the a_1^{m-1} locus. In this group, however, the controller of the time and place of occurrence of mutations is a component of the locus itself. Consequently, the changes of state that arise at these loci are reflected in changes in the control of time and place of mutations as well as in the type or types of mutation that may occur, and also in the time and place of occurrence of each such mutation if several types are produced. Thus there is a much more diverse

group of altered states associated with changes at any particular locus in the autonomous group. The general similarities between the autonomous and the *Ac*-controlled mutable loci are nevertheless striking.

Extent of Instability of Genic Expression in Maize

The discussion so far has mentioned a number of different mutable conditions at known loci in maize. In order to show that the phenomenon is much more prevalent than the particular cases described would indicate, some additional observations of changes in genic expression may be discussed briefly. Not only have *C*, *I*, *Bz*, *Wx*, A_1, and A_2 become mutable during the course of these studies, but instability of other known dominants has been noted. These are *R*, *Pr*, *Yg*, *Pyd*, *Y*, and possibly *B* and *Pl*, although the evidence for the last two is observational and not genetic. Some previously unknown dominant loci have also become unstable. These are associated with various chlorophyll-determining factors, endosperm-starch-controlling factors, aleurone-color factors, growth-controlling factors, etc. Instability has arisen at the loci of some of the known recessives such as *wx*, *yg*, and the special case a_1 described previously. Instability at the loci of the recessives *y* and *p* also appears to have arisen on several independent occasions. Genetic tests were not made, however, to determine the association of the instability with the known loci. Instability arising at recessive loci is recognized by mutations to or towards the expression of the dominant allele. It may be concluded, therefore, that many of the known recessive alleles are potentially capable of expressing action that is characteristic of the dominant alleles.

The expression of instability of various factors in maize is probably far more common than has been suspected in the past. Until recently, only a few such cases had been reported in the literature. One of the earliest recognized was that occurring at the *p* locus (pericarp and cob color, chromosome 1), studied by R. A. Emerson and his students (for literature citations, see Demerec, 1935) and recently being studied by Brink and his students (1951, and personal communication) and by Tavcar (personal communication). Reported cases of instability at the a_1 and the *wx* loci have been mentioned previously in this discussion. In addition, Rhoades (1947) has studied instability at the *bt* locus (brittle endosperm, chromosome 5) and has reported two cases involving chlorophyll characters that appeared in the progeny of irradiated seed (Rhoades and Dempsey, 1950). Fogel (1950) has been investigating instability at the *R* locus (aleurone, plant, and pericarp color, chromosome 10); and Mangelsdorf (1948) has reported instability at the *Tu* locus (Tunicate, chromosome 4). It is believed that critical examination will uncover many such cases in maize, and that they will involve many different loci.

Mutable Loci and the Concept of the Gene

It will be noted that use of the term gene has been avoided in the foregoing discussion of instability. This does not imply a denial of the existence within chromosomes of units or elements having specific functions. The evidence for such units seems clear. The gene concept stems from studies of mutation. That heritable changes affecting a particular reaction, or the development of a particular character, in an organism arise repeatedly and are associated with a change of some kind occurring at one specific locus or within one specific region of a chromosome, has been established. This knowledge has been responsible for the development of a concept requiring unitary determiners. It cannot be denied, in the face of such evidence, that certain loci or regions in the chromosomes are associated in some manner with certain cellular reactions or with the development of particular phenotypic characters. This is not the major questionable aspect of current gene concepts. The principal questions relate to the mode of operation of the components at these loci, and the nature of the alterations that affect their constitution and their action. Within the organized nucleus, the modes of operation of units in the chromosomes, of whatever dimensions these may be, and the types of change that may result in specific alterations in their mode of action, are so little understood that no truly adequate concept of the gene can be developed until more has been discovered about the function of the various nuclear components. The author agrees with Goldschmidt that it is not possible to arrive at any clear understanding of the nature of a gene, or the nature of a change in a gene, from mutational evidence alone. At present, the most we know about any "gene mutation" is that a heritable change of some nature has occurred at a particular locus in a chromosome, and that any one locus is somehow concerned with a certain chemical reaction, or with a certain restricted phenotypic expres-

sion, or even with the control of a complex pattern of differentiation in the development of a tissue or organ. The various types of known mutation, each showing unitary inheritance, obviously reflect various levels of control of reactions and reaction paths. It is necessary to consider these various widely different levels of unitary control and how they may operate in the working nucleus, and also to consider the nature of the changes that can affect their operation. It is with the nature of such heritable changes, the conditions that induce them, and their consequences, that this report has been concerned. Various levels of unitary control, as witnessed by inheritance behavior, are evident from the study. That genes are present in the chromosomes and that they function to produce a specific type of reactive substance will be assumed in this discussion, even though such a restricted assumption may prove to be untenable. The knowledge gained from the study of mutable loci focuses attention on the components in the nucleus that function to control the action of the genes in the course of development. It is hoped that the evidence may serve to clarify some aspects of gene action and its control. Some of the interpretations of the author, based on this evidence, have been stated or implied at various points in the previous discussion, and may now be summarized.

The primary thesis states that instability arises from alterations that do not directly alter the genes themselves, but affect the functioning of the genic components at or near the locus of alteration. The particular class to which a mutable locus belongs is related to the particular kind of chromatin substance that is present at or near the genic component in the chromosome. It is this material and the changes that occur to it that control the types and the rates of action of the genic components. Thus the basic mechanism responsible for a change at a mutable locus is considered to be one that is associated with a structural alteration of the chromatin materials at the locus. The mechanism that brings about these changes is related to the mitotic cycle; and it may involve alterations of both sister chromatids at the given locus. Some of these alterations may immediately result in the expression of an altered phenotype, a "gene mutation." Others produce modifications controlling the type of events that will occur at the locus in future cell and plant generations. Still others produce changes of a more extensive type, such as duplications and deficiencies of segments of chromatin in the vicinity of the locus. With regard to these conclusions, the evidence presented in the discussion of changes in state of c^{m-1} may be recalled.

Behavior and Action of *Ac* in Cell and Plant Generations

The interpretations given above deal with the organization and the kinds of events that occur at genetically detectable loci in the chromosomes. The next level of consideration deals with mode of operation of the nucleus in controlling the course of events during development. Do these studies suggest a mode of operation, or at least one component in the operative system? It is believed that the behavior of *Ac* may be of importance in such a consideration. With reference to c^{m-1}, for example, it has been shown that mutations to *C* occur at particular times and places in development, under the control of the particular state and dose of *Ac*. It has also been shown that *Ac* is unstable, for changes arise affecting its dosage action, and changes also occur in its location in the chromosome complement. It has not been explained, however, that the time of occurrence of these changes at *Ac* during development is also a function of the particular state and dose of *Ac* that is present in a tissue. With any particular *Ac*, and any particular dose of this *Ac*, or with any combination of different isolates of *Ac*, the time when these events will occur to *Ac* is a function of the single or combined action of the *Ac* factors. An example of this may now be given.

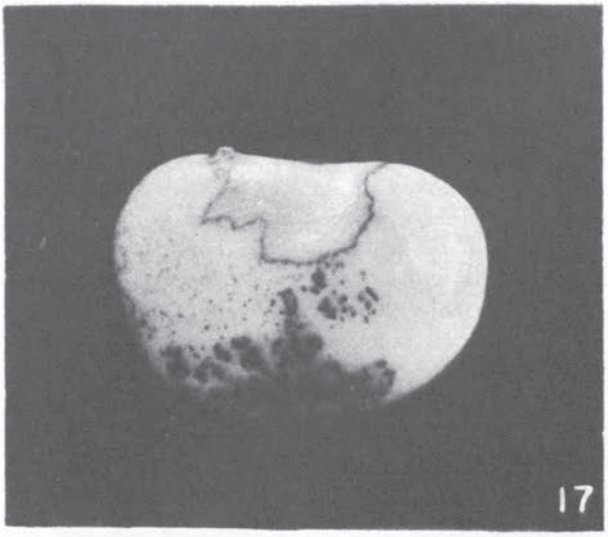
One plant having an *Ac* factor at the same location in each homologue of a pair of chromosomes, and carrying *I*, *Sh*, *Bz*, *Wx*, and *Ds* (standard location) in both chromosomes 9, was crossed to a number of plants homozygous for *C*, *sh*, *bz*, and *wx* and carrying no *Ds* or *Ac*. On all the many ears resulting from these crosses, approximately 90 per cent of the kernels were sectorial with reference to the time of occurrence of *Ds* events. Pollen from this same plant was placed on silks of plants having other combinations of markers in chromosome 9, and similar types of sectorial kernels appeared. The sectoring was produced by segregations, occurring in the earliest nuclear divisions of the endosperm, that involved the controller of the time of occurrence of *Ds* events, that is, the Activator. The action of *Ac* in these different sectors resembled that occurring either (1) when no *Ac* is present, (2) when a sharply decreased dose of *Ac* is present, or (3) when a sharply increased dose of *Ac* is present. Illustrations that will make this relation-

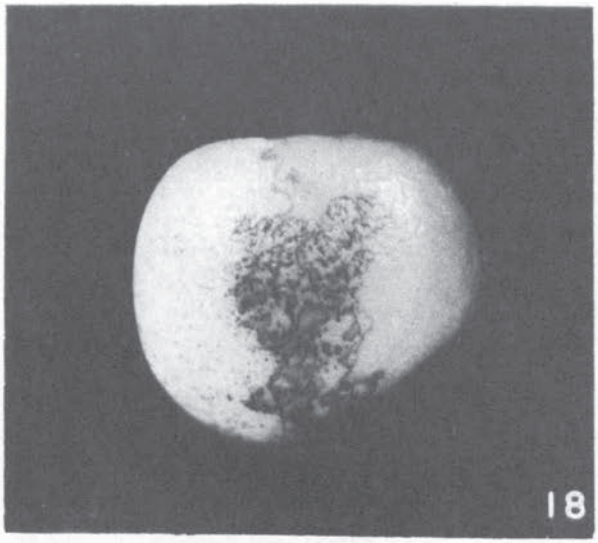
ship clear are shown in the photographs of Figures 17 to 22. In a definite fraction of the cases, a chromosome break at *Ds* was associated with segregations of *Ac* action. The mechanism responsible for this precise, somatically occurring segregation for *Ac* action was very likely the same as that responsible for the origins of germinal changes in action of *Ac*, as well as changes in its position in the chromosome complement. Such changes have been mentioned earlier in this report. It can be seen that if this same type of segregation occurred within some cells early enough in the development of a plant to be incorporated in a microspore or megaspore nucleus, the altered state or location of *Ac*, or both, would be recoverable in the plant that subsequently resulted from the functioning of the male or female gametophyte arising from such a spore. Such an early-timed event would allow for isolation and subsequent study of the transpositions and changes in state of *Ac*.

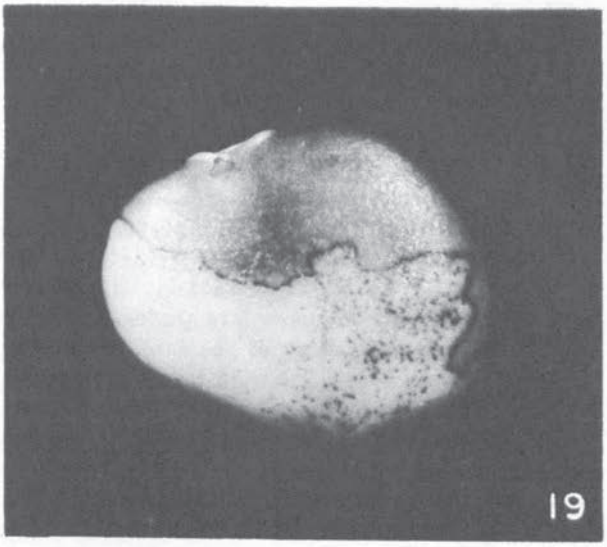
A study was initiated to determine the nature of the changes that occur at *Ac* by an analysis of the *Ac* constitutions in the gametes of plants having an *Ac* factor at the same location in each member of one pair of chromosomes, that is, in plants homozygous for *Ac* in allelic positions. In these plants the *Ac* factors were alike in state, and the homozygous condition was produced by self-pollination of plants having one *Ac* factor. Both chromosomes 9 in these plants carried the stable factors *c*, *sh*, and *Wx*. No *Ds* was present in these chromosomes. To test for *Ac* inheritance, these plants were crossed by plants having no *Ac* but carrying *C*, *Sh*, *wx*, and *Ds* in both chromosomes 9 (standard locations of *Ds* and identical states). If no changes had occurred to *Ac*, all the kernels on the resulting ears should be variegated. Similar variegation patterns, produced by sectors showing the *c*, *sh*, and *Wx* phenotype, should be present, because *Ac* would initiate chromosome breaks at *Ds* in the *C*, *Sh*, *wx*, *Ds*-carrying chromosome 9 contributed by the male parent. With the exception of a few kernels, just such conditions were realized on these ears. A photograph of one such ear appears in Figure 23. It will be noted that the majority of kernels on this ear show very similar patterns of variegation. A few kernels that differ from the majority are completely colored, with no colorless sectors of any size. In them, no *Ds* breaks at all occurred. A few other atypical kernels show an altered timing of the breakage events at *Ds*. In them such events occurred either much earlier or very much later in development of the endosperm.

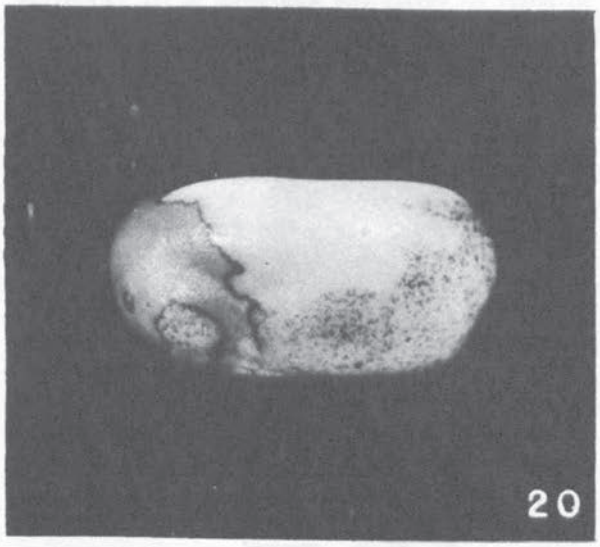
A study was made of the *Ac* constitution in plants derived from selections of all the different types of kernels appearing on such ears. In the plants coming from the kernels showing altered variegation patterns, it was necessary to determine the subsequent behavior of *Ac*; and in the plants coming from the nonvariegated kernels it was necessary to determine the presence or absence of *Ac*, and the presence or absence of *Ds* in the *C*, *Sh*, *wx*-carrying chromosome. The results of this study may be summarized. In the plants derived from the majority class of kernels, a single *Ac* factor was present. Its state was similar to that present in the parent plant (more than 25 cases studied). The *Ac* constitution in the plants derived from the nonvariegated kernels was most instructive. In 19 plants, no *Ac* was present. In 17 plants, two nonlinked *Ac* factors were present. In six plants, an *Ac* factor inherited as a single unit was present, but it gave a dose action equivalent to two doses of the *Ac* factor in the parent plant. In the plants derived from kernels that showed very late-occurring *Ds* events, either two nonlinked *Ac* factors were present (5 cases), or a single *Ac* factor was present giving a dose action greater than that of the *Ac* factor in the parent plant (3 cases).

