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

Edited by Lee B. Kass

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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. Beadle shared a Nobel Prize in 1958; McClintock was awarded an unshared Nobel Prize in 1983.

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).

Published by The Internet-First University Press

Ithaca, NY, USA

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Perspectives On
Nobel Laureate Barbara McClintock’s
Publications (1926-1984):
A COMPANION VOLUME
Volume II
Perspectives On
Nobel Laureate Barbara McClintock’s Publications
(1926-1984)
A COMPANION VOLUME
Volume II

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 (Increments) 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 updated editions from time-to-time (using dated, rather than numbered editions) that incorporate these increments. These digital collections may be freely downloaded for personal use, or may be ordered as bound copies, at user expense, from Cornell Business Services (CBS) Digital Services by sending an e-mail to digital@cornell.edu or calling 607.255.2524. In the body of the message include the URL for the book or article, and request contact regarding payment.

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 (45 of 106, or 42%), provided online links for free access to 32 (30%) more, and cited traditional sources for the remaining 29 (27%) articles. Only six (6) of the citations listed in the Contents are not presently accessible online. In this initial release we include fourteen essays (“perspectives”), which are paired with the original scholarship being discussed. Ten more perspectives are forthcoming, and will be offered as additional ‘increments’ 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 ‘Increments and Forthcoming Sections’ for Perspectives added to this eCommons collection. From time-to-time these ‘increments’ will be integrated into Volumes 1 and 2, which will then bear a new edition date.

The Internet-First University Press
December 2013

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Published by The Internet-First University Press
Ithaca, New York
Co-founders: J. Robert Cooke and Kenneth M. King

Scanning by the Digital Media Group of the Cornell University Library
Copyright discussions with Peter Hirtle
Proofread by Judy L. Singer, Marion Timothy and Kathleen Gale
Produced by J. Robert Cooke

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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.”

0.4
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
Volume I

CONTENTS

Barbara McClintock & Harriet Creighton at Cornell University 1929, Stone Hall, Cornell University  [0.2]  
Publisher's Note  [0.4]  
Dedication  [0.5]  
Foreword by Mark Sorrells  [0.16]  
Preface and Acknowledgments by Lee Kass  [0.17]  
Introduction by Lee Kass  [0.21]  
Barbara McClintock: Life & Work by Lee B. Kass  [0.23]  
The Publications of Barbara McClintock: Updated 2013 by Lee B. Kass  [0.26]  
Table I. Annotated Chronological List of The Publications of Barbara McClintock, Updated 2013  [0.27]  

NOTE: In the digital version, click a citation below to scroll to that section header; use ‘previous page view’ to return to the Contents.

References preceeded by an asterisk (‘) are not reprinted herein, but were collected and published previously in McClintock, B. 1987a. The Discovery and Characterization of Transposable Elements. The Collected Papers of Barbara McClintock. Genes Cells and Organisms; Great Books in Experimental Biology, J.A. Moore, series editor, Garland Publishing Co. New York. However, some of these are accessible online at the URL provided.

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

1926 Perspectives: forthcoming


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


1929 Perspective: Commentary on Barbara McClintock’s 1929 cytogenetic analysis of triploid maize: A cyto-logical and genetical study of triploid maize. by Mark E. Sorrells, Cornell University


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


**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. and H. E. Hill. 1929 [ABSTRACT #32]. The cytological identification of the chromosomes associated with the ‘R-golden’ and ‘B-liguleless’ linkage groups in *Zea mays*. *Anatomical Record* 44(3): 291.)


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


**Perspective:** forthcoming


**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


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


1935 Perspective: forthcoming


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

**Part II: ROAD TO TRANSPOSITION (1937-1945)** [2.1]

1937 See 1938


(McClintock, B. 1937a. [ABSTRACT] The production of maize plants mosaic for homozygous deficiencies: Simulation of the \(bml\) phenotype through loss of the \(Bml\) locus. *Genetics* 22(1): 200.)


Perspective: forthcoming [2.69]


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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 preceded 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]


1942 Perspective: forthcoming [2.125]


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


1947 Perspective: forthcoming [3.5]


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]


**Perspective:** forthcoming [3.27]


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


Perspective: forthcoming [3.67]


1957


1958


1959


1961

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


*McClintock, B. 1961b. Further studies of the suppressor-mutator system of control of gene action in maize: Control of *a1m-2* by the *Spm* system. A third inception of control of gene action at the *A1* locus by the *Spm* system. Control of gene action at the locus of *Wx* by the *Spm* system. Control of reversals in *Spm* activity phase. Nonrandom selection of genes coming under the control of the *Spm* system. *Carnegie Institution of Washington Year Book* 60, 1960-1961: 469–476. [3.91]

1962


1963


1978  **Perspective:** forthcoming [3.127]


1984  Perspective: The special character of McClintock’s Nobel Prize address. by James A. Shapiro, University of Chicago  [3.133]

(McClintock, B. 1983. [ABSTRACT]. Trauma as a means of initiating change in genome organization and expression. *In vitro* 19(3, Part II) [March 1983]: 283–284.)


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


Perspective: Barbara McClintock’s Cytogenetic Studies of Chromosome Constitutions of Races of Maize in the Americas. by David B. Walden, The University of Western Ontario  [4.7]


Perspective: Significance of McClintock’s pattern concept in the analysis of chromosome knob distribution in the races of maize. by T. Angel Kato Y, Colegio de Postgraduados, Montecillo, Mexico  [4.39]


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


*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_1902_through_2000

Note: The webpage title (Carnegie Institution Year Books Numbers 1 through 99, years 1902 through 2000) differs slightly.

APPENDICES (Volumes I and II)

A-1. Contributors Affiliations [App.1]

A-2. Contributors Biographical Sketches [App.3]

B. Manuscript Reviewers [App.6]
Foreword

Mark E. Sorrells

I had the good fortune to meet Dr. Barbara McClintock at the 75th anniversary of the Department of Plant Breeding and Genetics and the Synapsis Club reunion (1982). I first became acquainted with Dr. Lee B. Kass in the mid-2000s while we were planning the centennial celebration for the Cornell Plant Breeding Department. At that time, Dr. Kass was working with Dr. Royse P. Murphy on compiling the history of our department, founded in 1907. Their book called “Evolution of Plant Breeding at Cornell University” was completed and released to mark this milestone. Through this interaction, I came to appreciate the fact that Dr. Kass is the foremost authority on the life and work of Dr. Barbara McClintock. Dr. Kass has published four book chapters, six invited articles, and 12 articles in various other publications about the life and work of McClintock in preparation for her forthcoming intellectual biography, “From Chromosomes to Mobile DNA: The life and work of Nobel Laureate Barbara McClintock.” In addition, her training with Cornell’s Botany faculty, strong affiliation with Cornell’s Plant Breeding faculty, and over 30 years of teaching botany and genetics has eminently qualified her for this project. This companion volume “Perspectives on Nobel Laureate Barbara McClintock’s publications (1926-1984),” is a compilation of summaries and analyses of McClintock’s early work by experts familiar with her field of research. Consequently, this volume serves to provide unique perspectives that will complement Dr. Kass’ forthcoming book and highlight Dr. McClintock’s extraordinary talents as a scientist.

It has been nearly 30 years since McClintock was awarded the Nobel Prize for “her discovery of mobile genetic elements,” and nearly 90 years since her first publication. What can we learn by analyzing the published works of brilliant scientists? McClintock’s thinking was clearly decades ahead of her time, and perhaps we can gain some insight that would influence our own investigations. Was her genius simply dedication and hard work, or did she have a unique way of thinking or approaching her work? In his analysis, Shapiro points out that our knowledge of molecular mechanisms of sensing, regulation, DNA repair, and mobile genetic elements has grown tremendously, and yet molecular biology research is bringing us ever closer to McClintock’s cognitive view. These perspective analyses lead one to appreciate that McClintock understood much more about cell biology than just the basic ideas of chromosome mechanics and transposition. As early as 1929, McClintock described genome damage and made the observation that the chromatin had the ability to reorganize in some of the cells suggesting that they can sense and repair genome damage. As Phillips points out in his review of her 1930 paper on reciprocal translocations, McClintock’s genius probably allowed her to recognize the importance of chromosome landmarks such as knobs, and helped to guide her to important conclusions. Dr. Wayne reviewed her 1929 paper, where she described the morphology of the 10 chromosomes of maize applying her knowledge of chromosome landmarks. Once she was able to identify which chromosome appeared in triplicate in the cells of trisomic plants she could assign traits to a chromosome. In her 1932 paper, she took the mapping of traits a step further by using Stadler’s X-ray mutated corn plants that had a ring-shaped chromosome. But probably the most elegant application of her knowledge of chromosome morphology was in the 1931 paper by Creighton and McClintock, where they showed for the first time that exchange between genes was accompanied by exchange of physical parts of chromosomes. Coe and Kass (2005) published a fascinating analysis of that work that has been lauded as one of the great experiments in biology.

Perspectives on Nobel Laureate Barbara McClintock’s publications (1926-1984), is indeed a novel compilation of perspectives of leading scientists decades later on the many discoveries of Barbara McClintock. These new perspectives, along with Kass’ forthcoming biography of McClintock, will provide readers with valuable insights and interpretations of McClintock’s approach to genetics, offer an appreciation for the primitive state of that field in the early years, and reveal her unique approach to research.
Preface

This collection of papers and perspectives on the publications of Nobel Laureate Barbara McClintock emerged while I was preparing my forthcoming intellectual biography of McClintock’s life and work (Kass & Provine 1997a, Kass 2003). While reading McClintock’s primary papers, I recognized that many secondary sources did not provide an accurate representation of her scientific contributions (Kass 2002, Kass 2004, Kass 2005, Kass & Bonneuil 2004, Coe & Kass 2005a). I soon realized that starting at the beginning of her scientific career was essential for an understanding of her insights and an appreciation of McClintock’s unconventional approach to research.

History of Science Professor William B. Provine provided reprints of McClintock’s publications. Many of these reprints originally belonged to her major professor, Lester W. Sharp, and are included here. In this volume readers will also gain access to McClintock’s early research reports, first published in the *Maize Genetics Cooperation Newsletter (MNL)*. This cooperative publication was founded at Cornell in 1929 by R.A. Emerson, head of the Department of Plant Breeding (Kass & Bonneuil 2004; Coe & Kass 2005b, Kass et al. 2005, Murphy & Kass 2007, 2011), and continues publication to this day. I published my first annotated list of McClintock’s publications in the *MNL* (Kass 1999a), and offer here an updated and expanded account (Kass this volume, Table I).

Barbara McClintock was recognized by her scientific peers in Plant Biology and Genetics years before she had won the 1983 Nobel Prize for her discovery of “mobile genetic elements” and became a public figure (Fedoroff & Botstein 1992, Kass & Bonneuil 2004, Kass & Chomet 2009). As a graduate student at Cornell University in the early 1970s, I observed first-hand how McClintock had long been honored and revered by faculty, students and staff (Kass 2003, Murphy & Kass 2007, 2011).

McClintock was a Cornell University A.D. White Professor-at-Large (1965-1974; Cornell 2013a,b), when I first met her in the spring of 1972. She was hosted by the department where I was a graduate student, and where years before she had received her undergraduate and graduate degrees. Perhaps because I was a first year graduate student and did not know of her reputation for intimidation, I accepted the invitation (extended to all graduate students) to meet with her to discuss my research. I was quite impressed with her depth of knowledge in my subject area of plant chloroplast development. As we talked, we drew pictures together on the blackboard and talked about the recent literature in this field. She asked me about a paper recently published on the subject. When I said I had not read it she stopped talking and looked at me. “Come back after you have read the paper,” I recall her saying, “and we will continue the conversation.” So that is exactly what I did.

Years later, when I told this story to Harry Stinson, the former head of our Section of Genetics, Development and Physiology, and who had hosted McClintock’s visits to Cornell, he responded that this was an example of her daunting personality. I, however, felt no threat, but was grateful for her lead to the literature, which I eventually cited in my doctoral dissertation. Perhaps because we both grew up in Brooklyn, or because I was just too naive to know better, I truly enjoyed talking with Barbara McClintock about my research, attending her seminars, and especially joining her for dinner and beer, with my graduate student colleagues at local restaurants or in our homes. She taught us to be open minded, well informed, and to think independently -- A great legacy for a great mind.

In 1976, following McClintock’s visiting professorship at Cornell University (1965-1974), Professor Provine interviewed McClintock about her life and work. In 1980, Provine and Paul Sisco, then a graduate student in Cornell’s Department of Plant Breeding, interviewed McClintock again at her laboratory at Cold Spring Harbor, with the goal of writing about her early career at Cornell. Their project was supplanted by other commitments, and subsequently Provine invited me to take responsibility for this task. We secured funding from the National Science Foundation to support and expand the research for this project (Kass & Provine 1997a). Provine shared his McClintock interviews with me, and in 1999 the interviews became available to scholars at the Division of Rare and Manuscript Collections, Cornell University (Kass & Provine 1999; Kass 2003). Using the conversations as a guide, I searched for period documents to place in historical perspective the many reminiscences, recollections, and stories told by and about McClintock. This research led me to read McClintock’s primary papers, and determine that her seminal papers were crucial to an understanding of her later discoveries (Kass & Provine 1997b; Kass 1999b, 2000, 2002, 2003; Coe & Kass 2005a).
My own interviews with McClintock’s colleagues and friends, along with teaching her work in my genetics and biology courses at the college level, gave me an appreciation of McClintock’s early contributions. I quickly recognized that her early papers were not all readily available. It also inspired me to invite my colleagues to share their knowledge and understanding of McClintock’s primary papers with others by contributing perspectives on one or more of McClintock’s publications.

This book is a work in progress (an Incremental Book). The goal is to have perspectives (summaries and analyses) for all of McClintock’s published papers. The e-book format permits perspectives to be posted after they have been reviewed and revised as needed. Authors whose perspectives are currently in revision are listed as “forthcoming.” If one of the publications currently has no accompanying perspective, it may be available at a later date.

Lee B. Kass
20 September 2013

References cited


Acknowledgements

I am grateful to my colleague and friend Professor William B. Provine for inviting me to assume responsibility and expand upon his project to examine the work of Barbara McClintock, for unlimited use of his vast library and reprint collection, and for continued support and encouragement. I acknowledge partial research support from the National Science Foundation (grants SBR 9511866 and SBR 9710488); American Philosophical Society Library, Mellon Resident Research Fellowship; and Lilly Library, Helm Fellowship. I appreciate permissions granted from publishers, and the Cold Spring Harbor Laboratory Archives (Clare Clark and the Estate of Barbara McClintock) to reprint McClintock’s publications. For logistical support, I thank my colleagues in Cornell’s Department of Plant Biology (William Crepet, Robert Dirig, Sherry Vance, Peter Fraissinet), Department of Plant Breeding and Genetics (W. Ronnie Coffman, Royse P. Murphy, Mark E. Sorrells, Ron A. Anderson), and West Virginia University’s Division of Plant and Soil Sciences (Barton Baker, Joginder Nath, Vagner Benedito). I am grateful to colleagues who accepted my invitation to contribute their knowledge and understanding of McClintock’s work (see Appendix A), and to those who donated their time reviewing manuscripts (see Appendix B) and introductory chapters (Edward Coe, Robert Dirig, Peter Fraissinet, Robert Hunt, Randy Wayne, Paul Sisco, V. Betty Smocovitis, Clifford Weil), and proofreading (Judy Singer, Marian Timothy, Kathleen Gale).

Special thanks to the Cornell University Digital Media Group (Bronwyn J.Q. Mohlke, Danielle K. Mericle, Mira Basara) for their patience and excellent work scanning McClintock’s reprints, Edward Coe for scans of early *Maize Genetics Cooperation News Letters*, and the staff of Cornell’s Division of Rare and Manuscript collections (Elaine Engst, David Corson), Carl A. Kroch Library, for always being there for me. I owe special recognition to my publisher J. Robert Cooke who believed in this project from its inception and encouraged my efforts to make it a reality; and to Peter Hirtle for guidance on copyright law. Lastly, I gratefully acknowledge my husband Dr. Robert E. Hunt for his continued and tireless backing of my efforts to complete this project.
Introduction

Barbara McClintock’s Nobel Laureate lecture (McClintock 1984) described significant early investigations that were basic to an understanding of her discovery of “mobile genetic elements” (controlling elements, transposable elements, transposons) in maize, for which she received the Prize in 1983. McClintock’s publications relevant to her Nobel Prize-winning work were collected and reprinted in The Discovery and Characterization of Transposable Elements [The Collected Papers of Barbara McClintock] for the Great Books in Experimental Biology Series (McClintock 1987a). The reprinted papers begin in 1938, yet McClintock had published major contributions to cytology and genetics between 1926 and 1938 (See Kass this volume, Table I) that proved important to her most renowned work.

McClintock’s (1987b) introductory remarks to the anthology offered brief commentary on the significance of each contribution to her discovery of transposable elements. She also mentioned earlier studies that “proved to be highly significant for later studies” (p. vii), but the relevant papers are not reprinted. Included in that collection is a “numbered list” (p. xiii-xv) of McClintock’s published papers beginning with the earliest study (Randolph & McClintock 1926). Kass and Chomet (2009) recently expanded the list, based on Kass’ (1999) initial findings, and an update appears here (Kass this volume, Table I).

Previous anthologies of classical papers in genetics or biology have reprinted excerpts of McClintock’s work (Gabriel & Fogel 1955, Peters 1959). Some offered brief remarks and limited analysis, yet omitted details that might have fostered a complete understanding of the work. Others provided much commentary, but only reproduced sections of her papers (Phillips & Burnham 1977). A Festschrift published for McClintock’s 90th birthday by her friends and colleagues Nina Fedoroff and David Botstein (1992) also reprinted a limited number of her ground-breaking papers, and offered an excellent and informative review of the discovery of “Maize Transposable Elements” (Fedoroff 1992). The editors recognized that, “The influence of her early work is greater than that of any of her peers… Had she done no more, McClintock would have become a major figure in the history of genetics” (Fedoroff & Botstein 1992, p. 1; Kass 2003).

McClintock’s research builds on work she began in the 1920s, and the only way to understand how she arrived at her conclusions is to start at the beginning. This Companion Volume includes reproductions of McClintock’s earliest publications and abstracts, and her investigations on Neurospora and Races of Maize in Latin America, with accompanying “perspectives” (summaries and analyses) preceding individual articles or sets of papers. It also provides perspectives on papers previously reprinted (McClintock 1987a), without reprinting them here (See Kass this volume, Table I. Online links to the Carnegie Institution Year Books Numbers 1 through 99, years 1902 through 2000 are provided.)

The contributors of “Perspectives” essays are maize cytogeneticists or geneticists, experts in the field, who have taught the work or have cited it in their research. They were asked to write on the subject as if they were teaching it to a college class, summarizing the main points of the paper and giving an analysis of its importance. Many analyses include discussions of the impact of the paper on future work. They also try to clarify any genetic jargon or techniques that the reader may have difficulty understanding.

One goal of this work is to assist those who are hesitant to use McClintock’s primary papers for teaching. Many summaries and reviews of McClintock’s papers found in the published literature misrepresent work that she actually reported in her early papers (see Kass & Chomet 2009). I anticipate that these collections will clarify many of those inaccuracies, which apparently stem from authors (often non-scientists and even science text-book authors) relying on the literature without having read the primary papers.

With the publication of this work, all of McClintock’s published papers are now collected. The McClintock anthology, published previously (1987a), focused on papers relevant to Mobile Genetic Elements (1938-1984). My two volume collection reprints her earlier papers beginning in 1926 through 1938, and demonstrates their influence on her later work. We also reprint McClintock’s articles on the evolution of corn in the Americas (1957-1981), and her cytogenetic studies of the red bread mold Neurospora (1945-1954). We offer web-links to McClintock’s papers not reprinted in this two volume collection. Additionally, we present accompanying perspectives on many of her contributions. As a result of this effort, all but six of McClintock’s publications are now freely available online.
Literature Cited:


Barbara McClintock: Life & Work*

by Lee B. Kass, Visiting Professor, Cornell University  
*modified from Kass 2007b

Barbara McClintock (1902-1992), one of the foremost women scientists in 20th century America, is most noted for her pioneering research on transposable elements in maize. For this work she was awarded the Nobel Prize in Physiology or Medicine in 1983. She was the third woman to receive an unshared Nobel Prize in the sciences.

In this volume we focus on (and reprint) McClintock’s early contributions to plant genetics, which have often been overshadowed by her Nobel Prize-winning discovery. In the Dynamic Genome, a gift to McClintock on her ninetieth birthday, Nina Fedoroff commended McClintock’s early achievements: “The Influence of her early work is greater than that of any of her peers … . Had she done no more, McClintock would have become a major figure in the history of genetics.”

Born in Hartford, Connecticut, on June 16, 1902, Barbara McClintock was raised in Brooklyn, New York. After graduating from Erasmus Hall High School, she entered Cornell University at age 17, and in 1923 earned a B.S. in agriculture, concentrating in plant breeding and botany. She received both her master’s (1925) and doctoral degrees (1927) from Cornell’s College of Agriculture. She majored in cytology with Lester W. Sharp in the Department of Botany, and minored in genetics and zoology with Allan C. Fraser and Hugh D. Reed in the Departments of Plant Breeding and Zoology, respectively. As a graduate student, McClintock was a research and teaching assistant in the Department of Botany, Cornell University, College of Agriculture. During these years, Sharp referred both botany and plant breeding graduate students and post-doctoral researchers to her. Most notable were George Beadle (Ph.D. 1930), who learned cytology from McClintock, and went on to head the biology division at Caltech and win a Nobel Prize (1958); and L. J. Stadler (NRC Fellow 1926), later elected to the National Academy of Sciences.

McClintock’s career as one of the most prominent geneticists of the 20th century was launched while she was at Cornell. Upon receiving her doctorate, McClintock was made an Instructor. At that time, this appointment was the first step leading to tenure at colleges and universities like Cornell. Jobs in academia were scarce during the Depression, and jobs for women were limited. While employed at Cornell, Instructor McClintock continued to mentor and collaborate with graduate students. She befriended graduate student Marcus Rhoades (Ph.D. 1932), who also rose to preeminence in genetics and was McClintock’s lifetime supporter. From 1927 to 1931, she taught undergraduate and graduate courses in Cornell’s Department of Botany.

In early 1929, McClintock published her Ph.D. dissertation in Genetics, the foremost journal in the field. Within two years, she had published six other articles in major journals, all of which made important contributions to the newly emerging field of plant cytogenetics, and furthered the world’s knowledge about the location of genes on chromosomes. McClintock collaborated with students on the most notable of these investigations.

Instructor McClintock gave graduate students Henry Hill and Harriet Creighton two important projects for their thesis research, and co-authored these pioneering contributions with them. The first was a method to connect chromosomes with linkage groups in corn (McClintock & Hill 1931) and the second was the cytological proof for crossing over (McClintock 1931, Creighton & McClintock 1931). Creighton and McClintock’s significant study gave further confirmation to T. H. Morgan’s chromosome theory of inheritance, for which he won a Nobel Prize in 1933. These collaborative projects were based on important work that McClintock had pioneered: identification of corn’s ten chromosomes at mitosis (and later at meiotic pachytene stage), confirmation of Belling’s translocation hypothesis, and the sequence of the genes in chromosome 9. Creighton (Ph.D. 1933) became head of Botany at Wellesley College and President of the Botanical Society of America in 1956.

From 1931 through 1934, sponsored by two National Research Council Fellowships, and a prestigious Guggenheim Fellowship, McClintock traveled to a series of important research institutions across the U.S., Germany, and back to Cornell, where she worked in the Department of Plant Breeding as an assistant to R. A. Emerson, head of the department. There, she conducted research, funded by the Rockefeller Foundation, which would provide insights to an understanding of variegation, and would eventually lead to her Nobel award-winning investigations.
In 1936, McClintock accepted an appointment as Assistant Professor of Botany at the University of Missouri to join L. J. Stadler's genetics research group. Upon learning that the research unit might be eliminated, and preferring research over teaching, McClintock requested a leave of absence from Missouri in 1941 to seek employment elsewhere. In 1943, she accepted a position as a permanent staff member of the Carnegie Institution of Washington's Department of Genetics at Cold Spring Harbor on Long Island, New York. It was there, continuing work she began at Cornell and at Missouri, that she discovered “mobile genetic elements” in corn, for which she was awarded the Nobel Prize in Medicine or Physiology in 1983. She remained at Cold Spring Harbor for the duration of her career, accepting only short term appointments at national and international institutions elsewhere.

McClintock achieved considerable recognition within her lifetime. In 1944, prior to her most celebrated work, she was elected to the National Academy of Sciences, the third woman so honored. McClintock also became the first woman elected Vice President (1939) and President (1945) of the Genetics Society of America. By 1947, she received the Achievement Award from the American Association of University Women.

But it is for McClintock's work with maize, beginning in the mid-1940s, her meticulous observations of the dynamism of the genome, her communications of her theory of genetic transposition — the idea that genes could spontaneously change their position on a chromosome — that reinforced her reputation as a pioneering geneticist, which was widely acknowledged in later years. In 1957, the Botanical Society of America recognized her achievements with their esteemed Merit Award, and Cornell appointed her one of their first A.D. White Professor's-at-Large in 1965 (renewed in 1971).

McClintock also won a number of prizes during her later career. A few months before she formally retired in 1967, she received the Kimber Genetics Award from the National Academy of Sciences. In that year, the Carnegie Institution of Washington appointed her a Distinguished Service Member, one of their highest honors, which made it possible to continue working at Cold Spring Harbor Laboratory. During the 1970s she received the National Medal of Science (1970), the Lewis S. Rosensteil Award (1978), and the Louis and Bert Freedman Foundation Award (1978). A few years before receiving the Nobel Prize, she was honored with many awards; more notable were the Thomas Hunt Morgan Medal, the Wolf Foundation Prize in Medicine, a shared Albert Lasker Basic Medical Research Award, and the first Prize Fellow Laureate of the MacArthur Foundation.

McClintock’s life as a scientist was not always easy. Full appreciation of the implications of her work on mobile genetic elements, which challenged generally held beliefs that the chromosome had a stable structure, was not possible until molecular biologists found similar phenomena in bacteria and other organisms. Her work continues to influence and inspire the field of transposons and genomic dynamics in plants.

As one of the early women scientists in this country, McClintock earned timely recognition for her pioneering achievements, gaining a star in American Men of Science by 1944. Yet, as an aspiring young geneticist, she experienced injustice because of her gender. Determined to succeed in her chosen field, and respected and helped by devoted colleagues, McClintock eventually found a position at an institution at Cold Spring Harbor that gave her the freedom to pursue her love of science and which, she said, “fit her personality rather well.”

Bibliography:


The Publications of Barbara McClintock: Updated 2013

by Lee B. Kass
12 September 2013

Here I present an updated, annotated list of the publications of Nobel Laureate Barbara McClintock (Table I).

I reported my first annotated list of Barbara McClintock’s publications in the *Maize Genetics Cooperation Newsletter* (Kass 1999). In collaboration with Paul Chomet, I modified that list for an invited book chapter (Kass & Chomet 2009) in the *Handbook of Maize*. Since then, additional McClintock publications have been brought to light (Kass 2009).

**Notation:** This updated list also identifies McClintock’s publications, including abstracts, which are reprinted in this *Companion Volume*; these are followed by ➀. Papers identified as relevant to the discovery and characterization of transposable elements were previously collected and published (McClintock, 1987); these are followed by ➁. Citations to McClintock’s collected papers [previously cited as Moore, J. (ed.), 1987] are now cited here as McClintock (1987).

Thirteen reviews that McClintock contributed to *Biological Abstracts* between 1928 and 1957 are followed by ➂ in this updated and corrected annotated list of McClintock’s publications (Table I).

Many journal titles have changed over the years: *Stain Technology* is now *Biotechnic & Histochemistry*; *Zeitschrift fur Zellforschung und mikroskopische Anatomie* has undergone a number of name changes and is now *Cell and Tissue Research*; *Maize Genetics Cooperation Newsletter* is now *Maize Genetics Cooperation Newsletter*; *In Vitro* is now *In vitro Cellular & Developmental Biology – Plant*; and *Carnegie Institution of Washington Year Book* is now *Carnegie Institution for Science Year Book*.

**References:**


Table I. Annotated Chronological List of the Publications of Barbara McClintock [Updated 2013]

<table>
<thead>
<tr>
<th>No.</th>
<th>Title</th>
<th>Author(s)</th>
<th>Journal/Source</th>
<th>Year</th>
<th>Volume</th>
<th>Pages</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>A genetic disturbance of meiosis in Zea mays.</td>
<td>Beadle, G. W. and Barbara McClintock</td>
<td>Science 68(1766) [2 November 1928, received – no date given]: 433. [This became George Beadle’s dissertation research project.]</td>
<td>1928</td>
<td>68(1766)</td>
<td>433</td>
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<tr>
<td>9</td>
<td>A cytological and genetical study of triploid maize.</td>
<td>McClintock, Barbara</td>
<td>Genetics 14(2) [11 March 1929, received 11 July 1928]: 180–222. [Publication of 1927 Ph.D. thesis. Genetics was issued bimonthly (every two months) at this time.]</td>
<td>1929</td>
<td>14(2)</td>
<td>180-222</td>
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<tr>
<td>10</td>
<td>A method for making aceto-carmin[e] smears permanent.</td>
<td>McClintock, Barbara</td>
<td>Stain Technology IV(2) [April 1929, received - no date given]: 53–56. [In this publication carmine is spelled “carmin” in the title, throughout the text, and in the citation to Belling 1926.]</td>
<td>1929</td>
<td>IV(2)</td>
<td>53-56</td>
<td></td>
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<tr>
<td>11</td>
<td>A 2N-1 chromosomal chimera in maize.</td>
<td>McClintock, Barbara</td>
<td>Journal of Heredity XX(5) [May 1929, received - no date given]: 218. [McClintock annotated the reprint she sent to T. H. Morgan indicating that only one photograph was intended to be published. She apparently submitted two exposures with the intent that the best one would be printed. The citation to Blakeslee and Belling Science, 55, should be 60 (LX), not 55, and the year of publication, 1924, was omitted.]</td>
<td>1929</td>
<td>XX(5)</td>
<td>218</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Chromosome morphology in Zea mays.</td>
<td>McClintock, Barbara</td>
<td>Science 69(1798) [14 June 1929, submitted - no date given]: 629. [The first published ideogram of Zea chromosomes. The chromosomes were identified in the “first division in the microspore” (Mitosis) not at pachytene of Meiosis I as described by some text book authors. In the citation for McClintock Genetics, 14, the year, 1929, was omitted.]</td>
<td>1929</td>
<td>69(1798)</td>
<td>629</td>
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<tr>
<td>13</td>
<td>The cytological identification of the chromosomes associated with the 'R-golden' and 'B-liguleless' linkage groups in Zea mays.</td>
<td>McClintock, Barbara and Henry E. Hill</td>
<td>Anatomical Record 44(3) [25 December 1929]: 291. [The paper was “to be read by title” at the Joint Genetics Sections of the American Society of Zoologists and the Botanical Society of America, held with the AAAS, Des Moines, and</td>
<td>1929</td>
<td>44(3)</td>
<td>291</td>
<td></td>
</tr>
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</table>

1 The notations ① and ② are explained at the beginning of this list of publications.
Ames, Iowa, December 1929 – January 1930. Resulting manuscript submitted March 1930, and published one year later in *Genetics* 16: 175–190, March 1931. See McClintock 1933a (p.209) for correction of B-lg linkage group association with Chromosome 2 not Chromosome 4. ➀


15. McClintock, Barbara. 1930b. [ABSTRACT #9]. A cytological demonstration of the location of an interchange between two non-homologous chromosomes of *Zea mays*. *Anatomical Record* 47(5) [25 December 1930]: 380. [Paper presented on 30 December 1930, at the Joint Genetics Sections of the American Society of Zoologists and the Botanical Society of America, held with the AAAS, Cleveland, Ohio, December 1930 - January 1931. Two weeks prior to these meetings, the results were published in *PNAS* 16(12): 791–796, December 1930.] ➀

16. McClintock, Barbara and Henry E. Hill. 1931. The cytological identification of the chromosome associated with the R-G linkage group in *Zea mays*. *Genetics* 16(2) [16 March 1931, received 1 March 1930]: 175–190. ➀

17. McClintock, Barbara. 1931a. The order of the genes C, Sh, and Wx in *Zea mays* with reference to a cytologically known point in the chromosome. *Proceedings of the National Academy of Sciences* 17(8) [15 August 1931, communicated 7 July 1931]: 485–491. [Communicated the same date and issued as one reprint with Creighton and McClintock 1931. The results reported in McClintock 1931a are necessary for an understanding of Creighton & McClintock 1931, which follows directly in the Journal. These papers were intended to be read together. McClintock 1931a ends with the following statement: “It was desired to present briefly the evidence at this time, since it lends valuable support to the argument in the paper which follows.” Creighton & McClintock, 1931 state: “In the preceding paper it was shown that the knobbed chromosome carries the genes for colored aleurone” etc. Unfortunately the “preceding paper” (McClintock 1931a) is neither cited nor referenced.] ➀

18. Creighton, Harriet B. and Barbara McClintock. 1931. A correlation of cytological and genetical crossing-over in *Zea mays*. *Proceedings of the National Academy of Sciences* 17(8) [15 August 1931, communicated 7 July 1931]: 492–497. [Communicated the same date and issued as one reprint with McClintock 1931a; see annotation for McClintock 1931a.] ➀

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 [December, authorized 23 December 1931]: 1–30. [McClintock NRC Fellow at Missouri and Cal Tech, investigation conducted at Missouri beginning June 1, 1931; L. J. Stadler suggested the problem and furnished all the material in the growing state.] ➀


21. Creighton, Harriet B. and Barbara McClintock. 1932, [EXHIBIT/ABSTRACT]. Cytological evidence for 4-strand crossing over in *Zea mays*. *Proceedings of the International Congress of Genetics II* [24–31 August 1932, preface dated 26 July 1932]: 392. [This was an exhibit that was part of the section on “General Cytology: Cytological Evidence Bearing on Crossing Over” in the “General Exhibits.” The section was organized by Ralph E. Cleland.] ➀

22. McClintock, Barbara. 1932b. A correlation of ring-shaped chromosomes with variegation in *Zea mays*. *Proceedings of the National Academy of Sciences* 18(12) [15 December 1932, communicated 2 November 1932]: 677–681. [McClintock NRC Fellow at Missouri with L. J. Stadler; her address is given as U of Missouri; Contribution from Dept. of Field Crops, Missouri Agricultural Experiment Station, Journal Series No. 355.] ➀
23. McClintock, Barbara. 1933a. The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*, with 51 figures in the text and plates VII–XII. *Zeitschrift für Zellforschung und mikroskopische Anatomie* 19(2) [22 September 1933, received 21 April 1933]: 191–237. [McClintock NRC Fellow in the Biological Sciences, University of Missouri with L. J. Stadler and California Institute of Technology with E. G. Anderson; investigations conducted at Missouri and at Cal Tech.]


27. McClintock, Barbara. 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) [23 June 1934, received 2 March 1934]: 294–328. [McClintock NRC Fellow in the Biological Sciences, California Institute of Technology with E. G. Anderson; investigation conducted at Cal Tech. Paper written while McClintock was a Guggenheim Fellow in Berlin and Freiburg, Germany and submitted just prior to leaving Germany.]


32. Creighton, Harriet B. and Barbara McClintock. 1935. The correlation of cytological and genetical crossing-over in *Zea mays*. A corroboration. *Proceedings of the National Academy of Sciences* 21(3) [15 March 1935, communicated 9 February 1935]: 148–150. [Written while McClintock was a research assistant in the Department of Plant Breeding, Cornell University (address Botany Department).]

33. Rhoades, Marcus M. and Barbara McClintock. 1935. The cytogenetics of maize. *Botanical Review*. 1 (8) [August 1935, received - no date given]: 292–325. [Written while McClintock was a research assistant in the Department of Plant Breeding, Cornell University.]