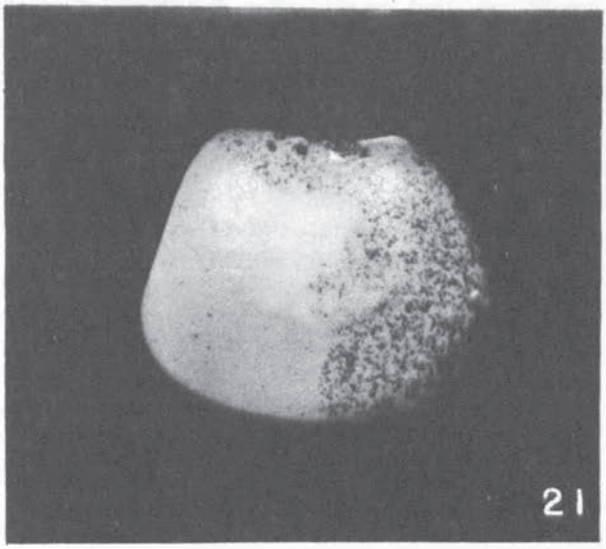
FIGS. 17 to 22. Photographs of kernels illustrating the somatic segregations of *Ac* that may occur very early in the development of a kernel. These kernels arose from the cross of plants (♀) carrying *C*, *bz* and no *Ds* in each chromosome 9 and having no *Ac* factor, by plants carrying *I*, *Bz*, and *Ds* (standard location) in chromosome 9 and also carrying *Ac*. For phenotypes expected from breaks at *Ds*, see descriptions accompanying Figures 10 to 15. In Figures 17 and 18 there are 4 large sectors in each kernel: one is *C bz* (above in Figure 17, to right in Figure 18), one is *I*, non-variegated (to right in Figure 17, upper left in Figure 18), one is characterized by late occurring *Ds* breaks, producing speckles of the *C bz* genotype (left in Figure 17, lower left in Figure 18), and one shows that numerous *Ds* breaks occurred earlier in the development of the kernel (lower segment in Figure 17, middle segment in Figure 18). The kernel in Figure 19 has 3 sectors: one that is *C bz*, one with few specks of the *C bz* genotype and one with many specks of the *C bz* genotypes. Figure 20 shows a kernel with 3 sectors: one that is *C bz*, one that is wholly *I Bz* and one having many specks of the *C bz* genotype. Figure 21 shows a kernel with two sectors: one with many specks of the *C bz* genotype and one with few such specks. Figure 22 shows a kernel with five sectors: a large *C bz* sector (lower left), a large sector having many specks of *C bz* (upper), a large sector showing many larger *C bz* areas (upper right), a small sector with few *C bz* specks (middle), and a sector of *I Bz* with no *C bz* specks (lower right).

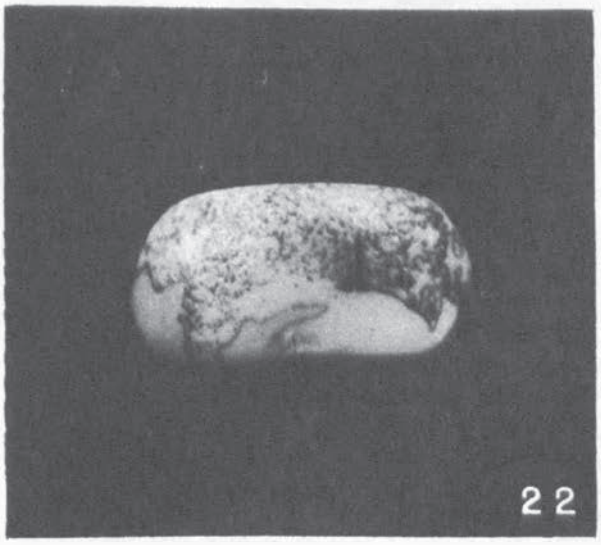

17


18


19


20


21


22

From this analysis it is clear that all the aberrant kernels on ears of the type shown in Figure 23 were produced because of some alteration of *Ac* that had occurred in cells of the parent plant. The reason that no *Ds* breaks were detected in some of these kernels is related either to the absence of *Ac* in the endosperm or to the presence of a marked increase in the dose of *Ac*. It will be recalled that the female parent contributed two gametophytic nuclei to the primary endosperm nucleus. If each nucleus carried two *Ac* factors, or a single *Ac* factor with a double-dose action, the endosperm would have either four *Ac* factors, or two *Ac* factors equivalent in action to four *Ac* factors. In such kernels, the high dose of *Ac* so delayed the time of occurrence of *Ds* breaks that none took place before the endosperm growth had been completed.

In order to verify the analyses of *Ac* constitution in some of these cases, tests were continued for another generation. For example, if a plant contains two nonlinked *Ac* factors, the gametic ratios approach 1 two-*Ac* : 2 one-*Ac* : 1 no-*Ac*—that is, a three-to-one ratio for the presence of *Ac*. On ears derived from crosses in which such plants are used as male parents, the kernels with one or with two doses of *Ac* may be distinguished because of clearly seen differences in the time of response of *Ds* to *Ac* doses. Therefore, some of the kernels considered to have two *Ac* factors and others considered to have only one *Ac* factor were selected from the test ears. The plants grown from them were again tested for gametic ratio of *Ac*. In each case, verification was realized. The gametic ratios produced by the latter plants approached 1 one-*Ac* : 1 no-*Ac*, whereas those produced by the former approximated 3 with one or two *Ac* factors to 1 with no *Ac*.

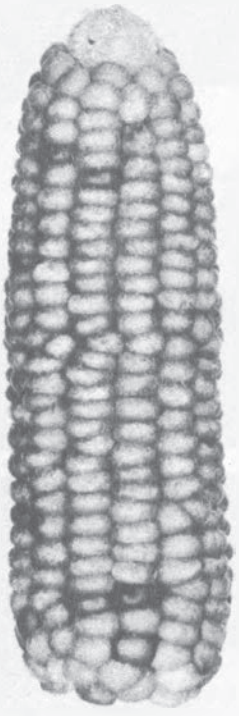


FIG. 23. Photograph of an ear derived from a plant having two identical *Ac* factors located at allelic positions in an homologous pair of chromosomes. This plant carried *c* in each chromosome 9. The ♂ parent, having no *Ac*, introduced a chromosome 9 with *C* and *Ds* (standard location). The majority of the kernels are similarly variegated for sectors of the *c* genotype due to breaks at *Ds* that occurred in the *C Ds* chromosome during the development of the kernels. Note the few fully colored kernels in which *c* sectors are absent, and also the several kernels that show large sectors of the *c* genotype.

The above-described series of tests, and still others that have been concerned with the time and type of changes occurring at *Ac*, have made it possible to understand the nature of its inheritance patterns. It has been found that, with any particular state or dose of *Ac*, the time of occurrence of changes of *Ac* is controlled by *Ac* itself. If, with a particular *Ac* state, the time of such changes is delayed until late in the development of the endosperm, then all the kernels should show this same late timing. This is known to occur with many of the isolates of *Ac*. As the photograph in Figure 23 has shown, however, a few aberrant kernels may be present on some of these ears. Some internal or external alteration in environmental conditions may have caused these few early-occurring changes at *Ac*. No attempts have been made, however, to study conditions that might alter the time of such changes.

If these tests for determining the inheritance behavior of *Ac* had not been made, considerable confusion might have arisen. This would certainly have been true had states of *Ac* giving relatively early changes been used in the initial inheritance studies. It must be stated that just such a situation has been observed. States of *Ac* giving aberrant gametic ratios have arisen. It is now realized that this is to be anticipated. It has been determined that the reason for the difference in patterns of inheritance between an *Ac* isolate that gives clear-cut mendelian gametic ratios and one of its modified derivatives, that gives aberrant gametic ratios, is related to the time in the development of the sporogenous or gametophytic cells at which such changes in *Ac* arise. With reference to the gametic constitutions that

will be produced, the time when these changes occur is most critical. If they occur in somatic divisions before the meiotic mitoses, or in the male or female gametophytes, an apparently unorthodox inheritance pattern for *Ac* will result. If they occur late, that is, in the endosperm tissues—which act in this connection like a continuation of the development of the gametophyte—then no such confused pattern of inheritance will arise. The gametic constitutions will then closely approximate those predicted for mendelizing units. In the study of *Ac* inheritance, it was necessary to make selections for these latter states of *Ac*. A few exceptions with regard to the time of changes at *Ac* may occur in some cells, even with such selected states of *Ac*. It was the analysis of *Ac* constitutions in plants derived, in cell lineage, from those cells in which such exceptional timing of changes at *Ac* had occurred, that provided the information leading to appreciation of the somatic origins of altered states and locations of *Ac*.

Confused patterns of inheritance behavior of mutable loci have been described in the literature many times. The ratios obtained have often been so irregular that no satisfactory formulation of the nature of the inheritance patterns could be derived. This would be just as true of some of the autonomous mutable loci in maize if attention had not been given to altered states and their behavior. Two examples may illustrate this. Both a_1^{m-1} and a_2^{m-1}, when first discovered, produced many mutations and changes in state very early in the development of the plant. The plant, therefore, was sectorial for the altered conditions at these mutable loci. The sectors were present in the tassel. When pollen was collected without reference to the sectors present, and placed on the silks of plants carrying the stable a_1 or a_2 alleles, the kinds of kernels appearing on the resulting ears, and their frequencies, were not readily analyzable in terms of mendelian ratios. No such difficulty arises, however, when similar tests are made for gametic ratios in plants derived from those kernels on the original test ears that show only very late-occurring mutations. The inheritance pattern is now of the obvious mendelian type, for mutations and changes in state are mainly delayed until after meiosis and gamete formation. As with *Ac*, the selection of states of autonomous mutable loci that produce very late-occurring mutations makes it possible to examine the inheritance behavior of such loci, freed from the apparent confusion resulting from early-occurring modifications at the locus, which can distort the expected mendelian ratios.

Is the Behavior of *Ac* a Reflection of a Mechanism of Differentiation?

We now return to the original question. What is the significance of the somatically occurring changes at *Ac*, and the changes in state that occur at the autonomous mutable loci? Do they suggest the presence of nuclear factors that serve to control when and where certain decisive events will occur in the nucleus? With regard to *Ac*, it is known that the events leading to its loss, to increase or decrease of its dosage action, or to other changes involving its action or position in the chromosome complement, are related; and that they appear as the consequence of a mitotic event, controlled in time of occurrence by the state and dose of *Ac* itself. Sister nuclei are formed that differ with respect to *Ac* constitution, as the photographs of Figures 17 to 22 illustrate. Because of this somatically occurring event involving *Ac*, the *Ac*-controlled mutable loci will differ markedly as to the time when mutational events will occur at them, or as to whether or not any such events will occur at all in the cells arising from the sister cells. This precise timing of somatic segregations effects a form of differentiation, for it brings about changes in the control of occurrence and time of occurrence of genic action at other loci, and does so differentially in the progeny of two sister cells. This likewise applies to the autonomous mutable loci; but in these cases the controller of the time and place of appearance of genic activity is a component of the locus itself.

The process of differentiation is basically one involving patterns of action arising in sequential steps during development and affecting the types of activities of definitive cells. The ultimate expression of component parts of an organism represents the consequence of segregation mechanisms involving the various cellular components. The part played by any one component of the cell in this segregation system can not be divorced from that played by any other component. It is possible, however, to attempt to examine the various components in order to determine their respective relationships and the sequential events that involve them. Embryological studies have contributed much to our knowledge of the segregation of cytoplasmic components. The segregation of nuclear components is less well understood, although some outstanding examples are known. These examples show segregations or losses of obvious components of the nucleus—that is, of whole chromosomes or easily seen parts of chromosomes. The segregation or loss of smaller components, not readily visible on microscopic

examination, may well be one of the mechanisms responsible for the nuclear aspects of control of the differentiation process. The phenomenon of variegation, as described here and observed in many other organisms, may be a reflection of such a segregation mechanism—exposed to view be- the timing of events leading to a specific type of genic action is "out-of-phase" in the developmental path. Variegation may represent merely an example of the usual process of differentiation that takes place at an abnormal time in development. Viewed in this way, it is possible to formulate an interpretation of the part played by the nuclear components in controlling the course of differentiation.

This interpretation considers that the nucleus is organized into definite units of action, and that the potentials for types of genic action in any one kind of cell differ from the potentials in another kind of cell. In other words, the functional capacities of the nuclei in different tissues or in different cells of a tissue are not alike. The differences are expressions of nonequivalence of nuclear components. This nonequivalence arises from events that occur during mitotic cycles. The differential mitotic segregations are of several types. Some involve controlling components, such as *Ac*, and produce sister nuclei that are no longer alike with respect to these components. As a result, the progeny of two such sister cells are not alike with respect to the types of genic action that will occur. Differential mitoses also produce the alterations that allow particular genes to be reactive. Other genes, although present, may remain inactive. This inactivity or suppression is considered to occur because the genes are "covered" by other nongenic chromatin materials. Genic activity may be possible only when a physical change in this covering material allows the reactive components of the gene to be "exposed" and thus capable of functioning.

A mechanism of differentiation that requires differences in nuclear composition in the various cells of an organism finds considerable support in the literature. The most conspicuous example is in *Sciara*, where a thorough cytological and genetical analysis has been made. (For reviews, see Metz, 1938, and Berry, 1941.) It is known, in this organism, at just what stage of development differences in nuclear composition will arise; and, with regard to the X chromosome, it is known what element in the chromosome controls the differential behavior. This element is at or near the centromere of the chromosome (Crouse, 1943). Furthermore, differential segregations of the B-type or accessory chromosome have been found to occur in a number of plants. (For reviews of literature to 1949, see Müntzing, 1949.) Numerous other examples are known of differential segregation involving whole chromosome complements, certain types of chromosomes of a complement, or, occasionally, a certain component of a chromosome. (For literature citations see Melander, 1950; White, 1945, 1950; Berry, 1941.) Whether the differential segregations involve whole complements of chromosomes, individual chromosomes, individual parts of chromosomes that can be seen, or submicroscopic parts of chromosomes, may well be a matter of degree rather than type. Certainly, the evidence for differential segregation is not wholly negative.

With regard to mechanisms associated with differentiation and genic action, an additional factor may be mentioned. The part played by the doses of component elements in the chromosomes appears to be of considerable importance. First, a number of genetic factors associated with known loci produce measurable quantitative effects that are related to dose: the higher the dose the greater the effect. Such dosage actions, probably reflecting rates of reaction, are familiar to all geneticists, and some of them have been reviewed in this study of mutable loci. Dosage controls of the *Ac* type, affecting the time of action of certain other factors carried by the chromosomes, has been less well appreciated. A third type of dosage action has made itself evident in these studies. In some aspects, however, it resembles the action of different doses of *Ac*. In the study of the autonomous a_2^{m-1} mutable locus, a number of mutants appeared, particularly on self-pollinated ears, showing a pale aleurone color. Study of the behavior of these pale mutants has revealed the following. Some of them produce pale-colored aleurone in one, two, and three doses and give no evidence of instability in the expression of the phenotype. (One and two doses are obtained by combinations of the pale-mutant allele with the stable a_2 allele.) That this stable expression may be deceptive is shown by the dosage effects of other similarly appearing pale-producing isolates derived from mutations at a_2^{m-1}. These may give pale aleurone color, and no indication of instability, in three and two doses; but with one dose something unexpected occurs. The kernels show a colorless aleurone in which mutations to deep aleurone color appear. Still other isolates give pale color in three doses, but in one or two doses produce the colorless background with deep-colored mutant areas. In these cases, it

is clear that some of the mutations at $a_2^{m\text{-}1}$ giving pale color and appearing to be stable are stable only because of some dosage action produced by a mutation of the original $a_2^{m\text{-}1}$ to the pale-producing type.

The study of dose-provoked actions in the pale mutants mentioned above and those of *Ac* have given some indication of the importance of dosage action in affecting genic expression. The original isolate of $a_2^{m\text{-}1}$ did not give evidence of such striking dosage action. When present in one, two, or three doses, it gave rise to colorless kernels in which mutations, mainly to a deep color and occasionally to a pale color, appeared. The graded series of dosage action exhibited by the various pale mutants derived from $a_2^{m\text{-}1}$ is very much the same as the graded series exhibited by the various isolates of *Ac*. In these cases, it appears as if each isolate is composed of a specific number of reactive subunits and that the dosage expressions are related to the total number of such units that are present in the nucleus. Although these graded dosage effects may be visualized on a numerical basis, it is not claimed that such an interpretation is necessarily the correct one. The large differences in dosage expression exhibited by the various isolates of *Ac*, and also the various isolates of the pale mutants derived from $a_2^{m\text{-}1}$, nevertheless appear to follow such a scheme.

Why different doses of components of the chromosomes function as they do in controlling developmental processes takes us to another level of analysis that is not under consideration here. A relation to rates of particular reactions can be suspected. It is tempting to consider that changed environmental conditions may well alter otherwise-established rates of reaction, and thus initiate alterations in the nuclear components at predictable times, leading to strikingly modified phenotypic expression. Just such effects have been observed by students of developmental genetics. They have shown that alterations of environmental conditions at particular times in development can lead to predictable changes in the subsequent paths of differentiation.

Consideration of the Chromosome Elements Responsible for Initiating Instability

It will be recalled that this study of the origin and behavior of mutable loci was undertaken because a large number of newly arisen mutable loci appeared in the progeny of plants in which an unusual sequence of chromosomal events had occurred—that is, the breakage-fusion-bridge cycle. Striking similarities in the patterns of behavior of these mutable loci were immediately noticed. It was the pattern of behavior, rather than the change in expression of the particular phenotypic character, that was obviously of importance. This pattern, revealed in all cases, stemmed from an event occurring at mitosis, which altered the time and frequency of mutations that would subsequently occur in the cells derived from those in which this event occurred. It was noticed that sister nuclei could differ in these respects—and sometimes reciprocally, as if the mitotic event had resulted in an increase in one nucleus of a component controlling the mutation time or frequency, and a decrease of this component in the sister nucleus. It was also noted that the change in phenotypic expression—that is, the mutation—likewise resulted from a mitotic event; and that the mutation itself and changes of the controller of the mutation process could result from the same mitotic event: one cell showing the mutation, the sister cell showing an altered condition with respect to control of future mutations in the cells derived from it.

Further, it may be recalled that the mechanism which resulted in the appearance of newly arisen mutable loci—that is, the breakage-fusion-bridge cycle involving chromosome 9—gave rise to numerous obvious alterations of the heterochromatic materials, in other chromosomes of the complement as well as in chromosome 9. It was also demonstrated that the effect of a known activator, *Dt*, located in the heterochromatin of the chromosome-9 short arm, and producing a very definite pattern of mutations of the otherwise stable a_1 locus in chromosome 3, could be recreated independently and on a number of different occasions in cells of a tissue in which the breakage-fusion-bridge cycle was in action. The combined observations and experiments point to elements in the heterochromatin as being the ones concerned with differential control of the times at which certain genes may become reactive. It is believed that somatic segregations of components of these elements may initiate the process of nuclear control of differentiation.

On the basis of these interpretations and those given in the previous section, it becomes apparent why a large number of newly arisen mutable loci appeared in the self-pollinated progeny of plants that had undergone the chromosome type of breakage-fusion-bridge cycle. This cycle induced alterations in the heterochromatin. These alterations changed the organization of the heterochro-

matic chromosome constituents and probably also, in many cases, the doses of their component elements. Changes were induced in these heterochromatic elements at times other than those at which they would normally occur during differentiation. This resulted in changes in the times in development when their action on specific chromatin material, associated with genic components of the chromosome, was expressed. The altered timing of their actions was consequently "out-of-phase" with respect to the timing that occurs during normal differentiation. This was made evident by the appearance of a "mutable locus." The "mutable locus" is thus a consequence of the alteration of an element of the heterochromatin produced by the breakage-fusion-bridge cycle. Once such an "out-of-phase" condition arises, others may subsequently appear because of the physical changes in the chromatin that occur at the mutable locus, leading, at times, to transpositions of this chromatin to new locations, as described earlier. In their new locations, these transposed chromatin elements continue their specific control of types of genic action but now affect the action of the genic components at the new locations.

Relation of "Mutable Loci" to "Position-effect" Expressions in Drosophila and Oenothera

In a previous publication (McClintock, 1950) the author has suggested that the position-effect variegations in *Drosophila melanogaster* and the variegations observed in many other organisms, including those associated with the mutable loci here described, are essentially the same. An adequate discussion of the interrelations would require more space than can appropriately be given here. Attention will be drawn, therefore, only to a few relevant facts, which may serve to indicate why this conclusion has been reached. In the first place, a number of different types of position-effect expression are found in *Drosophila* (for review and literature citations, see Lewis, 1950). In maize, comparable types of instability expression have appeared. In *Drosophila*, some of the variegations appear to result from loss of segments of chromosomes. This applies to those cases where the expression of the dominant markers, carried by the chromosome showing the "position-effect" phenomenon, is absent in some sectors of the organism. The extent of the deficiency varies, but it includes in each case the region adjacent to the heterochromatic segment with which many of the variegation types of position-effect expression are known to be associated. It may be recalled that such deficiencies are produced in maize when *Ds* is present.

That heterochromatic elements of the chromosomes of *Drosophila* undergo breakage events in somatic cells is suggested by the study of "somatic crossing over" in this organism (Stern, 1936). The appearance of the abnormally timed exchanges between chromosomes is conditioned by the presence of certain Minute factors, for example, *M(1)n*, much as the occurrence of structural aberrations at certain loci in maize (i.e., *Ds*, wherever it may be) is dependent on the presence of *Ac*.

Of particular significance for comparative purposes is the study of Griffen and Stone (1941) on the induction of changes in the position-effect expression in *Drosophila* of the white-eye variegation, w^{m5}. The w^{m5} case arose through an X-ray-induced translocation of the segment of the left end of the X chromosome at 3C2 (the w^+ locus) to the heterochromatic region of chromosome 4. Males carrying w^{m5} were X-rayed, and the progeny examined for changes in the variegation expression of the eye mottling. Many such changes were found. Studies of these cases were continued in order to determine the nature of the events associated with the changes. In all cases, the new modification in the phenotypic expression of the w^+ locus was found to be associated with a translocation, which placed the segment of the left end of the X chromosome, from 3C2 to the end of the arm, at a new location. In many cases, the new position was to a euchromatic region of another chromosome, and yet variegation persisted. Some of the new positions, however, gave rise to apparent "reversions" to a wild-type expression. Individuals having these "reversions" were X-rayed, and variegation types again appeared in the progeny. Here also the variegation was shown to be associated with a translocation involving the left end of the X chromosome at 3C2, from the location in the "reversion" stock to a new location—again, sometimes a euchromatic region. It may be suspected that the maintenance of variegation potentialities in all these cases was associated with the presence of a segment of heterochromatin of chromosome 4 that remained adjacent to the w^+ locus when the successive translocations occurred. This would not readily be detected in the salivary chromosomes. The presence of such "inserted" heterochromatin could be responsible for the continued expression of variegation at the w^+ locus in repeated translocations. If such was

the case, then the resemblance to the maize cases, described in this report, is obvious. The appearance of "reversions," and the subsequent appearance of variegation after X-radiation of individuals carrying such "reversions," might seem to present a contradiction. On the basis of an analysis of the cases described by Griffen and Stone, the writer believes that no contradiction is involved. This analysis has suggested that the timing of variegation-producing events during development is, in part, a function of the relative distance of the translocated segment—i.e., the left end of the X chromosome—from the centromere of the chromosome that carries it: the farther removed the segment is from the centromere, the later in the development the variegation-producing events will occur. In the "reversions," this segment has been placed close to the end of one arm of a chromosome. The reappearance of variegation occurs when the segment is translocated to a position closer to a centromere. Another factor is also associated with the timing of the variegation-producing events. This is the Y chromosome. When the Y is absent, the areas of altered phenotype are larger than when it is present, indicating an earlier timing of the variegation-producing events. It may be noted in this connection that some of the cases of "reversions" are only apparent reversions. In XY constitutions they appear to give a stable wild-type expression but in XO constitutions, the eyes show a light speckling of the altered phenotype. With the latter constitution variegation occurs, but only very late in the development of the eye. The similarity of this effect of the Y chromosome to that of dosage action of *Ac* is apparent in these cases as well as in many others in *Drosophila* that have been examined.

In a recent report, Hinton and Goodsmith (1950) gave an analysis of induced changes at the bw^D (Dominant brown eye) locus in chromosome 2 of *Drosophila*. This case was considered to be a stable-type position effect. It arose originally through the insertion of an extra band next to the salivary-chromosome band where bw^+ is located. Males carrying bw^D were irradiated and crossed to wild-type females. The offspring (9,757 individuals) were examined for changes in the bw^D expression. Twenty-one individuals showing the wild-type expression appeared in the F_1, and progeny was obtained from one-third of them. A study of the inheritance behavior of each modification was undertaken, and a study was also made of the salivary-gland chromosomes. From these studies it was clear that the modifications arose from changes that occurred in the vicinity of the bw^D locus and involved the inserted band. In four cases, restoration of the wild-type expression followed removal of this band. In two cases, it followed separation of this band from the bw^+ band by translocations. In one case, no obvious change in the salivary chromosomes was noted, but nevertheless a change in phenotypic expression had occurred. Of considerable importance, also, was the appearance, in some of these cases, of somatic instability of expression of the bw^+ phenotype. Variegation began to appear. It had never been observed in the brown-Dominant stock itself. It may be noted that the changes in the bw^D expression are associated with types of chromosomal alterations which are much the same as those proposed to account for changes in phenotypic expression at some mutable loci in maize.

A further resemblance between *Drosophila* and maize will be mentioned. In *Drosophila*, many of the translocations, inversions, and duplications are believed to be associated with the formation of dominant-lethal effects. In maize, a number of dominant lethals have arisen from transpositions of *Ds*. Some produce defective growth of the endosperm and embryo; others affect the development of the embryo but not the endosperm; and still others affect the capacity of the embryo to germinate, without affecting its morphological characters. Over half the newly arisen transpositions of *Ds* that are of this latter type have not produced viable plants, owing to lack of germination of the embryos in the kernels.

There are similarities between the maize cases and a case in *Drosophila pseudoobscura* described by Mampell (1943, 1945, 1946). In this *Drosophila* case, a heterochromatic element appeared to be associated with the initiation of instability at another locus, which in turn led to changes in chromosome organization and to numerous changes in genic action at various loci in the chromosome complement. These changes were expressed both somatically and germinally.

The position-effect behavior reported in *Oenothera* (Catcheside, 1939, 1947a, b) is much like that of *Ds*. The chromosomal events responsible for the observed types of change in phenotypic expression may be the same in the two organisms. In *Oenothera* as in maize, gross changes in chromosome constitution arise, such as duplications and deficiencies of segments of the chromosome involved. Similar cases in other organisms undoubtedly exist. It is probable, however, that

the lack of a critical mode of detection of a chromosome breakage mechanism has been responsible for the apparent delay in reporting such cases in connection with studies of somatic variegation and mutable loci. Also, because changes in state occur that involve reduction in the frequency of chromosome breaks, and because such breaks lead to lethal gametes, it is probable that states of a mutable locus producing some detectable breaks are rapidly eliminated from a population, leaving a state of the mutable locus that produces few or no such events to be propagated.

It has been argued that the variegation types of position effect in *Drosophila* usually do not give rise to germinal mutations, and that they belong, therefore, to a separate category of instability expression. Since some variegation position effects do give rise to germinal changes, this argument could in any case be only partially applicable. However, whether or not germinal changes arise is not considered relevant in the interpretation developed here. The time and place of occurrence of such changes is related to controls, existing in the nucleus. The differentiation mechanism described above should effect controls that would exclude the germ lines from undergoing many changes, but should allow numerous alterations in the soma that would lead to altered patterns of genic expression. Whether or not a particular somatically expressed pattern of genic action—for example, the distribution of pigment—arises from mutations at a "mutable locus" or from the action of a particular "stable" allele of the locus cannot be decided by using the criterion of presence or absence of germinal mutations. The important consideration is when, where, and how the patterns of genic action are controlled and eventually expressed.

The combined evidence from many sources suggests that one should look first to the conspicuous heterochromatic elements in the chromosomes in search of the controlling systems associated with initiation of differential genic action in the various cells of an organism; and secondarily to other such elements, which are believed to be present along the chromosomes and to be either initially or subsequently involved in the events leading to differential genic action. Evidence, derived from *Drosophila* experimentation, of the influences of various known modifiers on expression of phenotypic characters has led Goldschmidt (1949, 1951) to conclusions that are essentially similar to those given here.

The conclusions and speculations on nuclear, chromosomal, and genic organization and behavior included in this report are an outgrowth of studies of the instability phenomenon in maize. They are presented here for whatever value they may have in giving focus to thoughts regarding the basic genetic problems concerned with nuclear organization and genic functioning. Until these problems find some adequate solution, our understanding and our experimental approach to many phenomena will remain obscured.

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Controlling Elements and the Gene

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In a recent brief review (McClintock, 1956), a description was given of types of elements carried in the maize chromosomes that serve to control gene action and to induce, at the site of the gene, heritable modifications affecting this action. These elements were initially discovered because they do not remain at one position in the chromosome complement. They can appear at new locations and disappear from previously determined locations. The presence of one such element at or near the locus of a known gene may affect the action of this gene. In so doing, it need not alter the action potentials of the genic substances at the locus. Therefore, these elements were called controlling elements. It was also shown that controlling elements fall into groups, the members of each operating as an integrated system in the control of gene action.

In this report, some aspects of controlling elements will be considered that could not be discussed in the above-mentioned review. This will necessitate mention of some of the well known gene loci in maize, and symbols for them will be used in the discussion. So that a ready reference may be available, the pertinent information about each of these loci is given in the following list.

Chromosome 1: *P*, pericarp and cob color. A large number of alleles are known, including P^{vv}, which gives variegated pericarp and cob color.

Chromosome 3: A_1, anthocyanin pigment produced in kernel and plant; a_1, standard recessive allele, no anthocyanin in kernel or plant.

Sh_2, normal development of endosperm tissues; sh_2, recessive allele, shrunken endosperm. Very closely linked with A_1; less than a quarter of a crossover unit distal to it.

Chromosome 5: *Pr*, purple aleurone color; *pr*, recessive allele, red aleurone color. Located in long arm of chromosome 5.

A_2, anthocyanin pigment developed in kernel and plant; a_2, standard recessive allele, no anthocyanin developed in kernel or plant. Located in short arm of chromosome 5. Gives approximately 28 per cent recombination with *Pr*.

Chromosome 6: *Y*, yellow starch in endosperm; *y*, recessive allele, white starch.

Chromosome 9: *I*, dominant inhibitor of aleurone color in kernel.

C, allele of *I*, pigment produced in aleurone layer of kernel; *c*, recessive allele, no pigment in aleurone layer.

Sh_1, normal development of endosperm of kernel; sh_1, recessive allele, shrunken endosperm. Located approximately four crossover units proximal to *I*.

Bz, purple anthocyanin pigment in plant and aleurone layer of kernel; *bz*, standard recessive allele, bronze color in both plant and kernel. Located approximately two crossover units proximal to Sh_1.

Wx, amylose starch produced in pollen and endosperm, stains blue with solutions of I-KI; *wx*, recessive allele, starch is amylopectin, stains red-brown with I-KI solutions. Located approximately 15 crossover units proximal to *Bz*.

Distinctions Between Controlling Elements and Gene Elements

In maize, as in other organisms, a change at a particular locus in a chromosome is made evident by modification of a particular phenotype. Independently occurring alterations (mutations) at the same locus give rise to change in expression of this one particular phenotype, be it recognized by change in a particular enzymatic reaction or by means of some less well defined but identifiable modification of phenotypic expression. Because it is possible to predict which phenotypic character will be altered after modification at a particular known locus in a chromosome, it is inferred that some component is present there whose mode of action may be recognized within certain limits. These components appear to reside at fixed positions in the standard chromosome complement of maize, and they will be referred to in this discussion as the genes. Modification of action of a known gene component can result from insertion of a controlling element at or near the locus of the gene; and, in general, the types of change in phenotypic expression induced by its presence there are those that could be anticipated from previously acquired knowledge of mutant expressions that have resulted from modifications at this locus. Controlling elements, on the other hand, need not occupy fixed positions in the chromosome complement, and detection of the presence of one such element depends upon characteristics it exhibits that are independent of its position.

It is realized that our present knowledge of the gene does not allow formulation of a definition

of it based on structural organization, dimension, or primary type of activity. However, all the so-called gene loci with which we will be concerned are recognized because a change at the locus effects a change of some component that normally appears in the cytoplasmic region of the cell and therefore the alteration at the gene locus is reflected in this region. All the controlling elements so far identified, on the other hand, may have their area of activity confined within the nucleus itself, for they are known to serve as modifiers, suppressors, or inhibitors of gene action as well as mutators. They behave as if they were modulators of the genome. Each controlling element or system of interacting elements has its own mode of modulation, and this is expressed in an individualistic manner that is quite independent of the recognized type of action of the gene which it may be modulating. Our present knowledge would suggest that gene elements and controlling elements represent two different classes of primary components of the chromosome and that a close relationship exists between them.

The largest amount of evidence concerning the mode of behavior of controlling elements has been derived from study of the element called Activator (*Ac*), which by itself may control gene action at the locus where it resides, and also of another controlling element that responds to it. When this latter element is inserted at a known gene locus, changes in gene action may occur either immediately after its insertion or subsequent to it. Both the insertion of this element at the gene locus and the subsequent modifications in gene action it induces depend on the presence of the *Ac* element somewhere in the chromosome complement. In the absence of *Ac* no changes affecting gene action occur, and stability of gene expression will be exhibited as long as it is absent. Return of *Ac* to the nucleus through appropriate crosses will again effect activation of the second element of the system, and this will be expressed in a series of mutation-type changes at the locus of the gene where this element resides.