39. McClintock, Barbara. 1937a. [ABSTRACT] The production of maize plants mosaic for homozygous deficiencies: Simulation of the bm1 phenotype through loss of the Bm1 locus. [In: Abstracts of papers presented at the 1936 meetings of the Genetics Society of America, M. Demerec, Secretary.] *Genetics* 22(1) [January 1937, presented 29 December 1936]: 200. [Investigations funded by the Rockefeller Foundation and conducted in Department of Plant Breeding, Cornell University; McClintock’s address - Cornell University. In September 1936, McClintock left Cornell to begin her Assistant Professor appointment at U of Missouri. Results reported are part of a manuscript submitted February 1938 and published in *Genetics* 23: 315–376, July 1938. Note subheadings for sections V and VI in published paper are exactly the same as title of this abstract.] ①


41. McClintock, Barbara. 1938a. [ABSTRACT] A method for detecting potential mutations of a specific chromosomal region. [In Abstracts of papers presented at the 1937 meetings of the Genetics Society of America] *Genetics* 23(1) [January 1938, presented 28 December 1937]: 159. [McClintock Assistant Professor of Botany at U of Missouri; results reported here were based on investigations funded by the Rockefeller Foundation and previously conducted in Department of Plant Breeding, Cornell University.] ①

42. McClintock, Barbara. 1938b. The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. *Genetics* 23(4) [July 1938, received 25 February 1938]: 315–376. [Most of work undertaken at Cornell with aid of grant from the Rockefeller Foundation; original material supplied by L. J. Stadler.] ①

43. McClintock, Barbara. 1938c. The fusion of broken ends of sister half-chromatids following breakage at meiotic anaphase. *Missouri Agricultural Experiment Station Research Bulletin* 290 [July 1938, authorized 12 July 1938]: 1–48. [Continuation of investigations begun at Cornell University between 1934 & 1936; cites McClintock 1938b.] ②²


45. [1940 No Publications]


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² This notation ② is explained at the beginning of this list of publications.
48. McClintock, Barbara. 1941b. The association of mutants with homozygous deficiencies in Zea mays. Genetics 26(5) [September 1941, received 3 May 1941]: 542–571. [Both the journal article and reprints are dated inaccurately as September 1940; only reprint cover page is accurately dated.]

49. McClintock, Barbara. 1941c [Issued December 1941, Symposium held June 1941]. Spontaneous alterations in chromosome size and form in Zea mays. pp. 72–80. In: Genes and Chromosomes - Structure and Organization. Cold Spring Harbor Symposia on Quantitative Biology Volume IX [June 1941, Issued December 1941]. Katherine S. Brehme, ed. The Biological Laboratory, Cold Spring Harbor, Long Island, New York. [McClintock was appointed guest investigator for academic year 1941–42, Department of Botany, Columbia University. During the summer of 1941, and from December 1941 through December 1942, McClintock was also guest investigator, Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor. McClintock resigned from University of Missouri effective August 1942.]


51. McClintock, Barbara. 1942b [1 July 1941–30 June 1942]. Maize genetics: The behavior of “unsaturated” broken ends of chromosomes. Phenotypic effects of homozygous deficiencies of distal segments of the short arm of chromosome 9. Carnegie Institution of Washington Year Book, No. 41, 1941-1942 [Issued 18 December 1942, submitted June 1942]: 181–186. [In the text McClintock cites her work as “McClintock 1941; see bibliography.” The reprints do not include the bibliography, which lists three McClintock publications (1941a, b, & c, published in March, September, & December 1941, respectively.]


53. McClintock, Barbara. 1944a. Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor [sic], Long Island, N.Y. [This report is untitled in the MGCNL. This is a report on deficiencies in Chromosome 9]. Maize Genetics Cooperation News Letter. 18 [31 January 1944, submitted 1943]: 24–26. [The report concludes, “… the chromosomal breakage mechanism is a “mutation” inducing process which “induces” the same mutant time and again.” McClintock (1987) cites title as: “Breakage-fusion-bridge cycle induced deficiencies in the short arm of chromosome 9.” However, the term “Breakage-fusion-bridge cycle” is not used in this report.]


64. McClintock, Barbara. 1950b [©1952, Symposium held June 1951]. Chromosome organization and genic expression. Pgs. 13–47. In Genes and Mutations, Cold Spring Harbor Symposia on Quantitative Biology, Volume XVI [7–15 June 1951]. Katherine Brehme Warren ed. The Biological Laboratory, Cold Spring Harbor, Long Island, New York. ©1952. This reference has been cited as 1951 or 1952- see Citation Index; McClintock (1987) lists it as 1951; McClintock (1951b) cites it as 1951. Carnegie Institution of Washington Year Book No. 51, Department of Genetics Bibliography, lists it as McClintock 1951. ②


96. McClintock, Barbara. 1971 [1 July 1970–30 June 1971]. The contribution of one component of a control system to versatility of gene expression: Relation of dose of \(Spm\) to pattern of pigmentation with the class II state of \(a2m-1\). Distinctive phenotypes associated with activation of an inactive \(Spm\). An example of versatility of control of gene expression associated with component-2 of \(Spm\). *Carnegie Institution of Washington Year Book 70*, 1970-1971 [Issued December 1971, submitted June 1971]: 5–17. [This is the last report McClintock published in the CIW Year Book. The Genetics Research Unit closed 30 June 1971. McClintock was awarded the National Medal of Science that same year (Award year 1970, presented 21 May 1971).] 2


103. McClintock, Barbara, 1984a [Nobel lecture, presented 8 December 1983]. The significance of responses of the genome to challenge. Science 226(4676) [16 November 1984]: 792-801. [Footnotes indicate that “Minor corrections have been made by the author,” and that this lecture “will be included in the complete volume of Les Prix Nobel en 1984 as well as in the series Nobel Lectures (in English) published by the Elsevier Publishing Company, Amsterdam and New York.” The correct citations for Les Prix Nobel and Nobel Lectures are listed below (see McClintock 1984b and McClintock 1993).] ②


Part II: ROAD TO TRANSPOSITION (1937-1945)

The work that led to McClintock’s Nobel Prize-winning discovery of “mobile genetic elements” (transposable elements) and to their hypothesized mechanism of movement termed “transposition,” grew from earlier studies she had conducted at Cornell and the University of Missouri. McClintock described transposition as the movement of a genetic “locus from one location in the chromosome complement to another” (1949, p.143), and concluded that it was initiated by chromosome breaks and fusions occurring at such loci. Many of these mutations (mutable loci) were due to deficiencies or rearrangements of chromosome segments brought about by broken chromosomes. Studies of this process at meiosis led her to describe this cycle of chromatid behavior as the “bridge-breakage-fusion-bridge” cycle (1939). She soon changed the name to the “breakage-fusion-bridge cycle” (BFB; 1941a). The origin and behavior of these so-called mutable loci were caused by transposition and appeared to be the same as mutation-producing events she had observed in BFB studies she had conducted years earlier (1978, pp. 27-33; 1987a, p vii).

In this section we offer perspectives and reprints of McClintock’s early studies, which she reported as “highly significant for later studies” (1987a, p. vii) that resulted in her discovery of transposition. McClintock’s (1937, 1938a, b) early investigations of the behavior of broken chromosomes and their rearrangements led her to describe the chromatid type (1939, 1941a) and chromosome type (1942a) of BFB cycles. An “improved method” (1944a, b) of introducing broken chromosomes into maize plants induced new mutations caused by minute deletions in and rearrangements of maize chromosome 9 (1945a). McClintock (1987a, p. ix) explained that her new method of inducing mutations was a consequence of previous work (1937, 1938a, b, 1941b). Subsequent experiments (1942 through 1947) enabled her to describe the nature of these new mutant phenotypes and their correlation with broken chromosomes, and ultimately to discover their mechanism — transposition [see Part III].
Perspective: 1

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1 Perspective solicited.
2 For cross-reference purposes, this is the publication number in the annotated, chronological list of McClintock’s publications (Table I) in the Front Matter.
3 The symbol ① indicates that the McClintock publication is reprinted herein.
McClintock, Barbara, University of Missouri, Columbia, Mo.: A method for detecting potential mutations of a specific chromosomal region.—Plants having two deficient chromosomes-5 (the deficiency included the locus of \( Bm_1 \), allele of \( bm_1 \), brown mid-rib, producing a brown color in the lignified cell walls) and a small ring-shaped fragment covering the deficiency and carrying \( Bm_1 \) are mosaics of heterozygous and homozygous deficient tissues through frequent losses of the ring fragment during somatic mitoses. This homozygous deficient tissue is (1) much reduced in growth capacity, (2) contains no chloroplasts, (3) has the characteristics of \( bm_1 \) in its cell walls and (4) dries on exposure to sunlight. Relatively infrequently, during somatic mitoses, the ring chromosome increases or decreases in size through duplications or deficiencies of segments of chromatin composing the ring. Duplicated segments produce no obvious tissue modifications. If removal of specific regions from the ring results in homozygous deficient tissues having specific modifications, several types of mutant sectorials, depending upon the region removed, should be repeatedly encountered in large populations of such plants. The following types of “simple” mutant sectorials have been found: (1) transparent white with colorless cell walls, no plastids; (2) opaque white with colorless plastids, colorless cell walls; (3) deficiency-\( bm_1 \), similar in detail to normal \( bm_1 \); (4) pink colored tissue with colorless cell walls, colorless plastids; (5) blotched chlorophyll pattern, colorless cell walls. The following types of “compound” mutant sectorials have been found: (1) pink, deficiency-\( bm_1 \), viable in sunlight; (2) pink, deficiency-\( bm_1 \), dries in sunlight; (3) opaque white, deficiency-\( bm_1 \); (4) blotch, deficiency-\( bm_1 \), dries in sunlight; (5) blotch, dries in sunlight. On the theory that compound mutant sectorials are the product of losses of several adjacent regions of chromatin, the simple mutant effects are referred to the chromosome in the following order: pink, deficiency-\( bm_1 \), dries in sunlight and blotch, with translucent white removed from deficiency-\( bm_1 \) and opaque white close to it. Since a homozygous deficiency for the \( Bm_1 \) locus produces the same effect as the known gene \( bm_1 \), it is possible that these other mutants may eventually appear as “genes” closely linked to \( bm_1 \).
McClintock, Barbara, Cornell University, Ithaca, N. Y.: The production of maize plants mosaic for homozygous deficiencies: Simulation of the bm1 phenotype through loss of the Bm1 locus.—In two separate cases, two mitotically functional chromosomes, a deficient rod-shaped chromosome and its reciprocal, a small ring-shaped chromosome, were produced from a single chromosome-V by means of X-ray treatment (Rhoades and McClintock, 1935). Beginning at the spindle fiber attachment region, the section of the short arm composing the ring and inversely the piece lost from the rod was 1/20 and 1/7 the length of the normal chromosome respectively and included the locus of the Bm1 gene (allele of bm1, brown mid-rib, producing a brown color in the lignified cell walls). The larger ring-chromosome is characterized by frequent losses from the nuclei during mitotic cycles and less frequently by changes in size resulting in duplications and deficiencies. When two deficient rod-chromosomes, the smaller and the larger, plus the larger ring-chromosome which covers both deficiencies, are present in a plant, loss of the ring will give rise to tissues homozygous deficient for the region represented by the smaller deficiency. Changes in size of the ring should produce tissues homozygous deficient for sections within the limits of the smaller deficiency. Five types of homozygous deficient tissue have been distinguished. Through total loss of the ring: brown cell walls, colorless plastids, poor growth capacity. Through changes in size of the ring: (1) brown cells walls, green plastids, good growth; similar in detail to tissue produced by the normal bm1 gene. (2) Colorless cell walls, colorless plastids, normal sized cells, good growth capacity. (3) Colorless cell walls, colorless plastids, small cells, excessive proliferation at external surfaces. (4) Same as (3) but with brown cell walls.
THE PRODUCTION OF HOMOZYGOUS DEFICIENT TISSUES WITH MUTANT CHARACTERISTICS BY MEANS OF THE ABERRANT MITOTIC BEHAVIOR OF RING-SHAPED CHROMOSOMES

BARBARA McClintock
Cornell University, Ithaca, New York and University of Missouri, Columbia, Missouri

Reprinted from Genetics 23: 315–376, July, 1938
THE PRODUCTION OF HOMOZYGOUS DEFICIENT TISSUES WITH MUTANT CHARACTERISTICS BY MEANS OF THE ABERRANT MITOTIC BEHAVIOR OF RING-SHAPED CHROMOSOMES*

BARBARA MCCLINTOCK

Cornell University, Ithaca, New York and University of Missouri, Columbia, Missouri

Received February 25, 1938

TABLE OF CONTENTS

I Introduction .......................................................... 315
II The mitotic behavior of ring-shaped chromosomes ............. 318
III The nature of the Bm1-bm1 variegation ....................... 333
IV Types of functional gametes produced by the two original plants 336
V Production of plants mosaic for homozygous deficiencies ...... 346
VI Simulation of the bm1 phenotype through loss of the Bm1 locus 357
VII The production and appearance of plants homozygous for Def2 R2 360
VIII The phenotypic effect of altered ring-chromosomes .......... 365
IX Discussion ............................................................ 368
Summary ........................................................................ 373
Literature cited ............................................................ 375

I. INTRODUCTION

It is the purpose of this paper to describe the method by which viable tissues, homozygous deficient for a known region of a chromosome, may be produced in maize. The chromosomal region involved includes the locus of the gene Bm1 in chromosome V (allele of bm1, brown midrib, producing a brown color in the lignified cell walls). The lignified cell walls of the homozygous deficient tissue exhibit the features characteristic of the known recessive gene bm1 although the locus of this gene is absent.

The method of obtaining the homozygous deficient tissue is related to the unique behavior of ring-shaped chromosomes during somatic mitosis. This behavior has been briefly mentioned in previous publications (McClintock 1932; Rhoades and McClintock 1935). Ring-shaped chromosomes do not always maintain themselves unaltered through successive nuclear cycles in the maize plant. They may (1) increase in size through duplication and reduplication of segments of the original ring, (2) decrease in size by deletions of segments from the ring, (3) be totally lost from the nuclei or (4) be present in increased numbers in the different nuclei. Whatever the method by which a change in size occurs, only ring chromosomes are produced from ring chromosomes.

In maize it has been found that deficiencies in certain regions of the chromosomes have been produced by the Galton and Mendel Memorial Fund.

1 Contribution from the Department of Botany in cooperation with the Department of Field Crops, Genetics Research Project, Missouri Agricultural Experiment Station, Journal Series No. 370.

GENETICS 23: 315 July 1938
chromosomes may be transmitted successfully through the egg but not through the pollen (Burnham 1932; Stadler 1935). Pollen possessing a deficient chromosome plus a ring-shaped fragment chromosome should be functional if the ring-shaped fragment completely compensates for the deficiency. By utilizing a deficiency transmissible through the eggs and rendered non-lethal in the pollen by the inclusion of a ring fragment covering the deficiency, a zygote with two deficient chromosomes plus a ring chromosome can be produced. This zygote is heterozygous for the deficiency. This heterozygosity in the resulting individual would be maintained as long as an unaltered ring chromosome was present. Should the ring chromosome be lost in subsequent nuclear divisions, or should it change in size through loss of a segment within it, the tissues arising after such loss or alteration would be homozygous deficient for the entire deficiency in the first case or for regions within the limits of the deficiency in the second case.

Two cases of deficient rod chromosomes with complementary ring chromosomes were available for this study. The two cases arose in the
 progeny of X-rayed pollen containing a normal haploid complement with the dominant gene Bm 1. This pollen, when placed upon silks of bm 1 plants with a normal chromosome complement, gave rise, among a progeny of 466, to two individuals which were variegated for Bm 1 and bm 1 (figure 1). Aberrant behavior of a ring chromosome produced by the X-ray treatment and carrying the gene Bm 1 was suspected to be the cause of the variegation.

Figure 2.—a. Diagram of a normal chromosome V. The slightly bulging section represents the spindle fiber attachment region. The sets of arrows, 1 and II, point to the positions of breaks which gave rise to the two deficient rod chromosomes and their compensating ring chromosomes illustrated in I and II of b. The deficient rod and compensating ring chromosomes of I are referred to in the text as Def 1 and R 1 respectively, those of II as Def 2 and R 2 respectively.

Examination of synaptic configurations in sporocytes revealed not only the presence of a small ring-shaped chromosome in each plant but also a deficiency in one chromosome V. In each case, the size of the ring-shaped chromosome and the extent of the deficiency in the rod-chromosome were comparable. The deficiency in both cases involved a section of the short arm immediately adjacent to the spindle fiber attachment region. Since the ring fragment in both cases possessed a small but definite spindle fiber attachment region, these regions being clearly visible in meiotic prophases,
it was assumed that in each case the deficient rod and its compensating ring chromosome arose as the result of two breaks in the normal chromosome V, one break passing through the spindle fiber attachment region, the other breaking the chromosome at a distance from the spindle fiber attachment region equal to approximately \( \frac{1}{20} \) (Case I) and \( \frac{1}{7} \) (Case II) of the total length of chromosome V (figure 2). Fusions two by two of the broken ends resulted in a deficient rod and a compensating ring chromosome each with a section of the original spindle fiber attachment region. Since both the deficient rod and the ring chromosome possessed a section of the spindle fiber attachment region, both could be maintained through nuclear cycles.

Proof that the ring chromosome represented the region for which the rod chromosome was deficient was furnished by the synaptic configurations produced by homologous associations of the three chromosomes: the normal chromosome V contributed by the female parent, the deficient rod chromosome V and the small ring chromosome contributed by the male parent (figures 25 and 26 and photographs of the same, 17 and 18, Plate II). Cytological examination of different portions of the tassel disclosed the loss of the ring chromosome in several branches. Similarly, within a single anther, groups of cells were found lacking the ring chromosome. It was suspected, therefore, that the ring chromosome carried the locus of Bm 1, its loss during somatic mitoses being responsible for the presence of the bm 1 (brown) streaks in these plants. Conclusive proof for this was derived from the progeny of these two plants when crossed to normal bm 1 plants. The progeny included variegated (Bm 1 and bm 1) and bm 1 plants. Of the variegated plants, microsporocytes of 148 individuals were examined for the presence of the ring chromosome. The ring fragment was found in 146 of these individuals although in many plants several branches of the tassel lacked the ring fragment. In two plants no ring chromosome was found in the several branches of the tassel which were collected. Of the totally bm 1 plants, 47 were examined. In no case was a ring chromosome found. In a bm 1 tiller of a variegated plant, a considerably reduced ring chromosome was found. It is probable in this case that the Bm 1 locus had been deleted from the ring chromosome through somatic alterations to be described in the next section. Individual collections were made on the two sides of plants which were approximately half bm 1 and half variegated. In these cases, the presence of the ring chromosome could be established only on the variegated side.

II. THE MITOTIC BEHAVIOR OF RING-SHAPED CHROMOSOMES

The interpretation of the variegation and of the production of homozygous deficient tissues has been based on a knowledge of the behavior of
ring-shaped chromosomes in somatic nuclear cycles. A description of what has been observed regarding the appearance and behavior of the ring chromosomes in meristematic regions is therefore necessary before the individual cases can be considered. Although the primary cause of irregularities in the nuclear cycles is undoubtedly the same for large and small ring-shaped chromosomes, the subsequent behavior and the genetical consequences vary in these two extremes. The behavior of large ring-shaped chromosomes will be considered first; this will be followed by an account of the small ring-shaped chromosomes; finally, correlations and conclusions will be drawn regarding ring-shaped chromosomes in general.

*Mitotic behavior of large ring-shaped chromosomes*

Since the two ring-shaped chromosomes of cases I and II, figure 2, are both small, a large ring-shaped chromosome originally representing most of chromosome II has been examined (McClintock 1932). The observations were made on longitudinal sections of actively growing root tips. Observations at meiotic prophase in this plant had clearly indicated that changes in size and hence chromatin content of the ring chromosome were occurring in the premeiotic nuclei. Groups of related cells usually had similar ring chromosomes but the differences in unrelated cells were very great. In a few cells the altered ring chromosome was larger than the normal chromosome II. In some cells it had been reduced to only a few chromomerese. All gradations between these two extremes were found in different sporocytes of this same plant. The smallest ring chromosome has obviously undergone a great loss of chromatin. The original ring chromosome possessed a single knob. Evidence for duplication of segments other than the obvious increase in size of the ring chromosome was clearly registered in some cells by the increase in the number of knobs. Rings with two, three and four knobs were found.

It was suspected that the alteration in chromatin content of the ring was related to the division cycle of the chromosome. Observations of mitoses in root tip meristems suggested the manner in which the alterations occur without, however, revealing the primary cause. If one assumes that during the splitting process or after the split has occurred, a crossover took place between the two sister chromatids, a double-sized, continuous ring with two spindle fiber attachment regions would be produced. A second crossover between the two sister chromatids could result in an interlocking of the sister ring chromosomes provided the second crossover did not counteract the first. The presence of double-sized rings with two spindle fiber attachment regions at late anaphase and early telophase was clearly evident in a number of cells (figures 5, 7, 15, 16; photographs 4, 5, 8, Plate I). Unfortunately the presence of interlocking rings could not
be determined directly since the chromosomes of maize in somatic cells are relatively small. Many anaphase figures were suggestive but none could be definitely distinguished from double-sized rings with a twist at the mid-region. From the point of view of the origin of such configurations it would be important to know the relative percentage of each type. From actual counts it is certain that the double-sized rings are present in at least one-third of the aberrant figures. The actual number of late anaphase and early telophase figures with chromatin bridges produced by double-sized or interlocked rings amounted to approximately 8 percent of a total of 1145 figures recorded in roots whose ring chromosome had not materially reduced in size in most of the cells (D, table 1).

**Table 1**
The frequency of normal and aberrant somatic anaphase and early telophase configurations in plants with different ring chromosomes.

<table>
<thead>
<tr>
<th>RING CHROMOSOME</th>
<th>NORMAL</th>
<th>ABERRANT</th>
<th>% ABERRANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>A R1</td>
<td>605</td>
<td>1</td>
<td>0.16</td>
</tr>
<tr>
<td>B R2</td>
<td>1195</td>
<td>14</td>
<td>1.1</td>
</tr>
<tr>
<td>C R2 plus enlarged R2</td>
<td>1169</td>
<td>76</td>
<td>6.1</td>
</tr>
<tr>
<td>D Large ring chromosome II</td>
<td>1053</td>
<td>92</td>
<td>8.1</td>
</tr>
</tbody>
</table>

Since the fate of the double-sized or interlocked rings is not the same in all late anaphase and telophase figures, a number of types of behavior from anaphase to late telophase have been diagrammed in figure 3. Representative drawings from different cells are given in figures 5 to 24 and photographs of Plate I. In the diagrams, the behavior of double-sized rings has been emphasized since this type could be clearly recognized in many cells. They are either clearly open or show a twist at the mid-region. Some of the interlocked rings should produce figures resembling those shown in the diagram and would not be easily distinguished from them.

In most of the mitotic cycles the ring chromosome splits along a single plane, separation of the two halves proceeding normally at anaphase, figure 4. In the late anaphase figures the double-sized rings produce a double bridge the chromatin of which is pulled taut (figure 5, photograph 1, Plate I). It is suspected that breakage of the chromatin bridges sometimes occurs during this period (photograph 10, Plate I). Since such figures were not included in the counts mentioned above, the 8 percent of anaphase and telophase figures with bridges represent the minimum number of cells in which double-sized or interlocked rings occurred. Some of the telophase figures suggest an early breakage of one or both strands of the double bridge (figures 8 and 9 and photographs 5 and 6, Plate I).
Figure 3.—Diagrams illustrating the behavior in somatic mitosis of double-sized ring chromosomes with two spindle fiber attachment regions produced from the two split halves of a single ring chromosome.

a. Successive stages from mid-anaphase to mid-telophase of a medianly placed double-sized ring chromosome. The cell plate determines the positions at which breaks will occur in the two chromatin bridges.

b. Similar to a except that a twist is present in the bridge strands of the double-sized ring chromosome.

c. Appearance in early and mid-telophase of a double-sized ring which was non-medianly placed in the spindle figure. The components entering each daughter nucleus vary in chromosome length and constitution.

d. Similar to c except that the upper portion of the double-sized ring chromosome is not included in the reorganizing telophase nucleus. Such behavior results in the loss of a component of the ring chromosome from one of the daughter nuclei.

e. Appearance at mid-telophase of a double-sized ring chromosome with one broken bridge strand.

f. Appearance at mid-telophase of a double-sized ring chromosome with both strands broken.

g. Appearance at very early telophase suggesting an early breakage of bridge strands of a double-sized ring chromosome (or two interlocked sister ring chromatids).

h. Comparable situation as illustrated in d except that the strands of the bridges are twisted at the cell-plate region.

i. Fine bridge of chromatin between two resting nuclei suggesting that a breaking of the strands had not occurred at telophase.
In many cases, breakage of the strands composing the bridge does not occur at anaphase; compare photographs 9 and 10, Plate I. The moving apart of the spindle fiber attachment regions in the double-sized rings is retarded by the tension of the chromatin bridges. The subsequent behavior is conditioned by the position in the spindle figure of this retarded ring or of two retarded interlocked rings. As the telophase sets in there is an immediate release of tension on the chromatin bridges produced through the swelling of the forming nuclei (photograph 2, Plate I). As the nuclei continue to swell and approach the cell-plate, the chromatin of the ring within the nuclei is relaxed, allowing the form of the ring chromosome to be clearly defined (figures 8, 9, 11, 16; photographs 3, 4, 5 and 8, Plate I). At this stage the tension on the chromatin threads from the nuclear mem-

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**Figure 4.**—Normal separation of a ring chromosome in somatic anaphase.  
**Figure 5.**—A double-sized ring chromosome in early telophase.  
**Figure 6.**—A double-sized ring chromosome at a slightly later stage than that shown in figure 5.  
**Figure 7.**—Double-sized ring chromosome at mid-telophase. The bridge strands close to the cell-plate have become very thin. The shape of the chromosome within the nuclei has become discernible.  
**Figure 8.**—Mid-telophase. Early breakage at the cell-plate region of two bridge strands of the double-sized ring. See comparable figure, photograph 6, Plate I.  
**Figure 9.**—Mid-telophase. Early breakage at the cell-plate region of one bridge strand of a double-sized ring. See photograph 5, Plate I.  
**Figure 10.**—Late telophase. Breakage of bridge strands of a double-sized ring chromosome at the cell-plate region and withdrawal of the chromatin into the nucleus at the lower part of the figure.
brane to the cell-plate again increases. The threads become thin and taut as if being pulled into the nuclei (figures 7, 16, 17; photograph 8, Plate I). In a few cases, these fine chromatin threads are seen in relatively late telophase nuclei (figure 14). Since they usually do not persist into late stages, breakage must usually occur during the earlier telophase period. There were many sister telophase nuclei observed in which the ring chromosome in each nucleus was close to the region of the nuclear membrane lying nearest the cell-plate (figures 12 and 13). Such figures probably represent the last stage in the progress of the previously double-sized or interlocked rings. It should be emphasized that fusions of broken ends must occur after such breakage, since only ring chromosomes have been found to arise from ring chromosomes although rod chromosomes might be expected.

It sometimes happens that the passage of one spindle fiber attachment
region of a double-sized ring proceeds toward its pole in advance of the opposing spindle fiber region. Consequently, the double-sized ring is not medially placed in the spindle figure. The cell-plate then intercepts the chromatin bridges in a non-median position (figure 15, photograph 7, Plate I). As a result, the components of the double-sized ring entering sister telophase nuclei will be unequal in size and chromatin constitution. One segment of the double-sized ring is sometimes not included in the telophase nucleus on its side of the cell-plate (figures 16 and 17, photograph 8, Plate I).

If the chromosome is not split at anaphase, fusions of broken ends could give rise in the next division to normally disjoining sister ring chromosomes, or if twists are present in the chromonema before fusion, to a continuous double-sized ring or interlocked sister ring chromosomes when

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**Explanation of Plate I**

All magnifications are approximately $\times 1100$.

Plate I.—Individual cells from longitudinal sections of the growing points of roots. Photographs 1 to 10 are of the large chromosome II ring. Photographs 11 to 14 show an enlarged R2 chromosome. Photographs 15 and 16 are of the normal R2 chromosome.

Photograph 1. Late anaphase. Bridge produced by separation of the split halves of a ring-shaped chromosome which is in the form of a double-sized continuous ring. There is a twist of the strands at the mid-region.

Photograph 2. Early telophase. Beginning of relaxation of tension on the strands of the double bridge.


Photograph 5. Mid-telophase. A double-sized ring chromosome. The strand to the right appears to be broken.


Photograph 7. Late anaphase. Non-median placement in the spindle figure of a double-sized ring chromosome.

Photograph 8. Mid-telophase. The result of a non-median placement in the spindle figure of a double-sized ring chromosome. The strands adjacent to the cell-plate have become attenuated. The lower segment of the ring chromosome was excluded from the forming nucleus.


Photograph 10. Very early telophase. Figures such as this suggest an early breakage of the strands of a double-sized ring chromosome or of interlocked sister ring chromatids.

Photograph 11. Typical late anaphase position of a small ring-shaped chromosome which will be excluded from the reforming telophase nuclei.

Photograph 12. Early telophase. Excluded ring chromosome which was previously non-medianly placed in the spindle figure. The cell-plate has passed below it.

Photograph 13. Late anaphase. Stage in the process of exclusion of two closely associated ring chromosomes.


Photograph 15. Typical late anaphase position of a small ring-shaped chromosome which will be excluded from the telophase nuclei.

Photograph 16. Mid-telophase. The result of a previously excluded ring-shaped chromosome. The cell-plate has passed below the ring.
RING CHROMOSOMES IN MAIZE

2.19

simple assumptions are made regarding the method of splitting or re-
duplication of a chromonema along a single plane. If the chromosomes are
split at anaphase, two by two fusions of the two adjacent broken ends of
sister chromatids could result immediately in a continuous double-sized
ring. If fusions two by two took place between the non-adjacent broken
ends, continuous double-sized rings or interlocked sister ring chromatids
could result. If the single (no anaphase split) threads were very much
twisted or the double (split present at anaphase) threads coiled about one
another, complex configurations would appear in the next anaphase. Only
rarely was a figure found suggesting any complexity. If such behavior
were the secondary cause of double-sized or interlocked ring chromatids
(it cannot be the primary cause, see discussion), adjacent cells in the
longitudinal rows could be expected to show chromatin bridges in an
appreciable percent of the cases. They were present in a number of lon-
gitudinally adjacent cells. However, a very large number of such figures
would be necessary to allow a satisfactory statistical study to be made.
Although a large number of anaphase and telophase figures with aberrant
configurations have been observed, the numbers of these in adjacent cells
were insufficient for such a study.

The mitotic behavior of small ring-shaped chromosomes

The mitotic behavior of small ring-shaped chromosomes differs from
that of large ring-shaped chromosomes in (i) the reduced frequency with

ExPLANATION OF PLATE II

PLATE II.—All photographs are of pachytenes configurations in microsporocytes. X1100.
Photograph 17. Synaptic association of a normal chromosome V, a Def2 chromosome V and
an R2 chromosome. See text figure 25.
Photograph 18. Similar to photograph 17. See text figure 26.
Photograph 19. For description, see text figure 27.
Photograph 20. Synaptic association of a normal chromosome V and a Def2 chromosome V.
See text figure 28.
Photograph 21. From a sporocyte of a plant with a normal chromosome V, a Def2 chromo-
some V and two R2 chromosomes. The two rod-chromosomes have associated with one another
(note buckle at spindle fiber attachment region). The two ring chromosomes have associated with
one another.
Photograph 22. Synaptic association of two R2 chromosomes.
Photograph 23. Late pachytene. The arrow points to the tiny R1 chromosome.
Photograph 24. Collapsed R1 (upper) and R2 (lower) chromosomes associated at their
spindle fiber attachment regions.
Photograph 25. Collapsed R1 (left) and R2 (right) chromosomes associated at their spindle
fiber attachment regions.
Photograph 26. R1 chromosome (arrow). Its spindle fiber attachment region is stuck to that
of bivalent chromosome VIII.
Photograph 27. Collapsed R1 chromosome (arrow) whose spindle fiber attachment region is
associated with that of a normal chromosome V bivalent.
Photograph 28. Synaptic association of a normal chromosome V and a Def1 chromosome V.
See text figure 30.
which double-sized or interlocked rings arise; (2) the more frequent loss of the ring chromosomes from the nuclei; (3) the considerably less frequent occurrence of changes in size of the ring chromosomes and (4) the occasional increase in the number of rings in a nucleus.

The two small rings in cases I and II, figure 1, have been used to study the behavior of small ring-shaped chromosomes in mitosis. In the subsequent discussions these two ring chromosomes will be referred to as R 1 and R 2 respectively. Cytological examination of the sporocytes in different branches of the tassel in plants with either of these rings had indicated that loss of the ring chromosome was occurring far more frequently than changes in size of the ring. This is in direct contrast to the behavior of large ring chromosomes, where changes in size are more frequent than loss. To obtain evidence on the method of loss, examinations of the meristematic regions of the roots of such plants were made. The tiny R 1 ring chromosome is clearly visible in the prophase nuclei of these cells. However, the description will confine itself to the behavior of the larger of these two rings, R 2, and one of its enlarged derivatives, since anaphases showing aberrant configurations of the R 1 chromosome are found only very rarely.

The aberrant anaphase and telophase configurations are characterized by the median or nearly median position of the double-sized (or interlocked) ring chromosome in the spindle figure (photograph 15 for the normal R 2 and photograph 11 for the enlarged R 2, Plate I). However, they occasionally lie some distance from this position (figure 22 and photograph 12, Plate I). The ring chromosome in these configurations, as with the large ring chromosomes, frequently appears to be double-sized. In roots in which most of the nuclei contained the normal R 2 chromosome, 14 of the 1209 anaphase and telophase figures counted, or 1.1% percent showed these aberrant configurations (B, table 1). They were observed many times in roots where counts were not made.

The fate of the delayed double-sized ring depends upon its position in the spindle figure as the cell-plate appears. If it is in the middle, the cell plate passes through it, dividing it into relatively equal or decidedly unequal segments (figures 19 and 20). If it is not medially placed, the cell plate passes to one side and the double-sized ring remains in the cytoplasm of one of the daughter cells (figures 21 and 22, and photographs of same, 12 and 16, Plate I). If it lies rather far away from the mid-region, it may be included in one of the nuclei. If this occurs and if normal splitting of this double-sized ring with two spindle fiber attachment regions follows in the next division, two double-sized rings, each with two spindle fiber regions should then be found lying close together in the spindle figure when the two spindle fiber regions on the same chromatid pass to opposite
poles. Several configurations have been observed in which two rings were lying very close together (figures 23 and 24, and photographs of the same, 13 and 14, Plate I). The exact contours of the individual rings could not be accurately followed and therefore have not been shown in the drawings. Since the contours of the two ring chromosomes could not be accurately followed, such figures could represent two interlocked ring chromosomes from a similar condition to that described above, if instead of a double-sized ring, two interlocked sister ring chromosomes had been included in the preceding telophase nucleus.

Since anaphases and telophases with the ring chromosome lying in the middle of the spindle figure are the most frequent types of aberrant configurations, and since these rings are subsequently excluded from the
telophase nuclei, the frequently observed absence of the ring chromosome from branches of the tassel or from groups of cells in an anther can be explained.

In connection with the problem of the mechanism of movement of chromosomes in the spindle it would be of interest to explain why these small double-sized rings do not appear to be under greater tension as their spindle fiber regions pass toward opposite poles. It is possible that movement toward opposite poles is initiated at the spindle fiber region of the chromosome at early anaphase but that continued movement is made possible by other forces exerted on the chromosomes when they have reached a region in the spindle which is some distance away from the equatorial plate. These double-sized ring chromosomes may be too small to reach this region by the pull exerted at the spindle fiber region. The behavior of intermediate sized rings lends support to this assumption since these sometimes remain in the equatorial plate either with or without evidence of tension.

From cytological examination of sporocytes it was known that changes in size of the small ring chromosomes do occur though with relatively low frequency. The R 2 chromosome has been observed to decrease to several chromomeres and also to increase to seven or eight times its original size. Photograph 15, Plate I, represents a normal R 2 chromosome, photographs 11 and 12, an enlarged R 2 chromosome. It has also been seen that an increase in the number of these rings in a nucleus, usually with alterations in size, sometimes occurs. As many as six ring chromosomes in the nuclei of a sector of a plant which very probably possessed but one ring in the zygote have been observed in a single instance.