The second element of the system outlined above was originally given the designation Dissociation (*Ds*) because, in the presence of *Ac*, breaks appeared to be formed at the locus where it resided. It was later determined that the apparent "breaks" were produced because at this locus a dicentric chromatid and a corresponding acentric chromatid were formed. The acentric chromatid, composed of the segment of the chromosome from *Ds* to the end of the arm, was eliminated from the nucleus during a mitotic anaphase whenever such an event occurred. These dicentric-acentric chromatids were formed both in somatic and sporogenous cells, and the time during the development of a tissue when this took place was found to be a function of the dose of *Ac*: the higher the dose, the later the time of these occurrences. In the absence of *Ac*, however, no dicentric-acentric chromatids were produced nor was there any evidence that would suggest the presence of this *Ds* element at the locus. Return of *Ac* again initiated these dicentric-acentric chromatid formations. It is evident, therefore, that tests of the presence or absence of *Ac* in a plant may be made by crossing it with one that is homozygous for *Ds* but does not have *Ac*. These are the so-called *Ac* tester stocks, and descriptions of their usefulness for detecting the presence of *Ac* have been given elsewhere (McClintock, 1951, 1953; Barclay and Brink, 1954). If these *Ac* tester stocks carry recessive alleles of other known genetic markers, and if the plant carrying *Ac* is heterozygous for them, it is possible to determine the location of *Ac* and changes in location that may occur. By this means, various different positions of *Ac* have been detected. Evidence of its transposition from one known location to another has also been obtained.

In the presence of *Ac*, the *Ds* element undergoes transposition, and its insertion at various locations were detected. Sometimes *Ds* was inserted at or close to a known gene locus, and the effects of its presence on gene action were thereby discovered. In some cases both dicentric chromatid formations and changes in gene action were noted to occur at this gene locus, but only when *Ac* was also present in the complement; and the time of the occurrences in the development of a tissue reflected the dose of the *Ac* element that was present in the nucleus. In examining these cases, it was soon learned that the *Ds* element itself could undergo modifications that altered its mode of response to *Ac*. Some of them resulted in a reduced frequency of occurrence of dicentric chromatid formations, often correlated with an increased frequency of occurrence of change in gene action; and these latter were unaccompanied by gross change in chromosome morphology. Still other modifications of this *Ds* element resulted in almost complete elimination of dicentric chromatid formations, although mutations continued to occur at the locus of the gene where the *Ds* element resided. Thus, in such cases, the designation of "Dissociation" (*Ds*) for the element responsible for these mutations no longer appeared to be applicable. Nevertheless, this original designation has been retained because in the early studies it was possible to follow sequentially the changes in the *Ds* element that altered its type of response to *Ac*: from a high rate of dicentric chromatid formation to a low rate—and also the reverse, from a low rate to a high rate. Therefore, the designation "*Ds*" will be applied to any element at a known gene locus that responds to *Ac* in the following manner: in the absence of *Ac*, it undergoes no alterations that affect gene action; but in the presence of *Ac* such alterations occur, and the time of their occurrence during the development of a tissue, and cells of

the tissue in which they occur, are a function of *Ac*, particularly of its dose. The designation "*Ds*" for an element responding to *Ac* should not be construed to mean that this element will produce dicentric chromatids wherever it may be located. It may produce none. Nevertheless, its responses to *Ac*, as described above, are readily detected. It is also probable that there are different kinds of "*Ds*" elements, but all of them respond to *Ac* in this quite predictable manner.

It is clear that both the *Ac* and the *Ds* elements retain their characteristic modes of expression when located at various different positions in the chromosome complement, and that methods of detecting their presence in these different locations have been developed. The study of *Ac* and of the integrated *Ds-Ac* two-element system has been of considerable help in investigating other unrelated control systems. This applies particularly to the well known *Dt* (Dotted)-a_1 system originally discovered by Rhoades (1936, 1938, 1941, 1945). The standard recessive, a_1, is very stable in the absence of *Dt*. In its presence, however, mutations occur to the higher alleles of A_1 or to stable recessives that no longer mutate in the presence of *Dt*. The pattern of response to *Dt* is quite predictable. Dots of deep anthocyanin pigment appear in a nonpigmented background in the kernel, and streaks of anthocyanin appear in a nonpigmented background in the plant. The number of dots (mutations) that appear in the kernel is an expression of the dose of *Dt*: the higher the dose, the more frequent the mutations. The *Dt* element was located by Rhoades in the short arm of chromosome 9. Subsequently, Nuffer (1955) discovered the presence of *Dt* in two South American strains of maize. In one strain, it was located in chromosome 6 and in the other strain it was located in chromosome 7. The response of the standard a_1 allele to each of these newly detected *Dt* elements is the same as that expressed when the original *Dt* element, discovered by Rhoades, is present. Recently, Nuffer (personal communication) obtained evidence of transposition of the *Dt* element located in chromosome 7 to a new location in the chromosome complement. Thus, in this respect, also, *Dt* resembles *Ac*; both may undergo transposition without loss of identity.

The response of the standard a_1 allele to *Dt* is similar in many essential respects to that of *Ds* to *Ac* when the former is present at the locus of a known gene. It could be inferred, then, that a controlling element, responding to *Dt*, is also present at the standard a_1 locus and that it is this element which is responsible for the observed mutations and for their pattern of appearance in the plant and kernel tissues. Evidence of this is now available from studies aimed at analyzing the composition of the A_1 locus made by Laughnan (1952, 1955) and also by Nuffer (personal communication). When plants carrying an A_1 allele in one chromosome 3 and the standard a_1 allele in the homologue are crossed by plants homozygous for the latter recessive, some kernels exhibiting a mutant phenotype appear on the resulting ear. These express a lower level of intensity of pigmentation than that given by the A_1 allele. It has been shown that the majority of these mutants arise as a consequence of crossing over within a compound A_1 locus. When plants grown from these pale-colored kernels were crossed by plants carrying *Dt*, it was discovered that in about 5 to 10 per cent of them the mutant expression was unstable. Mutations to higher alleles of A_1 occurred, and the pattern of response (dots of deep pigmentation) was the same as that given by the standard a_1 allele. In these cases, however, the mutations were registered in a pale-colored background rather than in a colorless one. Plants that were homozygous for the A_1 allele also gave rise to pale mutants. It could be shown that many of these arose from crossing over within the compound A_1 locus. None of them, however, were unstable in the presence of *Dt*. From this it might be inferred that an element responding to *Dt* is not present at this A_1 locus. Because the pale mutants derived from crossing over in the heterozygote with a_1 do carry this element, it may be inferred that those crossovers that occur within a restricted region of the compound locus will introduce it. In the presence of *Dt*, it will respond in this new organization of the locus in the very same manner that it was responding before the crossover occurred.

On the self-pollinated ear of a plant that was A_1/a_1, *Dt*/*Dt* in constitution, a single kernel appeared that showed a striking change in pattern of mutation (Nuffer, 1951). A very large number of pigmented dots appeared in a colorless background, and the intensity of pigmentation in these dots varied from light to very dark. A plant was grown from this kernel, and an investigation of the nature of the change responsible for this altered mutation pattern was commenced. It proved to be a new allele of a_1 that responds to the presence of *Dt* by giving this strikingly different pattern of mutation: a high frequency of occurrence of mutation in plant and kernel in the presence of *Dt*, some of which gives rise to large sectors of mutant tissue and others to small areas. In the absence of *Dt*, however, no mutations occur and the mutant behaves as a stable recessive. By appropriate tests Nuffer (personal communication) was able to learn that the controlling element responsible for the dotted pattern of mutation and that responsible for this new pattern occupy different sites in the compound locus and that they can be separated by crossing over. It is now evident that the basic mechanism of control of gene action in the *Dt*-a_1 system is essentially the same as that in the *Ds-Ac* system. Interactions between the members of these two systems do not occur, however, for

Dt will not substitute for *Ac* in control of *Ds* (Nuffer, unpublished) nor will *Ac* substitute for *Dt* in control of gene action at a_1 (McClintock, 1953).

Recognition of other types of controlling elements is made possible by procedures that are essentially similar to those outlined above. This applies to the Suppressor-mutator system associated with control of gene action at a modified A_1 locus (a_1^{m-1}, McClintock, 1955, 1956), to a similar system operating to control gene action at A_2 (McClintock, unpublished), to several systems investigated by Dollinger (1955 and unpub.), and to still other systems now under investigation in several different laboratories. The presence of controlling elements in the maize chromosome complement is now well established. It is for the future to determine the extent to which such elements, and systems of interacting elements, operate in the over-all control of activity of the genome during development. The ever-increasing recognition of these elements and their modes of action suggest that they may perform a major function in this respect, and an understanding of the manner by which it is accomplished may be gained from examination of the modes of behavior of particular elements or systems of interrelated elements that are now known.

Detection of Transpositions of Controlling Elements

As stated earlier, the presence of elements, independent of the genes but controlling their action, was initially detected because they undergo transposition from one location to another within the chromosome complement, and the effects they produce when inserted at known gene loci are thereby made evident. Therefore, a description of some of the methods that have been used to detect such transpositions is of considerable importance for an appreciation of the nature of the behavior of controlling elements. A number of different methods have been used and they fall into two general categories, selective and nonselective. The nonselective method is more laborious than the selective method but its use may be required in the initial study of a controlling element. For example, in a cross of a plant having a single *Ac* element, whose location is unknown, to plants having no *Ac*, half the progeny can be expected to carry an *Ac* element as a result of meiotic segregations in the parent plant. If the individuals in this progeny are tested for the presence of *Ac* by the method described earlier, the expected ratio of presence and absence of *Ac* will usually be found. However, an occasional plant may be present in the progeny that has two *Ac* elements instead of the expected one. Again, if genetic markers have been introduced into the cross, and if the *Ac* element has shown no linkage with them in the parent plant, the progeny may include an occasional plant which has one *Ac*, as expected, but in which the *Ac* element now shows linkage with one of the markers (for an example, see Table 1, McClintock, 1951). If the progeny of this plant, in turn, is tested for the location of the *Ac* element, the majority of *Ac*-carrying individuals will show the same linkages of *Ac* with the given markers as exhibited by the parent plant. An individual may be found, however, in which this linkage is not expressed, and no linkage with the given markers will be exhibited in its progeny. In this manner, successive changes in location of the *Ac* element may be detected, but the method is quite unselective and it requires tests of a number of individuals in successive generations. Once the location of a controlling element has been determined, however, selective methods of detecting subsequent changes in its location may be applied, and these will be considered shortly.

A number of different selective methods of detecting transposition of controlling elements have been applied, and some of them take advantage of the particular mode of action of the controlling element under investigation. For example, the dose effects produced by the *Ac* element may be useful in this respect, for it is known that the higher the dose the later the time of occurrence of modifications affecting *Ac* itself or affecting *Ds* or *Ds*-type elements wherever the latter may be located. Changes in location of *Ac* usually occur rather late in the development of the somatic and sporogenous tissues, but occasionally they occur in a cell early in plant development and this can result in the appearance of sectors in which the *Ac* constitutions differ. If such a sector enters the ear, the altered *Ac* constitution in its cells is made apparent by the distinctive phenotypes of the kernels that develop within it after pollen from an *Ac* tester stock has been placed on the silks of the ear. They indicate either that *Ac* is absent in the cells that formed the sector or that it is increased in number. By selecting kernels from such a sector and by making test crosses with the plants derived from them, it is possible to verify the change in *Ac* constitution that has occurred in a somatic cell of the parent plant. Some sectors are twinned, in that the *Ac* element appears to be absent from one sector and increased in number in the twin; and verification of this is readily obtained from examination of the plants derived from the kernels in each component of the twinned sector.

The dose effects produced by *Ac* have also allowed detection of some of the changes in *Ac* location that occur late in the development of sporogenous cells and consequently are exhibited only in individual kernels on an ear. Their usefulness in this respect was described earlier (McClintock, 1951) in connection with tests devised to detect such changes in location of *Ac* that occur in plants carrying this element at allelic positions in a pair of homologous chromosomes. They also

allow detection of gametes carrying two *Ac* elements, produced by plants having only one. When such a plant is used in a test cross, the functioning of a gamete having two *Ac* elements gives rise to a kernel that exhibits a much delayed time of occurrence of the dicentric-acentric formations at *Ds* in chromosome 9 or of mutation at those gene loci where the *Ds-Ac* system is operating. Verification of the presence of more than one *Ac* element in these kernels may be obtained by testing the *Ac* constitution in the plants derived from them, provided, of course, that the change in *Ac* constitution occurred in a cell of the parent plant before gamete formation and thus provided for an endosperm and embryo that were alike in *Ac* constitution.

During studies of transposition of *Ds* and also of mutation occurring at those gene loci in which the *Ds-Ac* system of control of this operates, it has been noted that change in location of *Ac* often accompanies the event that affects the *Ds* element of the system. Such coincidences are so numerous that selection of individual kernels exhibiting modification of the *Ds* element is also useful as a method of selection for changes in location of *Ac*.

One of the most effective methods of selection for transposition of *Ac* utilizes those cases in which the *Ac* element resides at the locus of the gene whose action it is directly controlling: that is, the case of *Ac* (called Modulator and symbolized as *Mp*) at the *P* locus in chromosome 1, described by Brink and his collaborators (Brink and Nilan, 1952; Brink, 1954; Barclay and Brink, 1955; Fradkin and Brink, 1956), and that of *Ac* at the bronze locus in chromosome 9 (McClintock, 1956). Mutations at these loci are associated with events occurring to and instigated by the *Ac* element itself, and many of them are accompanied by removal of *Ac* from the affected gene locus and its insertion elsewhere. Therefore, if those kernels on an ear that exhibit germinal mutations are selected, and the plants grown from them are tested for *Ac*, it will be found that in a number of these plants *Ac* no longer resides at the mutant locus. However, *Ac* may still be present in the chromosome complement but located elsewhere. Tests of this type were made by Brink and his collaborators with regard to the *P* locus and by me with regard to the bronze locus. As an example of the type of result obtained from such tests, those conducted by me with the bronze locus will be given here.

The phenotypic expression produced when *Ac* is present at the bronze locus resembles that given by the standard recessive, *bz*. This latter recessive is completely stable in the presence of *Ac*, but mutations occur at the bronze locus where *Ac* resides. Some of these give the dominant *Bz* expression, and the majority of such mutants are thereafter stable in the presence of *Ac*. A larger fraction of the mutants express the recessive, *bz*, and this expression is thereafter stable in the presence of *Ac*. Some mutations, however, give rise to unstable dominants or to other types of change that will be considered later. Here we will consider only those changes that give rise to mutants that are stable in the presence of *Ac*. Kernels exhibiting mutant phenotypes were selected from ears produced by plants carrying only one *Ac*, and it was present at the unstable bronze locus in one chromosome 9. The homologous chromosome 9 in these plants carried the standard, stable recessive, *bz*. The short arm of each chromosome 9 carried other distinguishing genetic markers, to make it possible to ascertain that the observed mutations had occurred at the bronze locus where *Ac* resides. The ears from which the mutant-carrying kernels were selected were produced by these plants when they were crossed by plants having no *Ac* and homozygous for the recessive alleles of the selected genetic markers. On these ears, those kernels that were homozygous for the standard recessive, *bz*, were completely bronze; no *Bz* spots appeared in them. On the other hand, the majority of those kernels that received the chromosome carrying the bronze locus with *Ac* showed a number of deep purple (*Bz*) spots in a bronze (*bz*) background. A few kernels having this locus, however, showed an altered phenotypic expression and some of these, in turn, were either totally *Bz* or totally *bz*. Because of the constitution of these kernels with respect to the other genetic markers carried in chromosome 9, it was possible to refer the changed expression in each case to an event that had occurred, in a cell of the heterozygous parent plant, at the bronze locus where *Ac* resides. Some of these kernels were selected from the ears and plants were grown from them. These plants, in turn, were tested for presence or absence of *Ac*, for location of *Ac* if present, and for stability of the mutant expression. Among 16 *Bz* mutants selected, 14 proved to be stable. In six of the plants derived from these 14 *Bz* kernels, no *Ac* was present. In five plants, one *Ac* was present, but its position was altered. In four of these five plants, it was no longer linked with genetic markers carried in the short arm of chromosome 9, and in the fifth plant it was very closely linked with *Wx*. In the remaining three plants, *Ac* was present and its position was close to the locus of *Bz* but probably a short distance to the right of it. Thus, in at least 11 of these 14 cases of mutation to stable *Bz*, the association of mutation with removal of *Ac* from the bronze locus could be established with certainty, and in five of these cases the event could be related to transposition of *Ac* to a new location in the chromosome complement. It is probable that transposition of *Ac* to a new location was also associated with the mutation-inducing event in the six cases where *Ac* was absent in the plant derived from a *Bz* kernel. If this event occurred in a sporogenous cell before meiosis, segregation of the chromosome

carrying the *Ac* element in its new location at the following meiotic divisions could have resulted in the production of a gamete in which the *Bz* mutant was present but *Ac* was absent.

Twenty-four independent cases of mutation to a stable recessive were also examined. In nine of them, no *Ac* was present in the gamete that carried the stable recessive. In five cases, one *Ac* was present but it showed no linkage with markers in the short arm of chromosome 9. In nine cases, one *Ac* was present and showed linkage with these markers. In two of these nine cases, *Ac* was located close to *Wx*, and in one case it was located very close to *Sh*. In the remaining six of these nine cases, its exact location was not determined; it was linked with *Wx* and showed from 20 to 30 per cent recombination with it. In the remaining case of the 24 examined, two *Ac* elements were present, one located close to *bz*, and the other showing no linkage with markers in the short arm of chromosome 9. Thus, again, in this test, mutation could be associated with a change occurring to the *Ac* element at the bronze locus; for its removal from this locus was established with certainty in 17 of the 24 cases, and in eight of them the transposition of *Ac* to a new location could be determined.

It is obvious from the accounts given above that by selection of mutants one can also select for transpositions of *Ac*, and that this method is highly efficient. Somewhat similar results were obtained in tests of *Ds* in the case of c^{m-1} (*Ds* at the *C* locus). The majority of mutations to *C* were found to be associated with removal of *Ds* from the *C* locus, although its insertion at new locations could not be detected in most of the cases. Appropriate genetic markers that would have allowed detection of such insertion were not present in the tested plants.

Another useful selective method for detecting transpositions of controlling elements takes advantage of crossover techniques. For instance, a case was found in which *Ac* was inserted close to but to the left of *Wx*. Various tests were conducted to determine its location by means of crossover techniques. Recombinants appeared in about 10 per cent of the gametes of the plants tested; but this figure did not represent the true crossover value, as the example below will illustrate. In one test, the plants having *Ac* had the constitution *C Ac Wx* in one chromosome 9 and *C wx* and no *Ac* in the homologue; no other *Ac* element was present in these plants. When they were crossed with plants homozygous for *I*, *wx*, and *Ds* (located just to right of *wx*), and having no *Ac*, the majority of the *Wx* class of kernels on the resulting ear showed variegation for *C* areas in a colorless background. These kernels carried *Ac*. The *C* areas were produced by responses of *Ds* in the *I wx*-carrying chromosome to *Ac*, which resulted in dicentric-acentric formations at the locus of *Ds*. *I*, carried in the acentric fragment, was eliminated from daughter nuclei in the mitotic division which followed this event. The majority of the *wx* class of kernels, on the other hand, were totally colorless, since no dicentric-acentric chromatid formations occurred at *Ds* because no *Ac* was present in the nuclei of the endosperm. However, a few of the *Wx* kernels were nonvariegated (no *Ac*) and a few of the *wx* kernels showed *C* areas (*Ac* present). These last two classes of kernels represented the recombinants. When the plants grown from the variegated kernels in the *wx* class were tested for *Ac* location, its position, in about half of them, was no longer just to the left of *wx*, as might be expected from a crossover event. Instead it occupied a new location in the chromosome complement. In the other half of these plants, its location was that expected from crossing over. In other words, in this test, about half of the "recombinants" arose not from crossing over but rather from a transposition of *Ac*; and this probably occurred before the meiotic divisions and as a consequence allowed the *Ac* in the new location to be segregated at meiosis with a *wx*-carrying chromosome. Other examples illustrating this type of selective method will be considered in the following discussion of modes of detection of transpositions of the controlling element *Spm* (Suppressor-mutator) in the *Spm*-a_1^{m-1} system of gene control.

A series of studies has been made of transposition of the *Spm* element in the *Spm*-a_1^{m-1} system of control of gene action at the A_1 locus in chromosome 3, and it indicates that transposition of this element occurs with a high frequency. In this respect, it resembles the *Ds* and *Ac* elements, which also may undergo frequent transposition. The action of the *Spm*-a_1^{m-1} system was outlined in previous reports (McClintock, 1955, 1956), but its origin and mode of operation will be reviewed here. A modification at the standard A_1 locus in a sporogenous cell of a plant in a maize culture under investigation resulted in change in action of the genic materials at that locus. The change was discovered in a single kernel on an ear produced by the plant when it had been crossed by a plant homozygous for the standard recessive, a_1. Instead of being fully colored, as expected, this kernel was variegated for colored areas in a colorless background. The plant arising from this kernel also exhibited variegation for anthocyanin pigmentation. Subsequent tests indicated the mode of control of gene action at this modified A_1 locus. The basic mechanism involves two controlling elements: one resides at the modified A_1 locus (designated a_1^{m-1}) and directly controls gene action and the types of change in this action that may subsequently occur; and the other is an independently located element, designated Suppressor-mutator (*Spm*), which can modify this action in two distinctly different ways. When the *Spm* element is present, no anthocyanin pigment is formed. All action of the genic materials

at the a_1^{m-1} locus is inhibited by its presence until, in some cells, a mutation occurs at a_1^{m-1} that allows the genic materials to be active, the type of activity being a reflection of the type of modification produced by the controlling element at a_1^{m-1}. The expression of the gene induced by this modification is thereafter stable in the presence of *Spm* and a stable mutation is thereby effected. In the absence of *Spm*, on the other hand, the genic materials at the a_1^{m-1} locus are capable of some degree of activity and the kernels and plants are uniformly pigmented. This type of gene action at a_1^{m-1} is quite stable in the absence of *Spm*, and will be expressed without change in successive plant generations. When, however, *Spm* is again introduced into the endosperm and zygote nuclei having a_1^{m-1}, suppression of gene action is again made evident and the capacity of the *Spm* element to initiate stable mutation-type changes at a_1^{m-1} is also made evident. Unlike *Ac*, *Spm* does not show dosage effects, and the number of *Spm* elements present in a plant is indicated only by progeny tests. At least three and probably more independently located *Spm* elements were present in the initial plant having a_1^{m-1}, and because of this the majority of gametes produced by this plant, and by many of its progeny plants, carried *Spm*. Its detection was therefore obscured in the initial tests of a_1^{m-1}. Only after several generations of crosses to tester plants having no *Spm* was it possible to recognize this element, for individuals were then isolated that had only one or two *Spm* elements, and the part this element plays in the control of gene action and mutation at a_1^{m-1} was then clearly revealed. Clarification of the behavior of this system of control of gene action was thereafter readily accomplished.

When plants homozygous for a_1^{m-1} and having one *Spm* element are crossed by plants having no *Spm* but homozygous for the standard recessive, a_1, which is stable in its presence, the ratio of kernel types on the ears produced will usually approximate one uniformly colored (no *Spm*) to one variegated for deep colored spots in a colorless background (*Spm* present). If two independently located *Spm* elements are present, the ratio of kernel types approximates one uniformly colored (no *Spm*) to three variegated (*Spm* present); and if more *Spm* elements are present, the ratio of kernel types deviates in the expected manner. Again, tests of *Spm* constitutions may be made with plants that are homozygous for the standard a_1 allele (and therefore do not directly reveal the presence or absence of *Spm*) if these plants are crossed by ones homozygous for a_1^{m-1} but carrying no *Spm*. The ratios of uniformly colored to variegated kernels on the resulting ears reflect the *Spm* constitutions in the plants. Thus, plants homozygous for a_1^{m-1} and carrying no *Spm* can serve as tester parents in crosses made for the purpose of determining the presence or absence of *Spm* in individual plants and its numbers when present. If the plants being tested are heterozygous for other genetic markers, and if the tester stock carries the recessive alleles, linkage of an *Spm* element with one such marker may be detected after it has been inserted into the chromosome that carries this marker. By this means it was possible to detect linkages of *Spm* with genetic markers carried in chromosome 5, in chromosome 6, and in chromosome 9.

In plants that are a_1^{m-1}/a_1^{m-1} or a_1^{m-1}/a_1 in constitution and that have a single *Spm* element, large sectors may appear, some of which exhibit the pigmented phenotype that is characteristically produced in the absence of *Spm*. It can be shown that these sectors arise through loss of the *Spm* element from a cell early in development of the plant. The progeny of such a cell may contribute to the development of the ear, producing either all of it or only a part of it. When the ear is used in a cross with a plant that is homozygous for a_1^{m-1} but carries no *Spm*, in the former case all the kernels on the ear will be uniformly colored (no *Spm*), and in the latter case all the kernels within a well-defined sector will be uniformly colored (no *Spm*). If the plants derived from these uniformly colored kernels are again tested for *Spm* constitution, its absence in them may be verified. That they carry an a_1^{m-1} locus capable of responding to *Spm* may be shown by crossing them to plants that are homozygous for the standard a_1 allele and have one or more *Spm* elements. The typical variegated pattern of deeply pigmented areas in a nonpigmented background will appear in all kernels that have received a_1^{m-1} from one parent and *Spm* from the other.

In order to detect some of the changes in *Spm* constitution that may occur early in development of a plant, tests were conducted to determine its constitution in the cells that gave rise to an ear on the main stalk of the plant and also in those that produced an ear on one or more of its tillers (side branches). Tests of two ears per plant were obtained from 101 plants. In 95 of them, the number of *Spm* elements was the same in the cells that produced each ear (63 with one *Spm*; 26 with two *Spm*; 6 with three *Spm*). In six plants, the *Spm* constitution was not the same in the cells that gave rise to each ear (one case of one *Spm* in one ear and no *Spm* in the other; three cases of one *Spm* in one ear and two *Spm* in the second; two cases of one *Spm* in one ear, the second ear having a sector with no *Spm*). From twelve other plants, tests of three ears per plant were obtained; and correspondence in number of *Spm* elements was evident in each of the three ears of eleven of them (6 with one *Spm*; 4 with two *Spm*; 1 with three *Spm*). In one plant, the cells that gave rise to two ears carried one *Spm* element but the cells that gave rise to the third ear had two *Spm*.

Tests were also conducted to determine *Spm* constitutions in progeny of plants in which one, two, or three *Spm* elements were known to be

present. Those conducted with 249 individuals in the progeny of plants having one *Spm* will illustrate the type of result obtained. The parent plants carrying *Spm* had been crossed by plants that were homozygous for a_1^{m-1} but had no *Spm*. A ratio on the resulting ears of one uniformly pale-colored kernel (no *Spm* present) to one that showed spots of deep color in a colorless background (*Spm* present) indicated the presence of one *Spm* element in the cells that gave rise to the ears. Variegated kernels were selected from these ears, and the plants grown from them were again crossed by plants homozygous for a_1^{m-1} but having no *Spm*. From this test it was learned that one *Spm* element was present in the cells that gave rise to the ear in 215 of these plants. In 20 plants two *Spm* elements were present, and in six plants three *Spm* elements were present. In the remaining eight plants, no *Spm* was present in any part of the plant. It appears from these tests that many of the modifications affecting *Spm* constitution occur in individual cells relatively late in development and may occur even in the gametophytic cells of the plants.

In order to examine more precisely these changes in *Spm* constitution, tests were made of the progeny produced by plants having one *Spm* element at a known location in the chromosome complement. *Spm* may be located at various positions, and several different positions in chromosome 5, chromosome 6, and chromosome 9 have been identified. Tests of the progeny of plants having *Spm* at these different locations were conducted. Several examples will illustrate the kinds of information such tests can give.

One plant that carried a single *Spm* element was *Y*/*y* in constitution. The test cross made with it indicated that this *Spm* element was linked with *y*. On the ear produced by the test cross, one of the two recombinant classes of kernels was *Y* and exhibited the variegated phenotype (*Spm* present). Plants were grown from some of the kernels in this recombinant class, and these, in turn, were tested for *Spm* constitution and location. The results obtained from one such plant are given in Table 1. This plant, a_1^{m-1} Sh_2/a_1sh_2, *Y*/*y* in constitution, was used as a female parent in a cross with a plant whose constitution was a_1^{m-1} sh_2/a_1sh_2, *y*/*y* and which had no *Spm* element. Linkage of *Spm* with *Y* was clearly expressed in those kernels carrying a_1^{m-1} in both the Sh_2 and the sh_2 classes. As expected, kernels homozygous for the standard a_1 allele were completely colorless, for this allele is stable in the presence of *Spm*. Plants were grown from 22 of the variegated kernels in the Sh_2 *Y* class, and these in turn were crossed by plants homozygous for a_1^{m-1}, Sh_2, and *y*, and having no *Spm*. This test cross was made in order to determine whether or not *Spm* would continue to show linkage with *Y* and, if so, the number of individuals that would show it. In 19 of these 22 plants, one *Spm* element was found to be present, and it was linked with *Y* in each of them. The percentages of recombinant classes of kernels were similar on the ears produced by all of these plants. Among a total of 7569 kernels produced by these ears, there were 3847 uniformly pale-colored kernels (no *Spm*), of which 683 were *Y* and 3164 *y*. Among the 3717 variegated kernels (*Spm* present) on these ears, 3033 were *Y* and 684 were *y*. In addition, there were five deeply colored kernels, which may be attributed to germinal mutation at a_1^{m-1}, for a few such mutations are to be expected. The recombinants were 18.0 per cent of the total, and this percentage compares well with that of the parent ear (see Table 1) which was 16.3. In two of the 22 tested plants, two *Spm* elements were present and one of them was linked with *Y* (39 pale colored, *Y*: 118 pale colored, *y*: 315 variegated, *Y*: 181 variegated, *y*). The remaining plant had one *Spm*, but it showed no linkage with *Y* (117 pale colored, *Y*: 112 pale colored, *y*: 101 variegated, *Y*: 100 variegated, *y*; and in addition 2 deeply colored kernels attributable to germinal mutation).

A much more extended series of tests of the type just outlined was conducted with progenies of plants carrying *Spm* in chromosome 6 but at another location in the chromosome, and these tests were extended into the fourth generation. A description would require more space than is justified here; it need only be stated that, in general, the results obtained were similar to those described above. In the interest of further clarification of the behavior of *Spm*, however, we should present an example of this kind of test made with plants carrying the *Spm* element in a different chromosome of the complement. In the following case, it was located in chromosome 5.

The silks of one ear of a plant that was a_1^{m-1} Sh_2/a_1sh_2, *Pr*/*pr* in constitution and carried *Spm*, received pollen from a plant that was homozygous for a_1, sh_2, and *pr* and had no *Spm* (cross 1). Another ear of this plant was used in a cross with a plant of similar constitution except that it was

TABLE 1. TEST CROSS INDICATING LINKAGE OF *Spm* WITH *Y* IN CHROMOSOME 6

♀ $a_1^{m-1}Sh_2/a_1sh_2$; *Y Spm*/*y* × ♂ $a_1^{m-1}sh_2/a_1sh_2$; *y*/*y*; No *Spm*

	Phenotypes of Kernels						Totals
	Pale aleurone (No *Spm*)		Colorless aleurone with spots of deep color (*Spm*)		Colorless aleurone		
	Y	*y*	*Y*	*y*	*Y*	*y*	
Sh_2 class	20	113	111	30	0	0	274
sh_2 class	10	65	59	8	77	72	291
Totals	30	178	170	38	77	72	565

homozygous for *Pr* (cross 2). The kernel types appearing on the ear produced by cross 1 indicated linkage of *Spm* with *Pr*. Plants were then grown from some of the variegated kernels in the Sh_2, *Pr* class on each of these ears, and these plants, in turn, were tested for *Spm* constitution. Examples of the results of tests of several of these plants are given in Table 2 (plant 6684D-1 and some of its progeny from cross 1, plant 6685F-2 and some of its progeny from cross 2, and also progeny of plant 6685G-2 from cross 2). Plant 6684D-1 proved to be a_1^{m-1} Sh_2/a_1sh_2, *Pr/pr* in constitution, and it had one *Spm* that was located in the chromosome 5 carrying *Pr*. Its linkage with *Pr* was evident in all the test crosses (rows 1 to 3 under A of Table 2). Plant 6685F-3 was also *Pr/pr* in constitution, but the *Spm* element was linked with *pr* (B, Table 2). The location of *Spm* in the *pr*-carrying chromosome may be attributed to a crossover in the parent plant carrying *Spm*, which introduced it into a *pr*-carrying chromosome. Most of the variegated plants in culture 6685 (derived from cross 2) were *Pr/Pr* in constitution. The *Spm* element in most of them could be expected to be located in one of the two *Pr*-carrying chromosomes. The results of tests of one plant of the culture, 6685G-2, which was a_1^{m-1} Sh_2/a_1sh_2, *Pr/Pr* in constitution, were as follows. This plant was crossed by one that was homozygous for a_1^{m-1}, Sh_2, and also for the recessive, *pr*, and had no *Spm*. The kernel types on the ear produced by this cross (132 pale-colored kernels: 137 kernels that had deep-colored spots in a colorless background) indicated that plant 6685G-2 had one *Spm*. All the kernels, however, were *Pr* in phenotype. Twenty plants were grown from the variegated kernels on this ear, and each was tested for *Spm* constitution and for the linkage of *Spm* with *Pr*. *Spm* was found to be present in 17 of the 20 plants but absent in three of them. Fifteen of these 17 plants carried a single *Spm* element; in 14 of them it was linked with *Pr* (rows 1 and 2 under C of Table 2), and in one it gave no evidence of linkage with *Pr* (row 3, C, Table 2). The remaining two of the 17 *Spm*-carrying plants had two *Spm* elements, one of which was linked with *Pr* (rows 4 and 5, C, Table 2).