If a double-sized ring is included in one of the daughter nuclei, as described above, a start in the direction of increase in number of rings has been made. The chance of loss of this ring chromosome in subsequent divisions is great. However, as already shown, the ring chromosome may be directly broken in two by opposed poleward forces at anaphase, or the poles of the spindle may lie so close together that the spindle fiber regions of the ring are readily included in the two nuclei, after which the resulting chromatin bridges are cut through by the cell-plate and drawn into the nuclei. When two double-sized sister ring chromosomes each with two spindle fiber regions are present in an anaphase figure, and when each of these is subsequently broken and the broken ends drawn into the telophase nuclei, the initial event in the production of a sector of tissue with two altered ring chromosomes has occurred. It is apparent, on this basis, why increase in number of these small ring chromosomes is relatively rare: one infrequent event must be followed by another.
Conclusions regarding ring chromosome behavior

The foregoing account has indicated the probable method by which rings are altered in size and genic constitution or are lost from the nuclei altogether. From both cytological and genetical observations it has been concluded that the rate at which this occurs is dependent upon the length of the chromonema composing the ring: the longer the chromonema, the more frequent the occurrence of aberrant mitoses involving the ring. Counts of the aberrant ring chromosome configurations in late anaphase and very early telophase are given in Table 1. The counts are from roots in which the ring chromosome was present in most of the cells. In A, the R1 chromosome of case I, Figure 1, is represented. One aberrant configuration of the ring chromosome was observed in these roots which were counted. Judging from the relatively small amount of bm 1 tissue shown by plants with this R1 chromosome, loss of the ring chromosome must be relatively infrequent. In B, the small R2 chromosome of case II is represented. The observed aberrant configurations of the ring chromosome in these roots amounted to 1.1 percent of all the figures recorded. As stated above, these aberrant configurations result mainly in loss of the ring chromosome from both nuclei. Variegation, expressed by the bm 1 tissue in these plants, is considerably greater than in the plants from which the counts of Table 1, A, were obtained. Table 1, C, represents the counts from a plant which possessed two ring chromosomes, a normal R2 and an R2 enlarged approximately three times. In the roots from which the counts were made, both rings were present in many of the nuclei. Aberrant configurations involving the enlarged R2 chromosome were more frequent than those involving the normal R2 chromosome. The size of the enlarged R2 chromosome lies at the border line between those rings whose aberrant configurations lead mainly to exclusion from the telophase nuclei (small rings) and those whose aberrant configurations lead mainly to changes in size of the ring chromosomes (large ring chromosomes). For the enlarged R2, both types of configurations were frequently encountered. In Table 1, D, from a plant with a ring chromosome approximately twice the size of the enlarged R2 chromosome, the aberrant configurations amounted to 8 percent of the total number recorded. In this case, as described above, the figure is possibly too low. The telophase figures here were characterized mainly by changes in size of the ring chromosome rather than loss from the nuclei.

If a ring chromosome in a given individual carried a dominant gene and the two normal rod chromosomes carried a recessive gene, the expression of variegation produced by losses or changes in constitution of the ring chromosome would depend upon (1) the size of the ring chromosome and
(2) the position of the gene with respect to the spindle fiber attachment region. In relatively small ring chromosomes, which are mainly lost from the nuclei, the expression of variegation is directly dependent upon the actual size of the ring chromosome: the larger the ring chromosome the greater the amount of variegation exhibited. This is strikingly illustrated by the two ring chromosomes, R1 and R2. The variegation produced by R1 is very much less than that produced by R2. To check this conclusion without prejudice, cultures were obtained in which either R1 or R2 or both R1 and R2 chromosomes were expected to be present in individual plants.

Table 2
Comparisons of the predicted and observed ring chromosome constitutions in cultures segregating plants with one, two and three ring chromosomes.

<table>
<thead>
<tr>
<th>Prediction:</th>
<th>Prediction:</th>
<th>Prediction:</th>
<th>Prediction:</th>
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<tbody>
<tr>
<td>1R1</td>
<td>1R2</td>
<td>2 rings</td>
<td>3 rings</td>
</tr>
<tr>
<td>Correct</td>
<td>Deviation</td>
<td>Correct</td>
<td>Deviation</td>
</tr>
<tr>
<td>1 (R1 + R2)</td>
<td>15</td>
<td>3 (R1)</td>
<td>24</td>
</tr>
<tr>
<td>Correct</td>
<td>Deviation</td>
<td>Correct</td>
<td>Deviation</td>
</tr>
<tr>
<td>5 (1 ring)</td>
<td>1 (3 rings)</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

* Three showed one R1; one showed one R2 in an estimated two R2 plant; one showed one R2 in an estimated R1 plus R2 plant. Complete agreement in all cases could not be expected in two- and three-ring plants from sporocyte examinations since loss of one of the ring chromosomes in the developmental stages of the tassel is expected in some cases. This particularly applies to the R2 chromosome.

† The estimate for this plant was two R1. Some of the two R1 plants have practically no bm1 tissue. A two R1 plant could be difficult to distinguish from a three R2 plant.

of the culture. In some of these cultures plants with three ring chromosomes were expected. From the expression of the variegation exhibited by each plant a prediction was made as to the ring chromosome constitution of the plant. Cytological observations were subsequently made to determine the correctness of these predictions. Table 2 shows the correlation of these observations with the predictions. Cultures of plants with the R1 chromosome can readily be separated from cultures whose individuals possess the R2 chromosome through observations of the variegation alone (see following section for more complete discussion).

With relatively large ring chromosomes, which are characterized mainly by changes in size of the ring chromosomes, the expression of variegation would depend upon the nearness of the locus of the gene to the spindle fiber region. The farther away the locus, the greater is the amount of variegation that should be expressed.

The method of alteration of the ring chromosomes as suggested by the somatic anaphase and telophase configurations should produce rod-shaped chromosomes. Although thousands of microsporocytes have been examined in many of which an alteration of the ring chromosome has been ob-
served, no rod-fragments have been recognized. It can not be stated that
they do not occasionally occur, but certainly their frequency must be
exceedingly low. If the method by which ring chromosomes change in size
has been correctly interpreted from the study of somatic anaphase and
telophase figures, one is forced to conclude that the broken ends of the
chromosomes unite, thus re-establishing a ring.

It might be stated here that when two ring chromosomes are present in
the nuclei of a plant, it is rare that both rings show aberrant configurations
in the same cell (figure 18). Each ring chromosome apparently acts inde-
pendently with regard to the formation of double-sized or interlocked
rings.

That the behavior of ring chromosomes in maize is a consequence of
their form and not of their genic constitution can be definitely stated,
since a number of different ring chromosomes, each involving segments of
chromosomes not strictly comparable, have been found so far. These in-
clude segments from chromosomes II, V, VI, VIII and IX. Most of them
were detected by the variegation which they produced but three were
isolated independently of any visible effect.

III. THE NATURE OF THE *Bm 1–bm 1* VARIEGATION

The gene *bm 1* when homo- or hemizygous produces a brown color of
the cell walls. The color appears in the walls as soon as lignification sets in.
It is not present before this period. The depth of color, on external exam-
ination of *bm 1* plants, is greatest in those tissues which are composed
largely of thickened cell walls, such as the midrib of the leaf, the veins in
the leaf sheath and the stalk tissue. The brown color is not easily detected
in the leaf tissues other than the midrib since the cell walls are thin and
the color is masked by the chlorophyll.

As the plant matures in the field, the brown color has been noted to
fade considerably in exposed regions of the plant but remains deep in
those regions which are well protected from light. It was suspected that
direct sunlight was causing a change in the structure of the brown pigment
which resulted in loss of color. To determine if this was correct, black paper
was placed about exposed parts of several *bm 1* plants when the brown
color was intense. The bands of black paper remained about these parts
for a period of three weeks. When the paper was removed, the tissues
protected from light had retained their original deep brown; the brown
color in the tissues above and below the protected region had faded con-
siderably.

In plants possessing two normal chromosomes V with *bm 1* (or one
normal chromosome V with *bm 1* and one of the deficient chromosomes
V) and a ring chromosome with *Bm 1*, streaks of *bm 1* tissues are pro-
duced and can be seen by external examination of the plants (figure 1). Over 7000 variegated plants have been examined in the progeny of the two original variegated plants. Cytological observations have indicated that loss of the ring chromosome carrying Bm 1 is the primary cause for the appearance of the bm 1 tissues. Losses can occur anywhere in the ontogeny of the plant. The patterns of the bm 1 tissues should give some indication of where and when these losses occurred. Although wide or narrow bands on the stalk (figure 1) indicate an early or late loss of the ring chromosome, respectively, cross sections of the stem, where most of the cell walls are heavily lignified, give even a better indication of the time of loss. If loss occurred early in ontogeny, the whole plant would be bm 1. If the first loss occurred in one of the cells which is to give rise to the part of the plant above the ground, a wide sector of bm 1 would be produced. Still later losses would produce sectors of various widths in the stem. Very late losses would produce streaks or patches composed of a few cells only. All of these types of variegation patterns have been observed.

When a stalk with a relatively wide external band of bm 1 tissue is cross-sectioned and examined with low magnification, the brown-walled tissue is seen to be composed of a V-shaped sector with the tip of the V pointing toward the center of the stalk. Many narrow surface streaks are produced by similar sectors but the V is smaller and the tip considerably removed from the center of the stalk. Very narrow streaks may be composed of only a few cells. Such streaks are visible on external examination of the stalk only if they lie at or close to the surface. Patches of bm 1 cells not close to the surface cannot be seen by external examination.

Dilution of color in the brown (bm 1) cell walls on the side of the wall adjacent to the white (Bm 1) cell walls was a striking feature of the variegation in all plants. That it is a dilution produced by the adjacent Bm 1 cells and not a spreading of the brown color from the bm 1 cell walls is suggested by the considerable reduction in intensity of color in the brown walls of the very small patches composed of only a few cells, and by the dilution of color of a row of bm 1 epidermal cells on the side adjacent to inner Bm 1 cells.

The variegation in plants possessing an R 2 chromosome is expressed by a few totally bm 1 plants where the ring chromosome has been lost before the cells which are to produce the stem meristem have been differentiated, to plants which are composed of many bm 1 streaks of different widths. Cross sections of the stems of the average variegated plant show wide V-shaped sectors, smaller V-shaped sectors and many irregular patches of bm 1 composed of few to many cells.

The variegation patterns in plants with the R 1 chromosome were similar
RING CHROMOSOMES IN MAIZE

2.27
to those produced by plants with the R 2 chromosome but the total amount
of bm 1 tissue was very much less. There were fewer sectors of all types
in these plants, making cultures of the two types of variegated plants
readily distinguishable. This is expected from the cytological examinations
since the smaller ring chromosome is lost less frequently in somatic divi-
sions than the larger ring chromosome. The extent of variegation is a
direct expression of the rate of loss of the ring chromosome.

Plants with two ring chromosomes show considerably less variegation
than plants with one ring chromosome. Loss of one ring chromosome
followed later by loss of the second ring chromosome or simultaneous
losses of both ring chromosomes must occur before the bm 1 tissue could
be produced. The patterns of the bm 1 tissues in cross-sections of the
stem clearly show this relationship. These fall into three main types of
sectors: (1) solid V-shaped sectors, (2) spotted V-shaped sectors, and (3)
small patches of bm 1 tissue.

The solid V-shaped sectors are interpreted as relatively early losses of
one ring followed slightly later by loss of the second ring chromosome or
by occasional simultaneous losses of both rings. The spotted V-shaped
sectors reveal more closely the relationship between loss of one ring fol-
lowed considerably later by losses of the second ring. They are detected
as a cluster of bm 1 patches in an isolated region of a stem which other-
wise shows very few bm 1 patches. When each of the brown patches in
such a cluster is traced with a camera lucida and lines drawn joining the
outer boundaries of the outermost patches, the lines converge in the direc-
tion of the center of the stem. They clearly define a V-shaped sector.
Such spotted V-shaped sectors would be expected if loss of one ring carry-
ing Bm 1 is followed later in development by losses in different cells of the
the second ring chromosome carrying Bm 1.

The small patches of brown walled tissue, usually composed of only a
few cells, can be interpreted as relatively late, successive, or occasionally
simultaneous, losses of the two rings.

There are three types of plants with two ring chromosomes: (1) those
with two R 1, (2) those with one R 1 and one R 2 chromosome, and (3)
those with two R 2 chromosomes. Since somatic loss of the R 2 chromo-
some is considerably more frequent than the R 1 chromosome, the amount of
bm 1 tissue produced in each of these plants is progressively greater.
Plants with two R 2 chromosomes have considerable amounts of bm 1
tissue; those with one R 1 plus one R 2, very much less, and those with
two R 1 chromosomes exceedingly little bm 1 tissue. In this latter type
of plant it is often necessary to examine cross-sections of the stem to
determine if any bm 1 tissue is present. Such tissue, when not close to
the surface, cannot be detected from field examinations of the plants.
Plants with three ring chromosomes of the constitution two R 2 plus one R 1 chromosome or one R 2 plus two R 1 chromosomes, have been obtained. These plants frequently show no external evidence of bm 1 tissues. In all cases, however, careful examinations of the stalks have revealed small patches of bm 1 cells. The bm 1 cells could arise only after loss (mainly successive) of all three ring chromosomes from the nuclei.

For the sake of comparison, the stalks of a number of plants with a normal chromosome constitution carrying Bm 1 in one chromosome V and bm 1 in its homologue were examined. In no case was there any evidence of bm 1 tissue.

In conclusion it can be emphasized that the genetic expression of variegation is in full agreement with expectation on the basis of the cytological observations given in the previous section. In these plants with small ring chromosomes whose aberrant mitotic configurations are followed mainly by loss of the ring chromosome from the nucleus, the extent of variegation is a direct indication of the length of the chromonema composing the ring chromosome, the larger the ring chromosome the higher the rate of loss and thus, the greater the amount of exhibited variegation. Loss of the ring chromosome can occur at any stage in the development of the plant, early loss giving rise to a totally bm 1 plant, later loss to wide sectors of bm 1 and very late losses to small patches of bm 1 cells. The patterns exhibited by two and three ring chromosome plants are those expected from the cytological observations where it has been shown that simultaneous loss of the several ring chromosomes from a nucleus is rare. The cause of the aberrant mitotic configuration arises independently in each ring chromosome.

Knowledge gained from a study of variegation in these plants has been utilized in the analysis of tissues of plants mosaic for homozygous deficiencies (section V).

IV. TYPES OF FUNCTIONAL GAMETES PRODUCED BY THE TWO ORIGINAL VARIEGATED PLANTS

Each of the two original variegated plants possessed one normal chromosome V with bm 1, one deficient chromosome V and a ring-shaped fragment chromosome corresponding in size to the deficiency in the rod chromosome (figure 2). In case II (Def 2, R 2) prophase meiotic associations had indicated the homology of the ring chromosome with the region in the rod chromosome which had been deleted (figures 25 and 26; photographs of the same, 17 and 18, Plate II). Most frequently, the ring chromosome did not associate with its homologous section in the normal rod
chromosome but remained separate and collapsed (for meiotic prophase behavior of ring-shaped chromosomes, see McClintock 1933). In all cells the deficient rod chromosome V and the normal chromosome V were associated. The normal V had to buckle to compensate for the deletion in the deficient V. Figures 27 and 28, and photographs of the same, 19 and 20, Plate II, illustrate this association. In the plant from which figure 27 was drawn, two ring chromosomes were present. They are separate and collapsed. Another figure from the same plant, photograph 21, Plate II, shows the not infrequent association of the two R2 chromosomes to form a true ring-shaped configuration and also the association of the normal and Def 2 chromosomes.

In case I (Def1, R1), no figures were observed showing the association of the ring chromosome with its homologous section in the normal chromosome. It is probable that it occurred in a small percentage of the cases but
would be difficult to detect except in the most favorable figures because of the smallness of the deficiency and the ring chromosome. Figures 29 and 30 illustrate the pachytene association of the Def1 chromosome with a normal chromosome V. The small ring chromosome (R1) lies free and is collapsed. In figure 30 and photograph of the same, 28, Plate II, non-homologous associations about the deficient region have resulted in a separation of the spindle fiber attachment regions of the two chromosomes (for expected non-homologous associations, see McClintock 1933). The small buckle below the lower spindle fiber region or the distance between
the two spindle fiber regions represents the extent of the deficiency. Photographs 23, 26 and 27, Plate II, illustrate the appearance of the R1 ring at meiotic prophase. In photograph 23, very early diplotene, the

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure29}
\caption{Pachytene association in a microsporocyte of a plant with one normal chromosome V, one Def I chromosome V and an R1 chromosome. The ring chromosome is collapsed and is not associated with its homologous region (buckle) in the normal chromosome V.}
\end{figure}

ring shape of the chromosome is clear. In photograph 26 the spindle fiber region of the R1 chromosome is stuck to that of chromosome VIII. In photograph 27, the spindle fiber region of the collapsed R1 chromosome is adjacent to that of a normal chromosome V bivalent. Photographs 24

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure30}
\caption{Pachytene association of a normal chromosome V and a Def I chromosome V. Through non-homologous associations, the buckle which compensates for the deficiency, has shifted into the long arm of the normal chromosome V. Note the displacement of the spindle fiber attachment regions. See photograph of same, 28, Plate II.}
\end{figure}

and 25, Plate II, illustrate the relative sizes of R1 and R2 when both are present in the same sporocyte. In both photographs the collapsed ring chromosomes are associated by their spindle fiber attachment regions. In photograph 24 the R1 chromosome is above, the R2, below. In photograph 25 the R1 chromosome is to the left, the R2 to the right.

In plants heterozygous for either deficiency and its compensating ring
chromosome, the deficient rod chromosome and its normal homologue proceed quite normally during the meiotic mitoses, two spores of a quartet receiving the deficient chromosome, two the normal chromosome. The behavior of the ring chromosome, on the other hand is irregular. In the case of R1 chromosome the split halves separate and pass to opposite poles at anaphase I along with the disjoining bivalents (except where double-sized or interlocked ring chromosomes are formed). The split halves of the R2 chromosome likewise separate at I, either at the same time that the bivalents disjoin or slightly later. They are nearly always included in the first division telophase nuclei.

In the second meiotic mitosis, the behavior of the ring chromosome is variable. They do not divide again but either pass to one of the poles along with the other chromosomes of the complement or remain in the spindle figure and are excluded from the telophase nuclei. The behavior of the rings in the two sister cells is not always the same.

As a result of meiosis, four types of spores are to be expected. They carry the following chromosomes:

1. Normal chromosome V.
2. Deficient chromosome V.
3. Normal chromosome V plus the ring fragment.
4. Deficient chromosome V plus the ring fragment.

The percentage of each type in an anther would depend upon (1) the proportion of the sporocytes which lacked a ring chromosome, giving only types 1 and 2 above, and (2) the percentage of cases in which the ring chromosome, when present, was included in the second meiotic telophase nucleus.

Examination of the pollen has given some indication of the percentage of each of these four types which are present in an anther. Pollen from a bm1 sector of a plant known to have a normal chromosome V (carrying bm1), a deficient chromosome V (Def1), and an R1 chromosome, showed three types of grains: (1) large well filled grains, (2) small partially filled grains and (3) small totally empty grains. The proportions of each type are shown in table 3, A. In these bm1 sectors it is assumed on good evidence (see sections I and II) that the ring chromosome has been lost. Equal proportions of type 1 and type 2 grains should be present. If the small partially filled grains represent those with the Def1 chromosome, the large filled grains, those with the normal chromosome V, they should be present in equal proportions. A total of 5535 normal pollen grains to 5547 small partially filled grains clearly indicates this association. The 347 small empty grains represent 3.3 percent of the total. In all samples of
pollen from normal maize plants there is a small percentage of these empty grains. They probably represent the products of abnormalities in meiosis which are not infrequently observed in normal plants.