The kernel types produced by the crosses shown in A of Table 2 indicated that *Spm* in plant 6684D-1 was located in the chromosome 5 that carried *Pr*. In order to determine whether or not this linkage would be expressed in the following generation, plants derived from the *Pr* class of variegated kernels produced by the crosses entered in lines 2 and 3 of A of Table 2 were examined for *Spm* constitution and location. Among the eleven tested plants in the progeny of the cross given in line 3, one did not have *Spm*. Each of the remaining ten plants had one *Spm*, and its linkage with *Pr* was clearly expressed in nine of them. The phenotypes of the kernels on the ears produced by these plants, after the given test cross, are entered in row 1 under D of Table 2. The *Spm* element in one plant did not show linkage with *Pr* (row 2, D, Table 2). In the second test, all ten of the examined plants derived from the variegated kernels in the *Pr* class on the ear produced by the cross given in line 2 of A, Table 2, carried *Spm*. In nine of them, one *Spm* was present, and in eight of these it was obviously linked with *Pr* (row 3, D, Table 2); in the ninth plant no linkage with *Pr* was observed (row 4, D, Table 2). In the remaining plant, three *Spm* elements were present, as the ratio of kernel types entered in row 5, D, Table 2 will indicate.

In plant 6685F-3, which was *Pr/pr* in constitution, *Spm* was found to be linked with *pr* (B, Table 2). Most probably, it was introduced into the *pr*-carrying chromosome as a consequence of crossing over. The variegated kernels in the *Pr* class on the ear produced by the cross given in B, Table 2, represent recombinants, and plants derived from ten of them were examined for *Spm* constitution and location. *Spm* was found to be present in nine of these ten plants, and absent in one of them. One *Spm* was present in eight of the nine plants, but in only four of them was it linked with *Pr*. The combined ratios of kernel types on the ears produced by these four plants are entered in row 1 of E, Table 2. In the remaining four plants having one *Spm*, no linkage with *Pr* was exhibted (rows 2 and 3, E, Table 2). In one plant, three or four *Spm* elements probably were present, as the ratio of kernel types on two tested ears of this plant indicates (row 4, E, Table 2). It was mentioned earlier, in connection with transposition of *Ac*, that selection of recombinants is an effective method of detecting transposition of this controlling element. The example given above illustrates the usefulness of this method for detecting transpositions of *Spm*. Another illustration of the effectiveness of this selection method will be outlined below.

A variegated plant that was a_1^{m-1}/a_1^{m-1}, *Wx/wx* in constitution was crossed by a plant homozygous for a_1^{m-1} and for *wx* but carrying no *Spm*. On the ear produced as the result of this cross, there were 365 kernels; 196 of them were uniformly pale colored (no *Spm*), and 169 had deep-colored spots in a colorless background (*Spm* present). It may be concluded that one *Spm* element was present in the plant that produced this ear. Among the pale-colored kernels, 156 were *Wx* and 40 were *wx*, whereas the ratio in the variegated class of kernels was 29 *Wx* to 140 *wx* (A, Table 3). Linkage of *Spm* with the *wx* allele carried in one chromosome 9 is obvious. Nine plants derived from the variegated kernels in the *Wx* class (the recombinant class) were examined for *Spm* and for its location. All of them carried *Spm*, and in seven of them one element was present. In none of the nine ears obtained from test crosses of these seven plants, however, was there any evidence of linkage of *Spm* with *Wx*. The com-

TABLE 2. TESTS OF *Spm* CONSTITUTION AND LOCATION IN THE PROGENY OF PLANTS CARRYING *Spm* IN CHROMOSOME 5

See text for origin of plants in A to E below, for the constitutions of plants entered in B to E, and for test crosses made with them.

Crosses		Phenotypes of Kernels						
		Pale Sh_2 (No *Spm*)		Colorless with spots of deep color: Sh_2 (*Spm* present)		Colorless sh_2	Germinal mutant; Sh_2	Totals
♀	♂	*Pr*	*pr*	*Pr*	*pr*			
A. Types of kernels on ears produced by test crosses of plant 6684D-1 which was $a_1^{m-1}Sh_2/a_1sh_2$, *Pr Spm/pr* in constitution								
6684D-1	a_1sh_2/a_1sh_2 ; *pr/pr*; No *Spm*	50	90	91	48	321	2	602
a_1sh_2/a_1sh_2 ; *pr/pr*; No *Spm*	6684D-1	120	223	214	114	622	5	1298
$a_1^{m-1}Sh_2/a_1^{m-1}Sh_2$; *pr/pr*; No *Spm*	6684D-1	80	188	114	46	—	1	429

Spm Constitution of Plants	No. of Plants	Phenotypes of Kernels					
		Pale (No *Spm*)		Colorless with spots of deep color (*Spm* present)		Germinal mutant	Totals
		Pr	*pr*	*Pr*	*pr*		
B.							
1 *Spm*	1	275	91	67	214	4	651
C.							
1 *Spm*	6	434	1646	1512	415	9	4016
1 *Spm*	8	1798 (*Pr* and *pr*)*		1429	341	2	3570
1 *Spm*	1	281 (*Pr* and *pr*)*		114	132	0	527
2 *Spm*	1	49	105	209	148	1	512
2 *Spm*	1	128 (*Pr* and *pr*)*		132	87	1	348
D.							
1 *Spm*	9	429	1174	1133	366	3	3105
1 *Spm*	1	67	73	71	77	0	288
1 *Spm*	8	1089 (*Pr* and *pr*)*		616	251	1	1957
1 *Spm*	1	234 (*Pr* and *pr*)*		98	99	1	432
3 *Spm*	1	31	36	167	176	0	410
E.							
1 *Spm*	4	149	550	510	113	1	1323
1 *Spm*	3	321	290	248	254	1	1114
1 *Spm*	1	185	171	134	152	1	643
3 or 4 *Spm*	1	15	14	235	246	2	512

* In some crosses, difficulty was encountered in discriminating between *Pr* and *pr* kernels because of segregation of another factor that modifies pigment color in *pr/pr* kernels but only, however, in the pale class.

bined ratio of kernel types on these ears is given in row 1 under B of Table 3. One plant had two *Spm* elements, but linkage with *Wx* was not indicated in either of the two tested ears of this plant (line 2, B, Table 3). The ratio of kernel types on two ears produced by the remaining plant indicated the presence in it of three *Spm* elements, as shown in row 3, B, Table 3. When such a high number of *Spm* elements is present in a plant, evidence of linkage of one of them with a given marker is obscured. Thus it cannot be stated whether or not in this plant one of the elements was linked with *Wx*. It is clear, however, that there is no evidence among these recombinants of linkage of *Spm* with *Wx*. It may be concluded that the *Spm* element in the parent plant was located very close to *wx* and that the recombinants appearing on the ear of this plant arose mainly from a premeiotic transposition of the *Spm* element to a new location, which allowed it to be segregated at meiosis with the chromosome carrying the *Wx* allele.

In Table 2, the results of tests of 47 variegated plants are entered. The variegated plant was used as female parent in test crosses of 46 of them. In eight of these 46 plants, two ears per plant were used for such tests, and agreement with regard to *Spm* number and location was shown by the two ears in seven of the eight plants. In one plant, the kernel types on the ear produced by the main stalk indicated the presence of two *Spm* elements, one of which was linked with *Pr*, and these are entered in line 5 under C of Table 2. Tests of the tiller ear of this plant indicated the presence of only one *Spm* in the cells that gave rise to it, and this *Spm* was linked with *Pr* (9 pale colored, *Pr*: 84 pale colored, *pr*: 69 variegated, *Pr*: 11 variegated, *pr*). In two additional plants, three ears were used for the test cross, and agreement with regard to *Spm* number and location was shown in all three ears produced by each plant. The nine plants that gave the kernel types entered in B of Table 3 were all used as female parents in the test cross, and in four of them two ears per plant were so tested. Agreement with regard to *Spm* number was shown by both ears produced by each of the four plants.

Before we leave the subject of methods of detection of transposition of controlling elements, one other fact should be emphasized. If, in any one test, the controlling element is found to be linked to a known marker, and if other known markers are present in the same chromosome, linkage with them will also be exhibited, and in the expected manner. If genetic markers on other chromosomes are also present, and are followed in the tests, no linkage with them will be exhibited. In other words, the basic mode of inheritance is like that of other known genetic markers, because the controlling element resides at a particular locus until some event causes it to be transposed elsewhere, where its position may again be

TABLE 3

Spm Constitution of Plants	No. of Plants	Phenotypes of Kernels: Pale (No *Spm*) *Wx*	Pale (No *Spm*) *wx*	Colorless with spots of deep color (*Spm* present) *Wx*	Colorless with spots of deep color (*Spm* present) *wx*	Germinal mutants	Totals
A. Test cross indicating linkage of *Spm* with *wx* in chromosome 9 ♀ a_1^{m-1}/a_1^{m-1}; *Wx/wx Spm* × ♂ a_1^{m-1}/a_1^{m-1}; *wx/wx*; No *Spm*							
	1	156	40	29	140	0	365
B. Tests of *Spm* constitution in 9 plants derived from variegated kernels in the *Wx* class of A, above. All plants were used as females in crosses with plants that were a_1^{m-1}/a_1^{m-1}, *wx/wx*, and that had no *Spm*							
1 *Spm*	7	860	953	801	824	1	3439
2 *Spm*	1	52	66	197	208	0	523
3 *Spm*	1	46	36	402	377	0	861

identified. The time of occurrence of these changes in location, during development of a tissue, and the frequency of their occurrence, depend on several factors: dose (the *Ac* element and the control it exerts on transpositions of *Ds*), environmental conditions such as temperature and nutrition (Eyster, 1926; Rhoades, 1941; van Schaik, 1954), the genetic background (Brink, personal communication), and the location of the element in the chromosome complement (see account of nontransposing controlling elements in McClintock, 1956).

The Structure of Controlling Elements

Controlling elements and gene elements are alike in one important aspect, and this is related to the replication of their structural organization during chromosome reduplication. With respect to both types of elements, the newly constituted chromosome is a replica of the parent chromosome, provided no event occurs to alter the structure of the controlling elements or that of the locus where it may rseide; and in most division cycles such events do not occur. The most effective demonstration of maintenance of organization of a controlling element through successive mitotic cycles is provided by that element of a two-element system which undergoes modification only when the second element of the system is also present, such as *Ds* in the *Ds-Ac* system, or the element at the A_1 locus in the a_1-*Dt* system and the a_1^{m-1}-*Spm* system. In the absence of *Ac*, *Dt*, or *Spm*, in each of these systems the organization at the locus where the complementary controlling element resides must be replicated without altera-

TABLE 4. EXAMPLES OF TYPES OF ALLELES OF A_1 PRODUCED BY THE OPERATION OF THE *Ds-Ac* SYSTEM, THE a_1-*Dt* SYSTEM, AND THE a_1^{m-1}-*Spm* SYSTEM OF CONTROL OF GENE ACTION AT THE A_1 LOCUS IN CHROMOSOME 3

A. The *Ds-Ac* System Example: a_1^{m-3}

Phenotype produced in absence of *Ac*	Phenotype produced when 1 *Ac* is present
Allele 1 Colorless kernel. No anthocyanin pigment in plant. No mutations.	Allele 1 Dots of the full A_1-type expression in restricted regions of kernel. Few streaks of A_1 pigment in plant. Few germinal mutations to higher alleles of A_1 . Some chromosome "breaks" at a_1^{m-3} locus.
Allele 2 Uniformly light pale-colored kernel and lightly pigmented plant. No mutations.	Allele 2 Numerous spots of various sizes showing deep pigmentation in pale-colored background in kernel. Sectors of deep pigmentation in pale-colored background in plant. Many germinal mutations to higher alleles of A_1 . Few if any "breaks" at a_1^{m-3} locus.
Allele 3 Uniformly pigmented kernel and plant. Intensity of pigment much darker than that given by allele 2. No mutations.	Allele 3 Similar to allele 2 above except that mutant spots in kernels and sectors in plants appear in background coloration of medium intensity.

B. The a_1-*Dt* System

Phenotype produced in absence of *Dt*	Phenotype produced when 1 *Dt* is present
Allele 1 (standard a_1) Colorless kernel. No anthocyanin in plant. No mutations.	Allele 1 Few dots of deep A_1-type in pigmentation in colorless background in kernel. Fine streaks of A_1-type pigmentation in plant. Few germinal mutations to higher alleles of A_1 and to stable recessives. Most germinal mutants are stable in presence of *Dt*.
Allele 2 Colorless kernel. No anthocyanin in plant. No mutations.	Allele 2 Large number of pigmented spots or dots in colorless background in kernel. These show various grades of intensity of pigmentation from light to deep. Plant shows many sectors of mutant tissue in nonpigmented background. Many germinal mutations to alleles giving various grades of intensity of pigmentation, the majority of which are stable in the presence of *Dt*; a few, however, are unstable.

C. The a_1^{m-1}-*Spm* System

Phenotype produced in absence of *Spm*	Phenotype produced in presence of *Spm*
Allele 1 Colorless kernel. No anthocyanin pigment in plant. No mutations.	Allele 1 Many areas of different sizes showing various low levels of intensity of pigmentation in colorless background in kernel and in nonpigmented background in plant. Many germinal mutations to give alleles producing these low levels of pigment intensity. Mutants so obtained are stable in presence of *Spm*.
Allele 2 Very pale color in kernel. Intense anthocyanin pigmentation in plant. No mutations.	Allele 2 Small dots of full A_1-type pigmentation in colorless background in kernel. Fine streaks of $A1$-type pigmentation in nonpigmented background in plant. Very few germinal mutations. These are stable in presence of *Spm*.
Allele 3 Intense pigmentation in both kernel and plant. No mutations.	Allele 3 Many medium-sized dots showing full A_1-type pigmentation in colorless background in kernel, and many fine streaks of A_1-type pigmentation in nonpigmented background in plant. Few germinal mutations to higher alleles of A_1 . These are stable in presence of *Spm*.
Allele 4 Pale color in kernel. Intense pigmentation in plant. No mutations.	Allele 4 Many spots and large areas of full A_1-type pigmentation in nonpigmented background in kernel, and numerous sectors and streaks of A_1-type pigmentation in nonpigmented background in plant. Many germinal mutations to higher alleles of A_1 that are stable in presence of *Spm*.

tion in each mitosis in the germ line, and because of this it is maintained through successive plant generations. Only when, by appropriate crosses, the second element of the system is introduced, will alterations occur, and then only in some cells of the plant. Illustrations of the type of evidence that has revealed this are given below.

When *Ds* was first discovered, many dicentric-acentric chromatid-forming events were observed at the locus of *Ds* when *Ac* was also present in the nuclei of a plant. The frequency of their occurrence was high and they could be observed readily in both the plant and the endosperm tissues. Gametes having no *Ac* were produced by such plants, and in the next generation those plants having *Ds* but no *Ac* showed no dicentric-acentric chromatid formations at the locus of *Ds*, nor any other type of change that would reveal its presence. When, however, *Ac* was reintroduced in a subsequent generation by an appropriate cross, the presence of *Ds* was revealed, because dicentric-acentric chromatids were now formed at the previously determined position of *Ds*, and the frequency of their occurrence was the same, with the same dose of *Ac*, as that observed before *Ac* had been removed. Besides dicentric-acentric chromatid formation at the locus of *Ds*, other types of change in the presence of *Ac* were also noted, and some of them effected transposition of *Ds* to a new location. Occasionally, another type of modification occurred at the locus of *Ds*, but again only when *Ac* was also present, and this was recognized by a decided change in the relative frequency of occurrence of the above-mentioned types of response of *Ds* to *Ac*. The location of *Ds*, however, was not altered by the event that was responsible for this. If, by meiotic segregation, *Ac* was removed from the chromosome complement that carried such an altered *Ds*, the particular modification responsible for it was maintained without further change through successive plant generations. This was made evident because return of *Ac* in a later generation elicited from it the very same pattern of response that it had given before *Ac* was removed. Through selections based upon such clear-cut changes in the particular types of response of *Ds* to *Ac*, it was possible to isolate several different alleles of *Ds*, and to maintain them unaltered in successive generations in plants that did not have *Ac*. The behavior of each of them, when *Ac* was returned to the nucleus, could be predicted in advance if its behavior before the removal of *Ac* was known. Such predictions are possible only if the particular organization at the locus of *Ds* in each such case is replicated in each successive mitotic cycle in the germ line. It is difficult in the face of such evidence to avoid the conclusion that these alleles of *Ds* reflect organizational and thus structural differences either of the *Ds* element itself or of other components at the locus where it resides, even though the type and dimension of such differences are not yet known.

In all examined cases where a two-element system of control of gene action is known to operate, changes have been noted in the type of response of the element at the affected gene locus, and isolates showing different types of change have been made. The different isolates so far tested have behaved in inheritance as alleles of one another; and the type and pattern of response shown by each, when the second element of the system is present, is predictable. Table 4 was constructed in order to illustrate some of the kinds of differences these isolates may exhibit. Each of the alleles listed in this table demonstrates the presence of a particular type of organization at the gene locus where the controlling element resides. This organization is reproduced in each successive mitosis, and this is maintained through successive plant generations, provided the complementary element of the system concerned is absent.

It might be considered that a controlling element represents some kind of extrachromosomal substance that can attach itself or impress its influence in some manner at various positions in the chromosome complement and so affect the action of the genic substances at these positions. The modes of operation of controlling elements do not suggest this, however. Rather, they suggest that controlling elements are integral components of the chromosomes themselves, and that they have specific activities and modes of accomplishing them, much as the genes are presumed to have. Any proposed view of the structure and organization of controlling elements must consider not only the origin and maintenance of distinct alleles, as described above, and the modes of interaction exhibited by two-element systems, but also the nature of the change responsible for alteration in gene action that appears after either insertion or removal of a controlling element at a known gene locus. Insertion need not effect mere inhibition of gene action, as the analysis of the *Spm-*a_1^{m-1} system illustrates. Also, removal of the identifiable controlling element is not usually accompanied by exact restoration of the type of action shown by the genic substances before the controlling element appeared there, although sometimes it may give rise to a similar type of action. In many cases there is a clearly expressed difference, ranging from slight to marked. Thus, the presence of a controlling element at a gene locus need not effect mere inhibition of gene action, and its removal from the locus need not effect mere release of such inhibition. Other types of modification occur. Some form of organizational or structural change in chromosome materials must occur, both when a controlling element is present at a locus and also as a consequence of its removal. The available evidence suggests that the disappearance of an identifiable controlling element from a known location in a chromosome is associated with its appearance at a new location. The mechanism of transposition, then, is significant in any considera-

tion of the structures concerned, and our knowledge of this mechanism, inadequate as it is, should be evaluated.

The Mechanism of Transposition

Transposition of *Ds* from its first known position in the short arm of chromosome 9 to another position within that arm was detected very early in the study of the *Ds-Ac* system, and attempts were made to obtain information about the manner by which transposition is accomplished. Besides dicentric-active chromatid formation at the locus of *Ds*, other types of chromosomal aberration were noted in these early studies; they were made evident by the appearance of translocations, inversions, duplications, ring chromosomes, and deficiencies, but only when *Ac* was also present in the nucleus. In all such cases it was found that one of the two positions in the chromosome or chromosomes involved in the origin of the rearrangement was always at the previously identified locus of *Ds*. It was obvious, therefore, that the *Ds* element was primarily responsible for them. It was then decided to determine whether or not transposition of *Ds* accompanied such events, and for this purpose those cases that produced a duplication of a segment of the short arm of chromosome 9 were examined. They were chosen because the genetic markers located in this arm would allow the most precise analyses to be made. Three such cases were examined in detail, and from all three it was learned that transposition of *Ds* had accompanied the event that accomplished the duplication. A description of the origin and constitution of the chromosome having the duplication, in two of these three cases, was given in an earlier publication (McClintock, 1951) and need not be repeated here. It could be determined from a study of the three cases, however, that the *Ds* element that was inserted into the new location came from only one of two sister chromatids 9. It was also learned that the insertion of *Ds* at the new location was accompanied by its removal from its previous location. In each of the three examined cases, the involvement of sister chromatids in the origin of the duplication, the orientation of the duplicated segment, and the location of the two *Ds* elements suggested that contact of the locus of *Ds* with another locus in this chromosome preceded the event that produced the duplication and the transposition of *Ds*; and that the transposition could be associated with the mechanism of the subsequent chromosome reduplication itself. It was also learned from these cases that insertions of *Ds* into new locations could take place without effecting gross chromosomal rearrangements (see positions of *Ds* in diagrams given in McClintock, 1951).

The allele of *Ds* that gives rise to the types of chromosomal aberrations mentioned above is one that produces many dicentric-acentric chromatid formations at the locus of *Ds* itself, but only, of course, when *Ac* is also present in the nucleus. It might be considered that such configurations could arise either from lack of reduplication of the components at the locus where *Ds* resides, or from "stickiness" of these materials. The cases mentioned above indicate, however, that the *Ds* element is reduplicated when chromosomal aberrations occur, for the two *Ds* elements produced by the reduplication process can be accounted for even though one of them occupies a new location. This suggests that the dicentric-acentric chromatid formations might arise not from lack of reduplication at the locus but rather as a consequence of the reduplication mechanism itself. Some component of this particular allele of *Ds* may be so ordered that it will allow a reverse bonding between linearly arranged components during the reduplication process; this, in turn, would lead to the dicentric-acentric chromatid formations that are so clearly expressed in some cells of the somatic and sporogenous tissues of plants having the allele.

It should be emphasized, in this discussion of transposition, that the *Ds* element undergoes alterations which modify its type of action. These were mentioned earlier. Some of them give rise to an allele of *Ds* that no longer produces dicentric chromatids (or only a few of them) or other types of chromosomal rearrangement. Transpositions continue to occur, however. The controlling elements *Ac* and *Spm* also undergo frequent transpositions and these are not usually accompanied by chromosomal translocations. Thus it appears that transposition is not the consequence of "stickiness" at the locus where these elements reside; for, if it were, many cases of chromosomal aberration should have been detected in association with their transposition. The absence or infrequence of such cases is conspicuous.

One can conclude, then, that transpositions of controlling elements either arise from some yet-unknown mechanism or occur during the chromosome reduplication process itself and are a consequence of it. At present, the latter interpretation is favored, for it will account for many of the observations of change in number and location of controlling elements. If, in a plant having one such element at a known location, the element is transposed from one of two sister chromatids of one chromosome to a new location in one of two sister chromatids of another chromosome, segregation at the following mitotic anaphase could result in several different types of change in constitution of the element in the sister nuclei. Either one nucleus would have this element at the previous known location and the sister nucleus would have it at a new location, or one nucleus would have no element and the sister nucleus would have two, one at the previous known location and one at a new location. As a consequence of such segregations, cells could be formed having no such element, or one that was unchanged in its location,

or one at a new location, or two elements, one at the previously known location and one at a new location. In the gametes produced by plants having a transposable element, just such types of change in location and number of elements have been found; examples were given earlier. Changes in constitution of *Ac* occurring in somatic cells give rise to sectors that exhibit these changes, and in some of them it is clear that the dose of *Ac* has been increased. It is possible to observe that subsequent changes in *Ac* constitution may also occur, for subsectors showing them may appear within the larger sectors. If sequential transpositions of a controlling element occur in the cells of the germ line in the manner outlined above, gametes carrying more than two elements may be produced by plants whose zygote nuclei had but one; and examples of this have been found (see examples under D and E of Table 2).

If transposition occurs during the chromosome reduplication process, then the means by which it is accomplished is of considerable importance. It is conceivable that it is brought about by some mechanism similar to that proposed to account for transduction in bacteria (Demerec, Blomstrand, and Demerec, 1955; Demerec and Demerec, 1956) —a form of exchange or "crossing over" between the component contributed by the phage and that present in the bacterium, which requires a reduplication of both components. If such an event accounts for transposition, then reciprocal substitutions of components at the loci concerned should be produced as a consequence. There is evidence to suggest that such substitutions may occur. In the study of *Ac* at the bronze locus, described earlier, three cases of change in mode of control of mutation at this locus were noted. Each was associated with removal of *Ac* from the bronze locus, and in two of the three cases it could be established with certainty that *Ac* had been transposed to a new location. In two of the three cases, a recessive, bronze, phenotype appeared as a consequence of the removal of *Ac*; but instead of being stable in its presence, as in most such cases, this phenotype was stable only in its absence. In its presence, mutations to the higher alleles of *Bz* occurred, and the type of response was the same as that exhibited in other cases in which the *Ds-Ac* system of control of gene action operates. The mode of control in the third case was similar. In this case, however, removal of *Ac* was associated with partial expression of the genic substance at the bronze locus, for some *Bz*-type pigment appeared both in the plants and in the kernels having it. This expression was quite stable in the absence of *Ac*, but in its presence mutations occurred to give alleles that are associated with the appearance of higher or lower levels of intensity of the pigment. The origin of these three cases could be explained if a *Ds*-type element (one responding to *Ac*) had been substituted for *Ac* during the transposition process.

There is other evidence that should be mentioned in considering the possibility that substitution may accompany transposition, and this is related to the types of mutation that are produced when a known controlling element is removed from a locus. As stated earlier, the mutations so produced need not be alike; in some cases, wide differences in their mode of expression can be observed. It is possible that some of the differences they exhibit are the consequence of substitution of one type of controlling element for another. Only through further investigation, however, woult it be possible to verify this; and except in the three above-mentioned cases evidence is not yet available, although in several other cases there is evidence suggestive of it.

Positions in the Chromosome Complement at Which Controlling Elements May Be Inserted

It is known that controlling elements may be inserted at various locations within the chromosome complement. In order to learn whether these positions are randomly distributed or selectively located, a large number of independent transpositions of a particular element from a known location to new locations needs to be determined. Even though many sequential transpositions of the elements *Ds*, *Ac*, and *Spm* have been detected, the evidence obtained from any one of these elements is insufficient to allow definite conclusions to be drawn. The evidence does suggest a degree of nonrandomness, which, however, may merely reflect degrees of viability following upon insertions at particular positions rather than selectivity of positions at which the elements may be inserted. Such inviabilities were discovered in studies aimed at detecting the positions within the short arm of chromosome 9 into which *Ds* may enter. This arm carries genetic markers that allow easy detection in kernels of insertion of *Ds* between any two of them. Plants having *Ds* at a known location in this arm produce some gametes having *Ds* at new locations, some of which are also in the same arm. When plants carrying *Ds* and also dominant alleles of some of the known genetic markers in this arm were crossed to plants that were homozygous for the recessive alleles, kernels that arose from functioning of a pollen grain in which *Ds* occupied a new position in the arm were readily detected. The endosperm and embryo of a number of such kernels were quite normal in appearance, but other kernels were abnormal in various ways. Some of them were smaller than normal, others were germless or had defective embryos, and still others exhibited distorted growth in the endosperm tissues. Most of these kernels did not germinate. Some of the normal-appearing kernels also did not germinate. In other words, dominant lethality was exhibited among a number of kernels

having *Ds* at new positions within the short arm of chromosome 9. The new position of *Ds* could be verified only in plants derived from the kernels that did germinate. The positions occupied by *Ds* in these plants seemed not to be randomly distributed but to be clustered about certain locations within the arm. It is suspected that they represented only some of the positions into which *Ds* may enter, and that the insertion of *Ds* at other positions results in dominant lethality. This inference is supported by the results of examinations of cases that exhibited semidominant lethality of the following type. In endosperms with two normal chromosomes 9 not carrying *Ds* and a chromosome 9 with *Ds* at the new location, growth was so distorted that many kernels failed to mature. Only a few reached maturity but all of them were obviously aberrant in morphology. Plants could be obtained from some of the latter, however. Four independent cases of this type were examined, and in all four the semilethal effect was associated with a modification induced by *Ds* in a chromosome component located to the right of *Bz*. In these plants, the chromosome 9 carrying *Ds* was quite normal in morphology. The semidominant lethality was not due, primarily, to inhibition of gene action, for it is known that endosperms that are deficient for all of the short arm of one chromosome 9 develop normally. Some change in gene action other than localized inhibition was induced in these cases.

Because dominant lethality may be expressed when controlling elements are inserted at some positions in the chromosome complement, as described above, difficulties are encountered in attempts to determine whether a particular element can enter any site in the chromosome complement or is restricted to certain sites. At present, no definite statement can be made regarding this. It is known, however, that controlling elements may be inserted at a number of different positions. Those that seem to be preferred, on the basis of present knowledge, may represent only a selected number of possible sites at which the presence of the element does not induce inviability at some stage in development.

Influence of Controlling Elements in Modifying Gene Action

It is now known that controlling elements may modify gene action in a number of different ways. They may influence the time of gene action in the development of a tissue, and also determine the cells in which it will occur. Again, they may influence the type of action, with regard to either degree (quantitative aspects) or kind (qualitative) aspects). They may also act as inhibitors, suppressors, and modifiers, as well as inducers of types of change at a gene locus that resemble those often referred to in the past as point or gene mutation.

When a particular controlling element is inserted at a gene locus, its presence there may be detected by the changes in phenotypic expression that appear. Tests may then be conducted to determine the position in a chromosome at which the responsible changes are occurring, and thus the locus involved may be identified. Subsequent tests may be made to examine the mode of operation of the particular controlling system concerned with these changes and to determine its components. From such procedures it was learned that one system can operate at a number of different gene loci, and that the action at one gene locus may be controlled by different systems; the evidence has been presented in previous publications (McClintock, 1953, 1955, 1956).

When a controlling element is inserted at the locus of a known gene, a recognizable change in phenotypic expression may be observed as an immediate consequence, or no immediate change may result. In the latter case, the presence of a controlling element at the locus is detected subsequently, for it will initiate recognizable changes in gene action. The origins of a_1^{m-3} and a_1^{m-4} will illustrate this fact. Both arose from insertion of a *Ds*-type element (one responding to *Ac*) at the standard A_1 locus in chromosome 3, and the *Ds-Ac* two-element system controls gene action in both cases. In the case of a_1^{m-4}, inhibition of gene action probably occurred as an immediate consequence of insertion of a *Ds* element at the standard A_1 locus, as its presence there was made evident a few cell generations after that event occurred. In the case of a_1^{m-3}, on the other hand, insertion of the *Ds* element at the A_1 locus did not produce an immediate change in gene action. Its presence was revealed later, however, by altered expressions of the gene substance at the locus, which were recognized in some of the progeny of one particular plant of a culture. At least a full plant generation intervened between insertion of the *Ds* element at A_1 and recognition of its presence there. Had *Ac* been removed from the nucleus shortly after this insertion, the presence of the *Ds* element at the locus would not have been detected, for in the absence of *Ac* the phenotypic expression would have remained unaltered. Only by some fortuitous cross that again introduced *Ac* into a plant carrying this original state of a_1^{m-3} would the presence of *Ds* have been revealed, for only then would frequent change in gene action occur. Thus, the presence of a controlling element at a particular locus is revealed by the types of change that occur under given conditions, and if these conditions do not prevail its presence at the locus may not be recognized.

That the presence of a controlling element at a locus need not effect inhibition of gene action is also shown by studies of those cases in which *Ac* resides at the bronze locus in chromosome 9 and at the *P* locus in chromosome 1. The phenotype produced when these cases were initially recognized was that of the recessive, or null, expression. In both cases, mutations occurred, and the part

that *Ac* plays in these was reviewed earlier. It was found that removal of *Ac* from the locus concerned was associated in some cases with a change in gene action from one that gives the null expression to one that gives a high degree of activity, and also that the mutant so formed was subsequently stable in the presence of *Ac* when the latter was located elsewhere. Some of the mutants were not stable, however, and subsequent mutations occurred. In the case of *Ac* at the bronze locus, two of the 16 *Bz* mutants examined were of this type. From studies of both of them it was learned that the event that gave rise to the *Bz* expression did not result in removal of *Ac* from the immediate vicinity of the *Bz* locus and that *Ac* was responsible for the subsequent mutations that occurred. Some of these later mutations resulted in stability of the *Bz* expression, and in the three examined cases it was learned that *Ac* had been removed from the locus of *Bz*. Other changes were detected by the reappearance of the unstable recessive, and in the several examined cases it was learned that *Ac* was still present at the bronze locus. Still other types of mutant expression were noted, but these need not be discussed here. It is desired only to emphasize that the presence of a controlling element at a gene locus need not effect inhibition of action but may instead condition a mode of control of gene action in subsequent cell and plant generations, which will follow in a predictable manner. The ability to predict depends, of course, upon the extent of knowledge of the controlling system in operation in any one case.