Anthers from \textit{Bm1} regions of the plant, in which the ring chromosome is present, give a higher proportion of normal well-filled grains (table 3, B). The difference is interpreted as due to the presence of the ring chromosome in some of the grains which have a deficient rod chromosome (type 4, above). Since the ring chromosome, if unaltered, covers the deficiency, a normal appearing pollen grain is expected. On this interpretation, the number of each type in a particular anther can be estimated. Pollen types 1 and 2 should be present in equal numbers. Likewise, types 3 and 4 should be present in equal numbers. Type 2 grains can be directly recorded. An equal number of the normal appearing grains should belong to type 1. When this number is subtracted from the total number of normal appearing grains, the remainder can be equally distributed to types 3 and 4. The estimates of each type of grain from the \textit{Bm1} anthers in table 3 B, are: type 1, 1994; type 2, 1994; type 3, 743; type 4, 743, or 36.5 percent each of types 1 and 2 and 13.5 percent each of types 3 and 4.

In one plant, heterozygous for Def1 R1, four types of pollen grains were present, 619 large well-filled grains, 170 small but well-filled grains, 426 small partially-filled grains (type 2) and 49 small empty grains. If it is assumed that the small well-filled grains represent type 4 with an altered ring chromosome which does not completely cover the deficiency, pollen types 2 and 4 can be directly recorded. If, on the other hand, these grains are included in the normal appearing class, and calculations made as above, the proportion of types are: type 1, 426; type 2, 426; type 3, 184; type 4, 184. It is obvious that there is a close agreement in the calculated number of 184 for type 4 grains and the 170 grains which have been assumed to represent this type.

In plants heterozygous for Def2 and R2, the type 2 pollen grains are large but almost completely empty. These grains cannot be distinguished from the few empty grains produced by other causes than the presence of the deficiency in chromosome V. However, if these latter grains are assumed to represent two percent of all the grains, an approximate estimate can be made of the number of grains with each of the four chromosomal constitutions. The counts from the \textit{bm1} anthers are given in table 3 C, and similar counts from the \textit{Bm1} anthers, with estimates of proportions of types, in table 3 D.

The functional capacity of each of the four types of gametes can best be illustrated by reference to the types and numbers of individuals resulting from the crosses given in table 4. Section A in the table represents the
### Table 3

**A. Pollen counts from bm1 anthers of plants with the constitution Def1/bm1/R1.**

<table>
<thead>
<tr>
<th>PLANT</th>
<th>LARGE FILLED GRAINS</th>
<th>SMALL PARTIALLY FILLED GRAINS</th>
<th>EMPTY GRAINS</th>
<th>% EMPTY GRAINS</th>
</tr>
</thead>
<tbody>
<tr>
<td>598A-2</td>
<td>1181</td>
<td>1148</td>
<td>49</td>
<td>2.0</td>
</tr>
<tr>
<td>598A-2</td>
<td>832</td>
<td>917</td>
<td>38</td>
<td>2.1</td>
</tr>
<tr>
<td>596A-2</td>
<td>755</td>
<td>751</td>
<td>66</td>
<td>4.1</td>
</tr>
<tr>
<td>597A-2</td>
<td>610</td>
<td>611</td>
<td>21</td>
<td>1.6</td>
</tr>
<tr>
<td>598A-16</td>
<td>692</td>
<td>678</td>
<td>42</td>
<td>2.9</td>
</tr>
<tr>
<td>598A-3</td>
<td>523</td>
<td>526</td>
<td>70</td>
<td>6.2</td>
</tr>
<tr>
<td>597B-6</td>
<td>922</td>
<td>916</td>
<td>61</td>
<td>3.2</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>5535</strong></td>
<td><strong>5547</strong></td>
<td><strong>347</strong></td>
<td><strong>3.3</strong></td>
</tr>
</tbody>
</table>

**B. Pollen counts from Bm1 anthers of a plant with the constitution Def1/bm1/R1.**

<table>
<thead>
<tr>
<th>PLANT</th>
<th>LARGE FILLED GRAINS</th>
<th>SMALL PARTIALLY FILLED GRAINS</th>
<th>EMPTY GRAINS</th>
<th>% EMPTY GRAINS</th>
<th>ESTIMATES OF FOUR TYPES OF GRAINS IN PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>598A-2</td>
<td>1431</td>
<td>802</td>
<td>47</td>
<td>2.0</td>
<td>36 36 14 14</td>
</tr>
<tr>
<td>598A-2</td>
<td>1094</td>
<td>561</td>
<td>26</td>
<td>1.5</td>
<td>34 34 16 16</td>
</tr>
<tr>
<td>598A-2</td>
<td>956</td>
<td>631</td>
<td>28</td>
<td>1.7</td>
<td>40 40 10 10</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>3481</strong></td>
<td><strong>1994</strong></td>
<td><strong>101</strong></td>
<td><strong>1.8</strong></td>
<td></td>
</tr>
</tbody>
</table>

**C. Pollen counts from bm1 anthers of a plant with the constitution Def2/bm1/R2.**

<table>
<thead>
<tr>
<th>PLANT</th>
<th>NORMAL GRAINS</th>
<th>EMPTY GRAINS</th>
</tr>
</thead>
<tbody>
<tr>
<td>953B-6</td>
<td>681</td>
<td>733</td>
</tr>
<tr>
<td>953B-6</td>
<td>943</td>
<td>918</td>
</tr>
<tr>
<td>953B-6</td>
<td>864</td>
<td>962</td>
</tr>
<tr>
<td>953B-6</td>
<td>761</td>
<td>779</td>
</tr>
<tr>
<td>953B-6</td>
<td>1036</td>
<td>1079</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>4285</strong></td>
<td><strong>4471</strong></td>
</tr>
</tbody>
</table>

**D. Pollen counts from Bm1 anthers of plants with the constitution Def2/bm1/R2.**

<table>
<thead>
<tr>
<th>PLANT</th>
<th>NORMAL GRAINS</th>
<th>EMPTY GRAINS</th>
<th>ESTIMATE OF FOUR TYPES OF GRAINS IN PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1009-10</td>
<td>906</td>
<td>864</td>
<td>48 48 2 2</td>
</tr>
<tr>
<td>1009-8</td>
<td>925</td>
<td>833</td>
<td>38 38 12 12</td>
</tr>
<tr>
<td>1009-8</td>
<td>1281</td>
<td>847</td>
<td>38 38 12 12</td>
</tr>
<tr>
<td>1009-8</td>
<td>1109</td>
<td>822</td>
<td>41 41 9 9</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>4251</strong></td>
<td><strong>3156</strong></td>
<td></td>
</tr>
</tbody>
</table>

2.34
progeny from crosses of the two original variegated plants. Section B represents crosses of plants from A of this table, which were heterozygous for the deficiency and compensating ring, with normal bm1 plants. It can be seen that all four gametes produced by plants heterozygous for Def1 R1 can be transmitted through the eggs. Through the pollen, gametes of type 1 and 3 are readily transmitted but gamete type 2 is not transmitted and gamete type 4 only rarely in competition with gametes 1 and 3.

Table 4

<table>
<thead>
<tr>
<th>TYPE OF CROSS (Female parent to left)</th>
<th>CONSTITUTION OF PLANTS RESULTING FROM CROSSES</th>
<th>bm1 PLANTS</th>
<th>VARIEGATED PLANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bm1/bm1</td>
<td>Def/bm1</td>
<td>bm1/bm1/R</td>
</tr>
<tr>
<td>A. Def1/bm1/R1×bm1</td>
<td>218</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>bm1×Def2/bm1/R2</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>B. Def2/bm1/R2×bm1 reciprocal</td>
<td>352</td>
<td>1*</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>696</td>
<td>4*</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Def1/bm1/R1×bm1 reciprocal</td>
<td>300</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>C. Def1/bm1×bm1</td>
<td>155</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

* These bm1 plants probably arose through very early loss of the ring chromosome. The Def2 chromosome is not transmitted without the R2 chromosome.

In plants heterozygous for Def2 R2, gametes 1, 3 and 4 are transmitted through the eggs but gamete 2 is not transmitted. Through the pollen, gametes 1 and 4 are readily transmitted. The frequent transmission of the Def2 R2 combination through the pollen contrast with the very infrequent transmission of the Def1 R1 combination through the pollen. Both cases are in agreement in the lack of transmission of the deficient chromosome minus its compensating ring chromosome through the pollen.

To obtain high counts on transmission of the deficiency carrying gametes, the gene bt (brittle endosperm) was introduced into the normal chromosome. The bt gene is located in the long arm of chromosome V (Rhoades 1936) and gives very little crossing over with bm1. No positive case of crossing over between bt and the two deficiencies of chromosome V has been found. Thus, plants with a deficient chromosome carrying Bt and a normal chromosome with bt, when crossed to normal bt should give the percentage of deficiency-carrying gametes (Bt kernels) directly, without the necessity of growing large progenies and testing each indi-
individual plant for the presence of the deficient chromosome. The results of such crosses are given in table 5. In many of the crosses bm1 was likewise involved. The deficiency carrying plants were Def Bt/Bm1 bt with or without a ring chromosome carrying Bm1. These were crossed with normal bm1 bt individuals. The Bt kernels should give rise to variegated (Bm1—bm1) plants when the ring chromosome is present or totally bm1 plants when the ring chromosome is absent; the bt kernels should give rise to totally Bm1 plants. The results of a test of the Bt and bt kernels are summarized in table 6. If no crossing over occurred between the deficiency and Bt, bm1 could appear in these crosses only when a deficient chromosome was present. 177 of the 358 individuals showing bm1 were tested for the presence of the deficient chromosome. It was present in every case. Thus, the data in table 5 can be used as a direct means of determining the functioning of the gametes which carry a deficient chromosome.

<table>
<thead>
<tr>
<th>CROSS*</th>
<th>Bt</th>
<th>bt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. +Bt/+bt×bt</td>
<td>2893</td>
<td>2746</td>
</tr>
<tr>
<td>2. reciprocal</td>
<td>797</td>
<td>889</td>
</tr>
<tr>
<td>3. Def1 Bt/+bt/R1×bt</td>
<td>1217</td>
<td>6055</td>
</tr>
<tr>
<td>4. reciprocal</td>
<td>4</td>
<td>5544</td>
</tr>
<tr>
<td>5. Def1 Bt/+bt (no ring)×bt</td>
<td>927</td>
<td>12682</td>
</tr>
<tr>
<td>6. reciprocal</td>
<td>0</td>
<td>323</td>
</tr>
<tr>
<td>7. Def2 Bt/+bt/R2×bt</td>
<td>187</td>
<td>5735</td>
</tr>
<tr>
<td>8. reciprocal</td>
<td>363</td>
<td>1051</td>
</tr>
<tr>
<td>9. Def2 Bt/+bt (no ring)×bt</td>
<td>0</td>
<td>1319</td>
</tr>
<tr>
<td>10. reciprocal</td>
<td>0</td>
<td>4065</td>
</tr>
</tbody>
</table>

* A non-deficient chromosome V is represented by +. The pollen parent is placed at the right in each cross.

The results given in lines 3 and 5 of table 5 are particularly interesting with respect to the functioning of the Def 1 chromosome through the eggs. If there had been no megaspore selection in favor of the spore carrying the normal chromosome and if all the eggs (or zygotes) which carried the Def 1 chromosome functioned, the ratio of Bt to bt should be equal. In all cases the number of bt kernels was greater than Bt and in all cases the ears were incompletely filled. The presence of the abortive grains on the ear indicate that there has been little if any selection of normal chromosome carrying megaspores. Therefore, the percentage of Bt kernels,
when the \( bt \) kernels are taken as the standard of expectancy, indicates the extent of functioning of the deficiency carrying eggs (or zygotes). The \( 927 \) \( Bt \) kernels in line 5, table 5, represent 7.2 percent of the deficiency carrying eggs (or zygotes) which functioned. In the 74 ears which contributed to this count, the percentages ranged from 0 to 23.2 with half of the ears falling within the range of 2 to 8 percent. When the ring chromosome (R1) was present (line 3, table 5) the \( Bt \) kernels on the 30 ears contributing to this count ranged from 1 to 40 percent of expectancy on the individual ears and averaged 20.1 percent. That this increase can be attributed to the presence of the ring chromosome covering the deficiency in many of the eggs can be seen from line 1, table 6.

<table>
<thead>
<tr>
<th>CROSS</th>
<th>PLANTS FROM ( Bt ) KERNELS</th>
<th>PLANTS FROM ( bt ) KERNELS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( Bm1/bm1 ) variegated</td>
<td>( bm1 )</td>
</tr>
<tr>
<td>Def1</td>
<td>40</td>
<td>23</td>
</tr>
<tr>
<td>Def1</td>
<td>0</td>
<td>85</td>
</tr>
<tr>
<td>Def2</td>
<td>166</td>
<td>7</td>
</tr>
<tr>
<td>( bm1/bt ) ( \times ) Def2</td>
<td>95</td>
<td>2</td>
</tr>
</tbody>
</table>

That pollen containing the Def1 chromosome without its compensating ring chromosome does not function in competition with normal pollen can be concluded from line 6, table 5. By use of a 170 wire mesh screen these grains, which are small and partially filled with starch, can be segregated from the normal grains and the possible factor of competition with the grains carrying the normal chromosome eliminated. Some of the ears pollinated with sifted pollen gave no kernels at all, others a few \( bt \) kernels through passage of a few small but normal chromosome pollen grains through the wire mesh. It can be definitely stated, therefore, that these grains are incapable of producing an effective pollination when placed upon normal silks.

Line 4, table 5, suggests that the grains with a Def1 and an R1 chromosome normally do not effect a pollination in competition with normal chromosome carrying grains. The four \( Bt \) kernels were grown to determine the chromosome constitution of the resulting individuals. Two of these \( Bt \) kernels were definitely produced through contamination, one through functioning of a Def1 R1 grain and one could have been a crossover between \( Bt \) and the deficiency although contamination could not be excluded definitely. If this kernel represents a crossover, it is the only evidence so far obtained of crossing over between the deficiency and \( bt \).
From this evidence it could be concluded that (1) the ring-shaped chromosome, R1, does not completely cover the deficiency due to a loss of a small section either at the time of irradiation or during the development of the original plant or that (2) a mutation affecting pollen tube growth appeared at the time of irradiation or (3) the particular chromosomal modification (position effect) is responsible for the reduced pollen tube activity. If (1) above is correct, the deficiency, when homozygous, does not produce a visible effect in the tissues of the mature plant (see section V).

That the gametes with Def2 function only when the ring chromosome, R2, is present is evident from tables 5 and 6. The Def2 gametes with a complete R2 chromosome have an equal chance in competition with normal chromosome carrying gametes. The discrepancy in the percentages of Bt kernels in the reciprocal crosses, lines 7 and 8, table 5, can be understood when it is realized that the ear arises from a definite sector of tissue which originally may or may not have had the ring chromosome in its nuclei (6 of the 36 ears had no Bt kernels) whereas the pollen is shed from all parts of the tassel which is usually a mosaic of sectors with and without the ring chromosome. The percentage “expected” Bt kernels on the 36 individual ears in the cross summarized in line 7, table 5, ranged from 0 to 8.9 percent, those on the six ears summarized in line 8, from 4.5 to 21.6 percent.

As stated in section II, changes in size and genic constitution of the ring chromosomes occasionally occur during ontogenesis of a plant. This being so, it could be objected that the two original ring chromosomes could not be kept constant through successive generations. Small duplications within a ring chromosome are not phenotypically detectable. They must be determined through cytological examination. In contrast, the deficiencies within the ring chromosome can be detected through phenotypic appearances of certain plants (see section V) and through pollen transmissions which tend to eliminate gametes with the deficient ring chromosome. However, change in size of the ring chromosome is not frequent and with proper care, it is not difficult to maintain stocks with unaltered ring chromosomes.

V. PRODUCTION OF PLANTS MOSAIC FOR HOMOZYGOUS DEFICIENCIES

As shown in the previous section, the progeny of the crosses of the two original variegated plants by normal bm 1 included a number of individuals with chromosomal constitutions similar to the two original plans. Plants heterozygous for Def1 and R1 when crossed by plants heterozygous for Def2 and R2 should produce twelve types of plants, each with a different chromosomal constitution:
RING CHROMOSOMES IN MAIZE

1. \textit{bm1/bm1}  
2. \textit{bm1/bm1/R2}  
3. \textit{Def2/bm1/R2}  
4. \textit{Def1/bm1}  
5. \textit{Def1/bm1/R2}  
6. \textit{Def1/Def2/R2}  
7. \textit{bm1/bm1/R1}  
8. \textit{bm1/bm1/R1/R2}  
9. \textit{Def2/bm1/R1/R2}  
10. \textit{Def1/bm1/R1}  
11. \textit{Def1/bm1/R1/R2}  
12. \textit{Def1/Def2/R1/R2}

In the cross of heterozygous \textit{Def1 R1} by normal \textit{bm1} plants, a number of individuals with the constitution of plant type 4, above, were obtained. When these, in turn, are crossed by plants heterozygous for \textit{Def2 R2}, the first six types of plants listed above should be produced.

All of the plants except 6 and 12 from the first cross, and all the plants except 6 from the second cross can be distinguished by the presence or absence of variegation, the type of variegation exhibited and the type of pollen shown by each plant. Cytological examinations of a number of these plants were in agreement with the field determinations.

The appearance of plants of type 6 and 12 could not be predicted. In actual experience it proved very simple to identify them. Both types of plants possess two deficient chromosomes, \textit{Def1} and \textit{Def2}. Plant 6 has one ring chromosome, \textit{R2}, plant 12, two ring chromosomes, \textit{R1} and \textit{R2}. These two types of plants will be designated \textit{R2} double-deficient and \textit{R1 R2} double-deficient.

In the \textit{R2} double-deficient plants, the ring chromosome covers both deficiencies. Its loss in somatic nuclear divisions should result in cells homozygous deficient for the extent of the deficiency in the \textit{Def1} chromosome. If these cells were viable and continued to multiply at the same rate as the surrounding heterozygous deficient cells, which are close to normal in growth rate, both wide and narrow sectors of homozygous deficient tissues should be produced through early and late losses, respectively, of the ring chromosome during ontogeny. In the \textit{R1 R2} double-deficient plants, both ring chromosomes cover the homozygous deficient segment in the rod chromosomes. Simultaneous loss of both ring chromosomes or loss of one ring chromosome followed later by loss of the second ring chromosome must occur in order that homozygous deficient tissue can be produced. The total amount of homozygous deficient tissues produced in the \textit{R2} double-deficient plants should be considerably greater than that produced by the \textit{R1 R2} double-deficient plants. If tissues homozygous deficient for the extent of the deficiency in \textit{Def1} were visibly modified, the two types of plants should be readily distinguishable. However, no prediction as to the nature of the homozygous deficient tissue was possible before the appearance of these plants.

In addition to the \textit{bm1} and variegated plants resulting from the cross of \textit{Def1/bm1×Def2/bm1 R2}, one plant appeared (table 7) which was not \textit{bm1} and did not show the ordinary \textit{Bm1–bm1} variation. This plant was stunted in growth habit, the leaves and leaf sheaths were uniformly
streaked with fine bands of colorless tissue, as shown in figure 32 (compare with figure 31, a normal leaf). In the cross of Def1/bm1/R1×Def2/bm1/R2, besides the bm1 and variegated plants, two new types of plants appeared (table 8). One type was similar to the plant just described. The other type was considerably larger, approaching normal in growth habit, but was not a typical bm1 or variegated plant, and presented the same fine streaks of colorless tissues in the leaves and leaf sheaths as the first new type. However, the total amount of such tissue was markedly less and the pattern of this tissue was not uniform, figures 35 and 36.

### Table 8
Def1/bm1/R1×Def2/bm1/R2.

<table>
<thead>
<tr>
<th>CULTURE</th>
<th>bm1 AND VARIEGATED</th>
<th>Def1/Def2/R2</th>
<th>Def1/Def2/R1/R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>694</td>
<td>40</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>695</td>
<td>41</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>35-9</td>
<td>126</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>35-10</td>
<td>243</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>35-11</td>
<td>134</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>35-24</td>
<td>172</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td>856</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

It was suspected that the two new types of plants represented the two expected types of double deficiencies, and that the colorless streaks represented the homozygous deficient tissues. Since these streaks of colorless tissue were not wide, as some of them theoretically should be from homologies with the bm1 streaks in normal variegated plants, it was suspected that the cells of the homozygous deficient tissue were unable to multiply at the same rate as the surrounding heterozygous deficient cells. As stated in section II, loss of the R2 chromosome occurs much more frequently than loss of the R1 chromosome. If the rate of loss for each of these two chromosomes is uniform throughout development, a uniform distribution of colorless streaks should be present in the R2 double-deficient plants.
RING CHROMOSOMES IN MAIZE

In the case of the R1 R2 double-deficient plants, early loss of the R1 chromosome should give a sector of tissue with the pattern of colorless streaks characteristic of the R2 double-deficient plants, since the chromosome constitution within the sector is the same. If the R2 chromosome were lost early in ontogeny, a sector of tissue with a different pattern of colorless streaks should result. These colorless streaks should be considerably less frequent since the R1 chromosome is lost from the nuclei less frequently. Nevertheless, the distribution of such streaks should be uniform. If this hypothesis were correct, the small, uniformly but heavily streaked plants should be the R2 double-deficient plants, the larger, non-uniformly streaked plants, R1 R2 double-deficient plants. That these two types represented the expected R2 and R1 R2 double-deficient plants was established through cytological observations and confirmed by pollen examinations and appropriate crosses.

In the intercrosses of plants heterozygous for Def2 and R2, the union of a Def2 R2 gamete with a similar gamete results in a plant with two Def2 chromosomes plus two R2 chromosomes. (It is of theoretical interest to point out that the chromosome number has been increased in these plants without changing the genome complement, that is, these 22-chromosome plants are genomically equivalent to normal 20-chromosome plants.) Since these plants are markedly different from the double-deficient plants, a description will be postponed until section VII. The functional gametes produced by these plants contain the Def2 chromosome plus one or two R2 chromosomes. The gamete most frequently transmitted through the pollen contains but one R2 chromosome. In the crosses of Def1/bm1 and Def1/bm1/R1 by plants homozygous for Def2 and R2, all the eggs which carry the normal chromosome with bm1 should give rise to normal variegated plants, all those that carry a deficient chromosome to double-deficient plants. The results of these two types of crosses are given in tables 9 and 10. The test for the functioning of deficiency-carrying eggs is similar to the Bt and bt tests described in the previous section. The correlation between the proportions of functional

<table>
<thead>
<tr>
<th>CULTURE</th>
<th>NORMAL Bm1—bm1</th>
<th>Variegated*</th>
<th>Def1/Def2/R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>814</td>
<td>76</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>36-28</td>
<td>60</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>36-29</td>
<td>135</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>37-56</td>
<td>13</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>37-57</td>
<td>64</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>349</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

* A few plants were bm1, see page 364.
BARBARA McCLINTOCK

Table 10

<table>
<thead>
<tr>
<th>CULTURE</th>
<th>NORMAL Bm 1 — bm 1</th>
<th>Def1/Def2</th>
<th>Def1/Def2/R 1/R 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>36-25</td>
<td>106</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>36-26</td>
<td>56</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>37-52</td>
<td>154</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>37-53</td>
<td>175</td>
<td>22</td>
<td>31</td>
</tr>
<tr>
<td>37-54</td>
<td>107</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>37-55</td>
<td>162</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Totals</td>
<td>760</td>
<td>63</td>
<td>85</td>
</tr>
</tbody>
</table>

* A few plants were bm 1. See page 364.

eggs with a normal chromosome V, a deficient chromosome V, and a deficient chromosome V plus its ring chromosome, respectively, is similar in the two tests.

It remains to be shown that the colorless streaks in the double-deficient plants represent the homozygous deficient tissues produced after loss of the ring chromosomes during somatic mitosis. Adequate confirmation of this relationship is obtained from the patterns of such tissues in double-deficient plants with the following ring chromosomes: one R 1, one R 2, two R 2, one R 1 plus one R 2, two R 1 plus one R 2, one R 1 plus two R 2. Double-deficient plants with different combinations of ring chromosomes can be obtained from crosses of R 2 and R 1 R 2 double-deficient plants by plants homozygous for Def 2 R 2 and from intercrosses of the double-deficient plants. The results of the respective crosses are given in tables 11, 12, and 13. It should be noted that only plants homozygous for Def 2 R 2 and double-deficient plants result from these crosses.

Classification of these plants into the two categories of homozygous

Table 11

<table>
<thead>
<tr>
<th>CULTURE</th>
<th>Homozygous Def 2, R 2</th>
<th>Def1/Def2</th>
<th>Def1/Def2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Def 2, R 2</td>
<td>one R 2</td>
<td>two R 2</td>
</tr>
<tr>
<td>823</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>987</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>988</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>989</td>
<td>9</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>990</td>
<td>4</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>991</td>
<td>7</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>992</td>
<td>3</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>37-85</td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>37-86</td>
<td>3</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>37-87</td>
<td>0</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>37-89</td>
<td>1</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>37-90</td>
<td>4</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>37-91</td>
<td>4</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Totals</td>
<td>42</td>
<td>153</td>
<td>51</td>
</tr>
</tbody>
</table>
Def 2 R 2 and double-deficient was simple since the former type of plant has a peculiar growth habit (see section VII) and does not show the particular streaks which are always present in the double-deficient plants. The double-deficient plants, in turn, were classified as to their ring chromosome constitution on the basis of the patterns of the colorless streaks. If the colorless streaks represent the homozygous deficient tissue, then the pattern exhibited by the double-deficient plants with the R1 or R2 chromosome or various combinations of two or three of these rings should

### Table 12

<table>
<thead>
<tr>
<th>CULTURE</th>
<th>HOMOZYGOS</th>
<th>Def 1/Def 2 one R 2</th>
<th>Def 1/Def 2 two R 2</th>
<th>Def 1/Def 2 one R 1, one R 2</th>
<th>Def 1/Def 2 three rings</th>
</tr>
</thead>
<tbody>
<tr>
<td>37-58</td>
<td>11</td>
<td>30</td>
<td>9</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>37-59</td>
<td>6</td>
<td>10</td>
<td>11</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>37-60</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>37-61</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>37-62</td>
<td>1</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>37-63</td>
<td>3</td>
<td>14</td>
<td>3</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>37-64</td>
<td>3</td>
<td>14</td>
<td>0</td>
<td>33</td>
<td>4</td>
</tr>
<tr>
<td>37-65</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>37-66</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>37-67</td>
<td>4</td>
<td>11</td>
<td>2</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>37-68</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>37-69</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>37-70</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>37-71</td>
<td>2</td>
<td>8</td>
<td>6</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>37-72</td>
<td>14</td>
<td>16</td>
<td>3</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>37-73</td>
<td>12</td>
<td>16</td>
<td>5</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>37-74</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>37-75</td>
<td>6</td>
<td>15</td>
<td>1</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>82</strong></td>
<td><strong>152</strong></td>
<td><strong>61</strong></td>
<td><strong>254</strong></td>
<td><strong>67</strong></td>
</tr>
</tbody>
</table>

* Some of these plants had, in addition, an R 1 chromosome.

### Table 13

<table>
<thead>
<tr>
<th>CULTURE</th>
<th>HOMOZYGOS Def 2, R 2*</th>
<th>Def 1/Def 2 one RING</th>
<th>Def 1/Def 2 two RINGS</th>
<th>Def 1/Def 2 three RINGS†</th>
</tr>
</thead>
<tbody>
<tr>
<td>37-76</td>
<td>4</td>
<td>15</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>37-77</td>
<td>1</td>
<td>14</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>37-78</td>
<td>3</td>
<td>0</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>37-79</td>
<td>5</td>
<td>6</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>37-80</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>37-81</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>37-82</td>
<td>6</td>
<td>17</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>1000</td>
<td>0</td>
<td>8</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>1001</td>
<td>9</td>
<td>1</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>1003</td>
<td>2</td>
<td>7</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>32</strong></td>
<td><strong>71</strong></td>
<td><strong>178</strong></td>
<td><strong>87</strong></td>
</tr>
</tbody>
</table>

* Several of these plants had, in addition, an R 1 chromosome.
† Several of these plants were suspected to have four rings.
be predictable from the knowledge of their behavior in somatic mitosis (section II) and from the knowledge gained from a study of the patterns of bm1 tissues in normal variegated plants with these same combinations of ring chromosomes (section III). A number of these plants were examined cytologically to establish the value of the prediction. The results are summarized in table 14. The agreement between predicted and observed is obvious from the table.

**Table 14**

**Comparisons of predicted and observed chromosomal constitutions.**

<table>
<thead>
<tr>
<th>Predicted Constitution from Appearance of Plant</th>
<th>No. Plants Examined</th>
<th>Deviation from Expectation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous Def2, R2</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Def1/Def2+one R2</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Def1/Def2+two R2</td>
<td>10</td>
<td>1 (1 R1)</td>
</tr>
<tr>
<td>Def1/Def2+one R1 and one R2</td>
<td>24</td>
<td>1 (1 R1)</td>
</tr>
<tr>
<td>Def1/Def2+two R1 and one R2</td>
<td>5</td>
<td>2 (2 R2; 1 R1+2 R2)</td>
</tr>
<tr>
<td>Def1/Def2+one R1 and two R2</td>
<td>6</td>
<td>1 (R1+R2)</td>
</tr>
<tr>
<td>Totals</td>
<td>80</td>
<td>5*</td>
</tr>
</tbody>
</table>

* See footnote *, table 2.

The patterns of colorless tissue exhibited by the various double-deficient plants will be briefly described. Photographs of parts of leaves of double-deficient plants with various ring chromosome combinations are given in figures 31 to 38. To conserve space only a small part of a leaf is shown, which considerably limits the effectiveness of the demonstration.

The colorless streaks in the one R2 double-deficient plants are uniformly distributed throughout the leaf area and are relatively closely spaced (figure 32). Double-deficient plants with the R1 chromosome have been produced only by very early loss of the R2 chromosome in plants originally possessing both the R1 and R2 ring chromosomes. There is considerably less streaking but the distribution of these streaks is uniform (figure 34). Plants with two R2 chromosomes are clearly distinguishable from those with an R1 and R2 chromosome. In both types of plants the streaking is not uniformly distributed. The two R2 chromosome plants have a considerably greater total amount of colorless tissue. There are numerous sectors of various widths with a pattern similar to that shown by the one R2 plants (figure 33, sector to right). This is to be expected since loss of either ring chromosome would give rise to cells with the same chromosome constitution as the one R2 plants. Sectors with the R1 pattern, figure 34, are not found. The R1 R2 double-deficient plants have fewer streaks than the two R2 plants. The sectors in these plants are either of the one R2 type (figure 36, sector to left), or of the R1 type (figure 35, sector to right of mid-rib), through early loss of the R1 or R2 chromosome, respec-
tively. In the three ring double-deficient plants, very few colorless streaks were observed. This is particularly evident in the two R1 plus one R2 plants. Many of the leaves in these plants have no well defined sectors but only scattered streaks here and there. Sectors, when present, are usually narrow (figures 37, 38). The two R2 plus one R1 plants have more streaking and more well defined sectors.

That the colorless streaks represent the homozygous deficient tissues resulting from loss of the ring chromosome from the nuclei seems certain from the correlations of the patterns of this tissue in the six types of plants.

The amount of homozygous tissue in a double-deficient plant bears an
inverse relation to the size of the plant, the greater the total amount of homozygous deficient tissue present, the smaller the plant. The one R2 double-deficient plants are smaller than the two R2 plants, which in turn, are smaller than the R1 R2 plants. The three ring plants are practically equal in size and vigor to plants with a normal chromosome constitution.

Figure 34.—(upper) Surface view of a small region of a leaf of a plant with Def1, Def2 and one R1 chromosome. Note the distribution of streak of homozygous deficient tissue. Compare with figures 32 and 33.

Figure 35.—(middle) Surface view of a small region of a leaf of a plant with Def1, Def2 an R1 and an R2 chromosome. Note the R1 sector in the middle of the region to the right of the midrib and the sectors to either side of it which are comparatively free of streaks of homozygous deficient tissue.

Figure 36.—(lower) Surface view of a leaf of a plant with the same constitution as that in figure 35. Note the R2 sector (left) and the comparatively small number of homozygous deficient tissue streaks in the tissue to the right.

From homologies of the bm1 sectors in normal variegated plants (section III), wide sectors of homozygous deficient tissues in the double-deficient plants would be expected to be found if the cells of such tissues could grow and multiply at the same rate as the surrounding cells. It is
reasonable to assume that these cells, with a deficient chromosome complement, would be incapable of an equal growth rate. The juxtaposition of two tissues with unequal growth rates should cause considerable distortion of the cells about the boundaries of the two tissues. This is obvious from the microscopic observations of the two types of tissues in the double-deficient plants. The normal tissues appear to be pulling in the direction of the homozygous deficient tissues. The more rapid growth of the normal tissues may exert enough pull upon the homozygous deficient cells to cause separation at cell boundaries and the production of a hole in the midst of a patch of homozygous deficient tissue. In the one R2 double-deficient plants, which have the most homozygous deficient tissue, the unequal growth rates of the two types of tissue is reflected in a roughened, finely corrugated surface of the considerably reduced and narrowed leaf.

The evidence for considering the colorless streaks as tissues homozygous deficient for the extent of the deficiency in the Def1 chromosome can be briefly summarized:

1. Plants which show these colorless streaks must contain two deficient rod chromosomes, one of which must be the Def1 chromosome. This has been proven by cytological examination, pollen examination and appropriate crosses. Plants homozygous for Def2 R2 do not show the colorless streaks characteristic of the double-deficient plants. It will be shown in

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**Figure 37.**—(upper) Surface view of a small region of a leaf of a plant with Def1, Def2, two R 2 and one R 1 chromosomes. Note the two narrow sectors with streaks of homozygous deficient tissue.

**Figure 38.**—(lower) Surface view of a small region of a leaf of a plant with Def1, Def2, two R 1 and one R 2 chromosomes. Note the narrow sector to the left of the midrib with a few streaks of homozygous deficient tissue.
section VII that cells homozygous for the full deficiency in the Def2 chromosome (which is more than twice as long as that in the Def1 chromosome) are incapable of surviving.

2. Plants with these colorless streaks can arise only in crosses where double-deficient plants are expected. For confirmation, see tables 7 to 13.

3. When streaked plants are crossed to normal plants, no streaked plants should appear in the progeny. This has been fully confirmed.

4. The amount of streaked tissue present in a plant and the pattern exhibited should be correlated with the number and kinds of ring chromosomes present. (See table 14 and previous discussion.)

5. A reduced growth rate in the cells homozygous deficient for such a relatively large section of the chromosome is to be expected. This is reflected in the small size of the colorless streaks and the distortion of tissues produced by the juxtaposition of tissues with unequal growth rates.

In mentioning the crosses given in tables 11, 12 and 13, little was stated concerning the functional gametes produced by the double-deficient plants. The one R2 double-deficient plants produce four types of gametes (1) Def1, (2) Def2, (3) Def1 R2, (4) Def2 R2. As shown previously, the gamete with Def2 (2 above) will not function in pollen or ovule; (1) above will function in some but not all of the eggs in which it is present but will not function through the pollen. Gamete (4) is equally viable through the eggs and pollen (tables 11 to 13). Gamete (3) is a new type the functioning of which had to be tested. It was found to function readily through the eggs. That it does not function through the pollen in an appreciable amount in competition with (4) is shown by the following test. Pollen of R2 double-deficient plants was placed upon silks of normal bm1 plants. The resulting plants should be variegated (Bm1 and bm1) and heterozygous for either Def1 or Def2. Since both types of plants can be distinguished through pollen examinations (see section IV), the pollen of these plants resulting from this cross was examined. All showed the presence of the Def2 chromosome. Cytological verification was obtained from 28 of these plants. From this evidence, it can be concluded that type (4) pollen grain is the only normally functioning grain produced by these plants.

The functional gametes of the R1 R2 double-deficient plants have been determined. Of the eight possible gametes: (1) Def1, (2) Def1 R1, (3) Def1 R2, (4) Def1 R1 R2, (5) Def2, (6) Def2 R1, (7) Def2 R2, (8) Def2 R1 R2, the egg can transmit all except (5) and possibly (6). The pollen transmits only (7) and (8).

The dissimilarity in functional capacity of the different types of gametes in pollen and ovules is reflected in the Pr and pr ratios (Pr, purple aleurone; pr, red aleurone) in reciprocal crosses (table 15). Pr is located in the long arm of chromosome V, 18–24 units from Bm1 (Emerson, Beadle and
RING CHROMOSOMES IN MAIZE

Fraser, 1935; the percentage of crossing over varies within this range in different strains). As is obvious from the discussion in this paper, Bm1 is very close to the spindle fiber region in the short arm of chromosome V. A measure of the crossing over between the deficiency (or spindle fiber region) and Pr can be obtained directly from the crosses shown in table 15.