The presence of a particular controlling element at a known gene locus can influence gene expression in different ways, which may range from complete suppression to various degrees of action. Moreover, the types of action may differ not only quantitatively but also qualitatively. There is evidence to suggest that in some cases these various types of mutation are reflections of modifications affecting different components of a compound locus, and that each component of the locus is concerned in its own way with development of one particular phenotype. Extensive evidence based on crossover studies of the compound nature of the A_1 locus in chromosome 3 has been presented by Laughnan (1949, 1952, 1955a, b) and evidence regarding the *R* locus in chromosome 10 has been obtained by Stadler and colleagues (Stadler and Nuffer, 1953; Stadler and Emmerling, 1954, 1956). Instability of expression of A_1 has appeared rather frequently, and cases of this have been examined by several maize geneticists (Rhoades, 1936, 1938, 1941, 1945; McClintock, 1951, 1953, 1956; Nuffer, 1955, 1956; Laughnan, 1956; Peterson, 1956; Richardson, 1956). On the basis of our present knowledge of the origin and behavior of such "unstable loci" it is inferred, when not determined with certainty, that a controlling element resides at the A_1 locus in each such case. The modifications in gene action it induces there can affect the action of one or another of the known components of this locus, or it may affect all of them simultaneously. Other gene loci, such as that of *C* in chromosome 9, also appear to be compound. Evidence of this was obtained by study of several distinguishable types of mutation that occur in the case of c^{m-2}. (For origin of this case and the controlling system involved, see McClintock, 1951.) These are associated with the production of at least two different diffusible substances, both of which are required for pigment formation. It was also noted that the dose expression given by the *C* allele commonly used in genetic studies is related to the limited production of one of these substances by this allele: the more *C* alleles present, the greater the amount of this substance formed, and, consequently, the denser the pigmentation. It is conceivable, then, that some of the qualitative differences in expression of mutants of a given locus reflect alterations in action of different components of a compound locus, and that a controlling element as the consequence of one event, may affect the action of only one component or of more than one; or the modification induced by any one event may affect the action of all of them.

It is now known that the presence of a controlling element at a known locus can effect change in gene action not only of the genic components located close to it, but also of other genic components located some distance to either side of it. A number of examples of this kind affecting gene action in a particular segment within the short arm of chromosome 9, extending over a region 5 or more crossover units in length have been examined; and these have been reviewed in previous publications (McClintock, 1953, 1954, 1955, 1956). Recently, Richardson (1956) found a case of "spreading effect" that appeared to be induced by a controlling element located at A_1 in chromosome 3. The nature of the changes responsible for such spread of mutation along the chromosome is of considerable importance for an understanding of the manner by which controlling elements can induce their effects, and those involving the segment of chromosome 9 that includes the loci of *I*, *Sh*, and *Bz* are particularly useful for this purpose. The "spreading effect" in these cases is known to be induced by the presence of a *Ds* element that is located just to the left of *Sh*; and it is also known that the mutation-inducing process is not accompanied by change in location of *Ds*. One might consider that the "spreading effect" is merely the expression of a deficiency for the loci involved, even though most such cases give viable homozygotes, or, barring this, that the organization of the chromosome segment concerned or the structure or organization of components within it is altered in some particular manner by the *Ds* element. Therefore, tests of some of these cases are being conducted in order to determine whether or not crossing over occurs within the

affected segment. It has been learned, in several of these cases, that either crossing over does not occur or its frequency of occurrence is very low. In others, however, crossing over takes place within the affected segment and the frequency of occurrence differs among the several examined cases. Plants derived from reciprocal crossovers are now under investigation in order to determine if mutant expressions of genic components within the affected segment will appear in their progeny, and if so, the types each may give. That separation by crossing over can occur between the individual genic components whose modification is responsible for the compound mutant expression has already been shown by Richardson in the case of the "spreading effect," mentioned above.

Examination of the mode of operation of two-element controlling systems has revealed the breadth of influence of such systems in modifying gene action. The study has concerned not only the number of different genes that such systems control, but also the manner of this control, which is of greater significance. It has been possible to examine the mode of operation of the *Ds-Ac* system at seven different gene loci (for references, see McClintock, 1953, 1956), and to learn that the system operates in essentially the same way in each case. The mode of control of gene action resides in the system itself. It is the *Ds*-type element at the locus of the gene that is directly responsible for control of gene action and for the changes that occur in gene action; and it is the *Ac* element of the system that is responsible for initiating these modifications where the *Ds* element resides, and also for the time of their occurrence. In some of the cases examined, the change in gene action is usually associated with removal of *Ds* from the gene locus, and stability of mutant expression in the presence of *Ac* is thereby effected (c^{m-1}, bz^{m-1} are examples). In other cases, the *Ds* element is not usually removed when a change in gene action is initiated, and, in the presence of *Ac*, subsequent changes may occur (c^{m-2}, wx^{m-1}, a_1^{m-3} are examples). Any one of the mutants so produced, however, will be stable in the absence of *Ac*. Thus, by removing *Ac* from the nucleus, it is possible to isolate a number of alleles that are distinguishable from each other by different modes of gene expression (see Table 4 for examples). Extensive examination of the operation of this *Ds-Ac* system as well as other systems has thus provided a large body of knowledge, from which it is possible to conclude that controlling systems are composed of distinct and well-defined entities in the nucleus, that these are independent of the gene elements as defined earlier, that they need not reside at fixed sites in the chromosome complement, that they retain their identities when transposed from one location to another, and that they operate in much the same general manner wherever they may be located.

The mode of operation of the *Spm*-a_1^{m-1} two-element system, which was considered in some detail earlier in this discussion, is impressive because one aspect of control of gene action expressed by the *Spm* element of the system is suggestive of the mode of operation of suppressors, inhibitors, and modifiers that have been identified in other organisms. In its presence, all action at the a_1^{m-1} locus is suppressed, but in its absence all but one of the alleles of a_1^{m-1} are active to some degree, and some of them produce kernels and plants that exhibit intense pigmentation (see Table 4). The *Spm* element undergoes many transpositions without suffering loss of identity, for its mode of control of gene action at a_1^{m-1} has been found to be the same when variously located. In contrast to *Ac* in the *Ds-Ac* system, increments of *Spm* do not effect modification in time of mutation at a_1^{m-1}. The same phenotypes appear when either one or more *Spm* elements are present in the nuclei of a plant.

Another two-element system has been examined in which the mode of control of the independently located element resembles, in certain respects, those of both *Spm* and *Ac*. The element in this system that is comparable to *Ac*, *Spm*, or *Dt*, exhibits a suppressor-mutator type of control of gene action and mutation at a modified A_2 locus, designated a_2^{m-1}, and in a manner that is similar to that of the *Spm* element of the a_1^{m-1} system. For example, in its absence, gene action at the a_2^{m-1} locus in one of the isolates (one of the alleles of a_2^{m-1}) resembles that given by the standard A_2, for the plants and kernels are intensely and uniformly pigmented and no mutations occur. In its presence, however, all gene activity at a_2^{m-1} is suppressed until the occurrence there of a mutation-inducing event that allows pigment to be produced; and the time of occurrence of such events during the development of a tissue depends on the dose of this suppressor-mutator-type element that is present in the nucleus: the higher the dose, the later the time of occurrence. In this respect, it very much resembles *Ac*.

Differences in phenotype produced by increments of *Ac* or of the *Spm*-type element just described are sharply defined. However, the effects of additions of elements that exhibit this type of dosage expression need not be so contrasting. It is known from other evidence that these effects may be expressed in a somewhat different manner, and an example will be given here. It involves two elements, one of which resides at the A_2 locus in chromosome 5; the other element is independently located. A low dose of the independently located element of this system effects early-occurring mutations at the locus of A_2, from a nonactive allele to one that gives an apparently standard A_2-type expression. Added increments of this element effect step-wise delays in time of occurrence of these mutations, until mere specks of pigment appear in a nonpigmented background in the kernel and only very fine streaks of an-

thocyanin pigment appear in a nonpigmented background in the plant. Increments of the element above that which gives this speckled pattern result in a striking change in phenotypic expression. Now, pigment is produced in both plant and kernel. Although the intensity of color is low, the pigment is uniformly distributed and no mutations occur. This change in gene action is not the consequence of mutation at the modified A_2 locus, but rather the expression of a high dose of the independently located element of the system. This may be shown by crossing the pale-colored plants to standard a_2 tester stocks in which the element is absent. Doses of it are thereby reduced in some of the progeny, and this reduction is evidenced by the reappearance in kernels and plants of the variegated phenotype: a nonpigmented background in which pigmented spots or areas appear. It has also been determined that this element undergoes transposition. This may sometimes be exhibited in sectors in the plant or kernel, which express the change in number of the element that occurred in the ancestor cell giving rise to the sector.

It was stated earlier that the presence of a controlling element at a particular gene locus may not be recognized unless favorable conditions for revealing it are also present; and examples were given. The origin of a number of mutants might well be traced to effects produced by controlling elements, but this might be as difficult as it initially appeared to be for the standard a_1 mutant in maize. This mutant responds to *Dt* by producing higher alleles of A_1, but in the absence of *Dt* the recessive mutant expression is most stable. The mutant was used in genetic studies long before *Dt* was discovered or its control over the element at the a_1 locus recognized. Had *Dt* not been discovered, the a_1 mutant would still be considered an example of stable gene change, just as many of the other known mutants are now considered. In order to identify readily the presence of controlling elements and to be able to examine their different modes of behavior, only those cases were selected, originally, that gave clear-cut evidence of their presence by modification of gene action in both somatic and sporogenous cells. These are the so-called "mutable genes" or "mutable loci." It is quite evident, however, that the standard a_1 mutant is as good a member of the "mutable gene" class as any other that has been described in this or previous reports. How many other known mutants, whose behavior appears to be stable in the existing experimental cultures, also belong to this class? Can conditions be altered so as to expose the presence of a controlling element? Efforts in this direction have not yet been made, except with the standard a_1 mutant itself (McClintock, 1951a, b). The method used gave positive results in that case; and this suggests that positive results might also be obtained if similar experiments were conducted with other known mutants which appear to be stable in the genetic stocks now being used.

Controlling elements appear to reflect the presence in the nucleus of highly integrated systems operating to control gene action. The modes of operation of the known two-element systems bring into sharp relief one level of this integration. Other levels are now under investigation, and these are related to the effects produced by modifiers of particular systems that have appeared within the cultures under examination or been introduced by strain crosses. For example, in the *Spm*-carrying cultures, a certain type of modifier arises with rather constant frequencies. In its action it resembles, in certain respects, the *Spm* element itself, and it may be derived from this element in some manner. It differs from the original *Spm*, however, in quite recognizable ways. In the absence of *Spm*, this modifier effects suppression of gene action at a_1^{m-1}, but only in the aleurone layer of the kernel. The kernels may be totally colorless or they may show one or several small dots of deep color. The plants, on the other hand, show the pigmented phenotype that is exhibited in the absence of *Spm*. In the presence of *Spm*, the modifier behaves as a recessive, and in test crosses of plants carrying both of them typical segregation ratios for both elements are exhibited. The modifier probably undergoes transposition just as *Spm* does, for it has been located at several different positions in the chromosome complement. It is a controlling element and also it is a component of this system. Still other types of modifiers of this system have appeared, each expressing a characteristic type of modification of the system, or, in other words, a characteristic type of integrative action within the system as a whole. Recognition of the presence and operation of a so-called two-element system, then, represents only recognition of the lowest integrative level of those elements in the chromosome complement that are directly concerned with modification of the genome as a whole.

Mendelizing units associated with phenotypic expressions that are similar in many essential respects to those produced by known controlling elements in maize, such as modifiers, suppressors, and some types of inhibitors, have been described in a number of organisms. Are many of their effects also attributable to the activities of controlling elements; and what kinds of criteria may be used to discriminate between the proposed two classes of genetic elements, that is, gene elements and controlling elements? Transposability, which made possible the recognition of controlling elements in the chromosome complement of maize, may not serve in all cases as a reliable criterion for discrimination between the two classes of elements, because the frequency of its occurrence may be so low, under certain conditions, that detection may be difficult (McClintock, 1956). Nevertheless, as far as my knowledge goes, little

if any effort has been made to detect transposition of mutators, modifiers, suppressors, or some types of inhibitors in other organisms, and the degree to which it may occur is not yet known. It would be surprising, indeed, if controlling elements were not found in other organisms, for their prevalence in maize is now well established.

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DISCUSSION

CATCHESIDE: I would like to suggest that Dr. McClintock has in the transposition of controlling elements the explanation of the phenomenon which has been referred to by several speakers as negative interference. It would follow that evidence for controlling elements in organisms other than maize should be sought in those cases which show apparent negative interference.

Harriet Creighton and Lee B. Kass

at the Wellesley College Greenhouse in 1994

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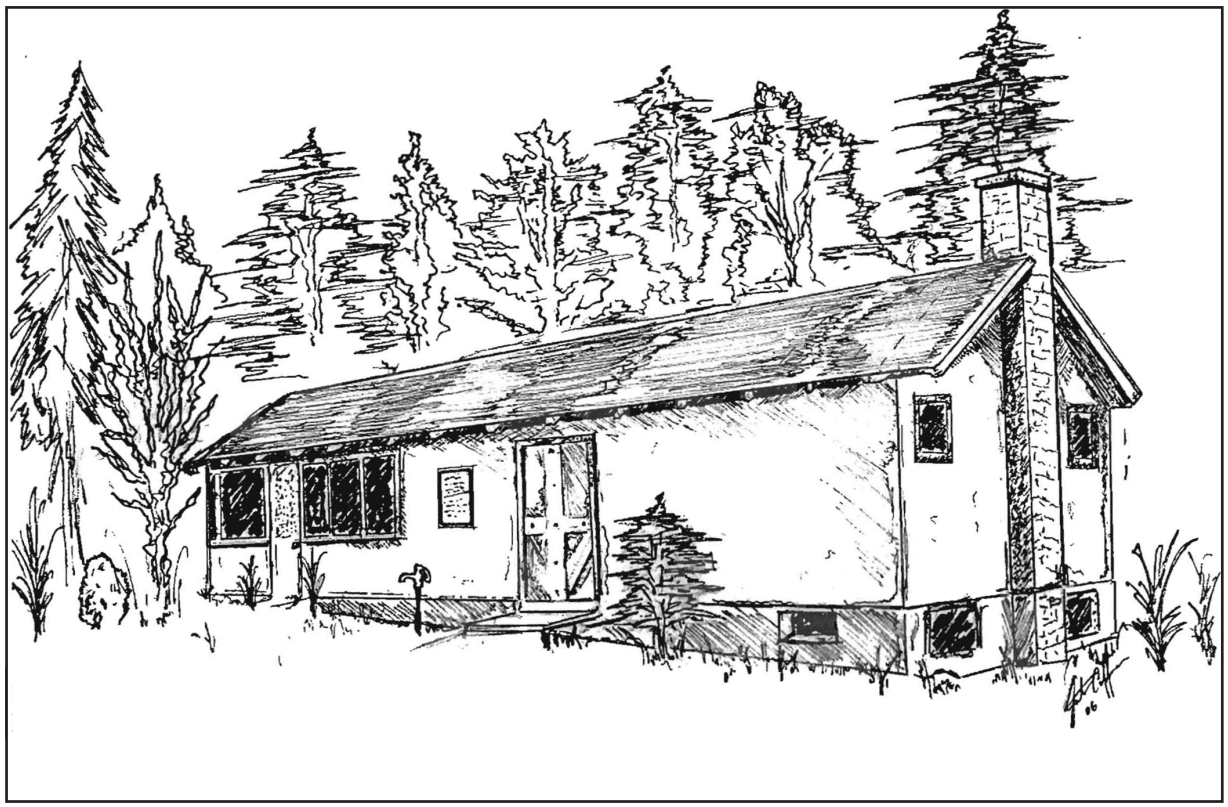

Justin Coffman

Emerson Garden Field Laboratory
Fondly known as the "McClintock Shed"

Emerson Garden "McClintock Shed" *(Image used with permission of Robert Dirig).*