<table>
<thead>
<tr>
<th>CROSS</th>
<th>Pr</th>
<th>%</th>
<th>pr</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Def1 pr/Def2 Pr/R2×pr reciprocal</td>
<td>5102</td>
<td>81.3</td>
<td>1198</td>
<td>18.7</td>
</tr>
<tr>
<td>Def1 Pr/Def2 pr/R2×pr reciprocal</td>
<td>495</td>
<td>19.0</td>
<td>2106</td>
<td>81.0</td>
</tr>
<tr>
<td>Def1 pr/Def2 Pr/R1/R2×pr reciprocal</td>
<td>177</td>
<td>27.8</td>
<td>458</td>
<td>72.2</td>
</tr>
<tr>
<td>Def1 Pr/Def2 pr/R1/R2×pr reciprocal</td>
<td>4964</td>
<td>83.7</td>
<td>973</td>
<td>16.3</td>
</tr>
</tbody>
</table>

Since no ring chromosome is present, only the pollen grains carrying the normal chromosome function. Crossing over is not altered in plants heterozygous for Def1 and very little in plants heterozygous for Def2.

In double-deficient plants, with one Def1 and one Def2 chromosome, crossing over is similar to that in plants heterozygous for the Def2 chromosome, “reciprocal” crosses (table 15).

VI. SIMULATION OF THE bm1 PHENOTYPE THROUGH LOSS OF THE Bm1 LOcus

When one-ring and two-ring double-deficient plants were closely examined, fine streaks of brown tissue were seen in the leaf sheath, the midrib and the veins of the leaf. These fine streaks were many times more frequent in the one-ring plants than in the two-ring plants. The shade of color and its association with cells having thickened walls were strikingly similar to the effect produced by brown midrib (bm1). In some of the streaks it was obvious that the brown color of the vein was associated
with adjacent parenchyma cells lacking chlorophyll. Since it was known that normal bm l produces a brown color in the lignified cell walls, which can be seen in sections of any lignified tissue, the leaf sheaths with brown streaks were removed, sectioned fresh and examined microscopically. It was immediately observed that the brown color was in the cell walls. It was similar in its range of color and in its deposition in the cell wall to that of ordinary bm l. When brown patches appeared in regions where plastids are normally lacking, that is, heavy-walled sclerenchyma cells, the adjacent parenchyma cells, when too thin-walled to show the brown clearly, frequently lacked plastids. When the adjacent plastid deficient parenchyma cells possessed thickened walls, a brown color could be seen in these walls. The brown-walled and plastid deficient cells formed a definite unit of tissue. It was suspected that these small sectors represented homozygous deficient tissues and that a brown pigmented cell wall would accompany all such tissues. However, the walls of the cells in the colorless streaks in the leaf, except about the veins, are too thin, and the concentration of brown pigment insufficient for a visible effect. If the homozygous deficient cells, produced by loss of the ring chromosome from the nuclei, have brown cell walls, cross-sections of the stem of the single-ring double-deficient plants should show many small, uniformly distributed patches of cells with brown cell walls, just as the leaf is uniformly streaked with fine stripes of colorless tissue. This proved to be true for each of the many R 2 double-deficient plants examined. The sections of the stem were advantageous in relating the brown-walled cells to those which lacked plastids since the normally plastid-carrying parenchyma in the outer region of the stem is thick walled and has a sufficient concentration of brown to be readily seen. Considerable distortion of the bundles and cells was present, particularly in the regions about relatively large patches of brown-walled cells. The types of distortion suggested that the brown-walled cells had grown at a slower rate than the surrounding white-walled cells.

In contrast to the single-ring plants, cross-sections of stems of the two-ring plants showed fewer brown patches. Such would be expected if the brown color were limited to cells which were homozygous deficient; loss of one ring followed by loss of the second ring must occur before the homozygous deficient cells could be produced. Frequently a cluster of brown patches was present in a restricted region of the stem. When the extent and position of these brown patches were traced with a camera lucida and a boundary drawn about the cluster, it was clear that they formed one continuous sector. In the two R 2 double-deficient plants, the number and distribution of the brown patches in such a sector were similar to those of the one R 2 plants. In the R 1 R 2 plants, some of such sectors were similar to the above and some had considerably fewer patches in a cluster.
RING CHROMOSOMES IN MAIZE

In the former plant, the sectorial clusters of brown patches correspond to the one-ring sectors seen in the leaf through early loss of one of the R2 chromosomes. In the latter plant, the two types of sectorials correspond to early losses of the R1 or R2 chromosome, respectively.

If the brown-walled, plastid-deficient patches of cells represent the homozygous deficient tissue, the three-ring double-deficient plants should show very few such patches. When present, their distribution should correspond to that observed in the leaves of these plants. The results obtained were in complete agreement. There were very few such patches in these three-ring plants. This is especially true of the two R1 plus one R2 plants.

It might be stated here that plants homozygous for Def2 R2 do not show these patches of brown-walled, plastid-deficient cells. The homozygous deficient cells in these plants, as will be shown in the next section, are inviable.

(1) The correlation of the brown cell walls with the cells lacking plastids, (2) their presence in narrow streaks, (3) the restricted growth capacity of these cells, (4) their uniform distribution in one-ring double-deficient plants, (5) their reduced frequency in two R2, R1 R2, and three-ring plants, respectively, and (6) the distribution patterns in sectorials in these two- and three-ring plants summarize the homologies of the brown-walled cells with the streaks of colorless cells in the leaf which were shown to be homozygous deficient in the previous section.

The brown-walled cells are homozygous deficient for the full extent of the deficiency in Def1. At this point it should be emphasized that the locus of Bm 1 is carried by the ring chromosome and that there is no locus for Bm 1 or bm 1 in the deficient rod chromosomes. If the brown color in the walls of these homozygous deficient cells is similar to bm 1, it should appear in the development of the wall at the time lignification sets in, as has been shown for normal bm 1 (section III). Furthermore, the brown color of the walls in such cells, when adjacent to white-walled cells, should be diluted on the side adjacent to the white-walled cells, as observed in normal variegated plants. Thirdly, when exposed to intense light, the brown color should fade just as normal bm 1 fades on exposure to light.

Immature cells are present at the base of each leaf sheath. These cells rapidly merge into fully mature cells just above this region. If a leaf showing a prominent brown streak is removed and serial sections made to trace the brown streak as it emerges from the immature cells, it becomes obvious that the brown color appears as the cell walls become lignified in a manner fully comparable to normal bm 1. Thus, the time of appearance of the color in the development of the wall is similar to that in normal bm 1 plants.

When examining the brown patches in sections of the stem it was ob-
served that the color of the brown in the walls of the plastid deficient cells was considerably diluted on the side adjacent to the white-walled, plastid containing cells. This, in turn, is comparable to the observations in normal variegated plants.

To test the third correlation, fading of the color when exposed to light, black paper was placed over part of a conspicuous brown streak in a leaf sheath or midrib of a leaf. Upon removal, several weeks later, the brown color under the paper had retained its intensity, that above and below had lost much of its intensity. In this respect, the brown of the homozygous deficient tissues is similar to normal bm1.

The range in color of normal bm1 varies in some plants from a deep wine red to a light orange, the deep red color being present in the stem toward the basal nodes, the light orange in the regions toward the top of the plant. This same gradation of color in comparable regions was found in the brown patches of the double-deficient plants.

To summarize: The two browns, the normal bm1 and the brown produced in cells possessing no locus for this gene, are comparable in (1) time of appearance of the color in the development of the cell walls, (2) in dilution of the color in regions adjacent to white-walled (Bm1) cells, (3) in loss of intensity of color when exposed to light, and (4) in range of color variations in specific regions of the plant. No differences could be detected in the expression and behavior of the brown color in the two cases. Although it has not been proven that bm1 is due to a deficiency in chromosome V, it can be stated that absence of the locus of Bm1 will duplicate the phenotypic expression of bm1.

VII. THE PRODUCTION AND APPEARANCE OF PLANTS HOMOZYGOUS FOR Def2 R2

Mention of plants homozygous for Def2 R2 has been made in the previous section. The first of these plants appeared in the progeny of sib crosses of Def2/bm1/R2 through the union of two gametes each containing Def2 and R2. Such plants were to be expected. However, as in the case of the double-deficient plants, no prediction could be made as to appearance other than that they should not be typical bm1 or variegated plants. Nevertheless, they are readily recognizable. They are short, usually deep green in color, with thickened, erect leaves and do not show normal Bm1–bm1 variegation. The streaks of colorless tissue, so characteristic of double-deficient plants are absent from the leaves.

In later generations many plants homozygous for Def2 R2 were obtained. Cytological examination of microsporocytes at pachytene has confirmed the accuracy of the phenotypic classification (table 14). The two deficient chromosomes V synapse homologously throughout their length.
The changed arm ratio produced by the deficiency is clearly evident. In the normal chromosome V, the chromomereres adjacent to the spindle fiber region on the short arm are relatively large and deep-staining. With the removal of this section in the production of the ring chromosome, small, light-staining chromomereres are brought adjacent to the spindle fiber region, making this deficient chromosome V readily recognizable at pachytene. The two ring chromosomes either synapse to form a ring-shaped bivalent, similar to the rings in photographs 21, 22, Plate II, or remain unsynapsed and appear as two collapsed rings, similar to the rings in photograph, 19, Plate II. There is no tendency for the ring chromosomes to synapse with any part of the deficient rod chromosomes V.

The pollen of these plants is always highly abortive. Only those grains possessing a ring chromosome are normal in appearance and capable of functioning.

The loss of one ring chromosome followed by loss of the second ring chromosome should give rise to cells homozygous deficient for the full extent of the deficiency in the rod chromosomes. If these cells were viable and capable of multiplying, evidence of such tissue would be expected in the leaves of these plants from homologies with the double-deficient plants described in the previous two sections. Since evidence of such tissue did not appear in the leaf, it was suspected that the cells whose nuclei were homozygous deficient for this relatively long section were inviable or incapable of further multiplication. Longitudinal sections of growing points of roots of these plants were made with the view of finding evidence of the fate of these cells since such cells necessarily are formed.

In all root meristems of plants homozygous for Def2 R2, the following peculiar cell type was found. It was confined to roots of these plants, not being present in normal or double-deficient plants. Very much enlarged, heavily vacuolate groups of two or more cells in positions indicating relation in descent, were observed in regions of the root where enlarged cells are not normally encountered (figures 39, 40, 41). When a mitotic figure was observed in one of these cells, the chromosomes were short, thickened and sometimes irregularly placed in the spindle. Daughter telophase nuclei were sometimes joined by a connecting nuclear bridge. In older regions of the root, degeneration processes in these cells, depicted first by an aberrant staining reaction of the nucleolus followed by a pycnotic condition of the cytoplasm (figure 42), and finally by a collapse of these cells due to the pressure of the normal surrounding cells (figure 43). Should the space formerly occupied by these cells be extensive, the surrounding cells divide in planes other than normal, filling in this space which might otherwise have remained as a hole in the tissue (figure 43). The numbers of such patches of enlarged cells varied considerably in different roots. Some had many, others relatively few.
The conditions depicted conform strictly to expectancy if these cells represent the homozygous deficient cells resulting from loss of the ring chromosomes from their nuclei. Loss of one ring chromosome would cause no obvious tissue alteration since tissues heterozygous for Def2 are normal in appearance. Loss of the second ring chromosome, such loss taking place at anaphase by being left at the equatorial plate of a mitotic figure, would result in two adjacent cells whose nuclei would be homozygous deficient for the extent of the deficiency in the Def2 rod chromosomes. The occurrence of pairs of enlarged cells has been mentioned. In many cases, it was possible to see the cast-out ring chromosome in the cytoplasm of one of these cells but also in many cases, degeneration processes in the cytoplasm had advanced too far for such a determination. No ring chromosome was observed in the few cells which had mitotic figures but the small ring chromosome could have been obscured by one of the rod chromosomes so that evidence of the homozygous deficient conditions of these cells from direct observations of the chromosome constitution was not considered satisfactory.

The numbers of such patches of cells and their distribution in the different roots lend strong supporting evidence for the homozygous deficient
Figure 41.—Longitudinal section of a root tip of a plant homozygous for Def2 and R2. Note the row of very much enlarged (homozygous deficient) cells. Mag. ×160.

Figure 42.—(left) Longitudinal section immediately below the actively meristematic region of a root of a plant homozygous for Def2 and R2. Note the row of four enlarged, degenerating (homozygous deficient) cells. Mag. ×160.

Figure 43.—(right) Longitudinal section of a root tip of a plant homozygous for Def2 and R2. Note that there has been a proliferation of cells about the degenerated streak. Mag. ×160.

nature of these cells. They were present in all roots but the frequency was variable. In a root in which only one ring chromosome is present in the nuclei of the normal cells, a high frequency of such patches should be
observed. In this latter case, every loss of the ring chromosome would be reflected in a patch composed of two or more such cells. In roots where two ring chromosomes are present in many of the nuclei, loss of one ring chromosome followed by loss of the second ring chromosome or the very occasional simultaneous loss of both ring chromosomes would have to occur to produce such a patch. Thus, the frequency of such patches in these roots would be considerably less than in the former type of root. Since a root could contain but one ring chromosome in its normal cells or be a mosaic of one- and two-ring sectors of various sizes, variations in the numbers of patches of abnormal, enlarged (homozygous deficient) cells in the different roots of the homozygous Def 2 R 2 plants is to be expected.

(1) The presence of these patches of abnormal cells only in plants homozygous for Def 2 R 2 and their absence in double-deficient and normal plants, (2) the observed presence of the R 2 ring in the cytoplasm of one of these cells in many cases, (3) the frequency and distribution of these patches in different roots, (4) their arrangement in descent, that is, rows of two or more, (5) the rapid distintegration of these cells, and finally (6) the absence of evidence of homozygous deficient tissues in the mature cells of the stalk and leaves of these plants as contrasted with double-deficient plants, strongly support the view that they represent the homozygous deficient cells since such cells must be produced in these plants. Since early death is the fate of these cells, it is clear why the leaves of these plants are not streaked with modified tissues which could be interpreted as representing the homozygous deficient tissues.

At this point it might be mentioned that a third deficiency (Def 3), not previously considered in this paper, which is outside the limits of Def 1 but within the limits of Def 2 and therefore covered by the R 2 chromosome, produces the same pattern of homozygous deficient tissues as that exhibited by the R 2 double-deficient plants when the constitution of the plant is Def 2/Def 3/R 2. However, in this case, the homozygous deficient tissue has an even poorer growth capacity than tissues homozygous for the deficiency in the Def 1 chromosome. To return to the Def 2 chromosome, the deficiency is apparently too long, the loci deleted too important in cell physiology for survival of cells which are homozygous deficient for this region.

When pollen of plants homozygous for Def 2 R 2 is placed upon silks of normal bm 1 plants, the progeny should all be variegated for Bm 1 and bm 1 except in those cases where the ring chromosome has been lost sufficiently early in development to be absent from that part of the embryonic tissue which produces the visible part of the plant. In this latter case,
the plant would be \textit{bm}1. The progeny of 13 such crosses totalled 1,829 variegated to 68 \textit{bm}1 plants. To exclude the possibility that this 3.5 percent of \textit{bm}1 plants represented contaminations, 42 of them were examined for the presence of the deficient chromosome. In 41 of these plants the deficient chromosome was present; one represented a contamination.

The number of kernels which develop on the ear of a plant homozygous for Def2 R2 is always very small, ranging from 0 to 30 kernels with the average about 10. This would be expected since only those gametes which possess a ring chromosome are functional. Many of the ears should arise from one-ring sectors and others should be composed of both one- and two-ring sectors. Since, in the two-ring sectors, the two small ring chromosomes frequently do not synapse, of if so, do not remain together at the first meiotic mitosis, their elimination in the two meiotic mitoses is frequent. Relatively few ovules with eggs containing a ring chromosome would be expected and thus only a few kernels should be expected on an ear. Since the exertion of anthers and shedding of pollen is dependent upon the presence of a number of well-formed grains in the anther sac, pollen collected from such plants usually contains enough functional grains (those with a ring chromosome) to produce a complete set of seed when placed on silks of normal plants. Since both deficient chromosomes are similar, there is no selection in favor of one or the other chromosome either through the pollen or eggs. Reciprocal backcrosses of \textit{Pr}/\textit{pr} plants give 1:1 ratios.

\textbf{VIII. PHENOTYPIC EFFECTS OF ALTERED RING CHROMOSOMES}

As stated in section II, ring-shaped chromosomes not only are lost from nuclei during somatic mitoses but also change in size. They may increase in size through duplications of segments composing the ring or decrease in size through losses of segments. The frequency of such changes depends upon the size of the ring chromosome. In the case of the small R2 chromosome, such changes are relatively infrequent. Cytological examinations of large numbers of plants and many nuclei within each plant have given abundant evidence, however, of such changes. The R2 chromosome has been seen to increase to approximately seven times its original size and to have decreased to several chromomeress. The duplicated segments do not result in tissues showing extensive modification. Slight modifications are visible with higher multiples resulting in smaller plants with thickened, erect leaves.

In the case of the double-deficient plants, loss of the ring chromosome

\textit{In this case, the tube nucleus would contain the ring chromosome and could be expected to function normally in pollen germination. Thus, sperm nuclei lacking an R2 chromosome, could be introduced into the embryo sac.}
results in viable tissues which are homozygous deficient for the extent of Def1, as shown in the previous sections. This tissue is poor in growth capacity, has brown cell walls similar to normal bm1, possesses no plastids and, on continuous exposure to sunlight this tissue in the leaves dries and shrivels. In Def1 the four chromomeres adjacent to the spindle fiber attachment region on the short arm of chromosome V have been removed. The R2 chromosome includes these four chromomeres plus the next five chromomeres. Should changes in size of the ring chromosome delete one or more of the four chromomeres covering the deficiency in Def1 or fractions of them, tissues homozygous deficient for sections within the limits of the deficiency in Def1 should result. Since tissues homozygous for the full extent of the deficiency in Def1 are viable, fractional deficiencies within this region might be expected to have even better viabilities and should reveal themselves by wider sectors of homozygous deficient tissue having a specific modification, this modification should be repeatedly encountered as a sectorial in large populations of such plants. In other words, the nature of these sectorials should vary depending upon the fraction of the four chromomeres which has been deleted from the ring chromosome. The same region should be independently deleted in a number of instances and therefore the same type of sectorial should be produced in a number of different double-deficient plants.

A large number of sectorials have been found. These are classified as simple mutant sectorials, involving a single recognizable change, and compound mutant sectorials, which are combinations of the simple mutant types. Only those sectorials which are readily recognizable because of their good growth capacity are included in this classification. The simple mutant sectorials are as follows: (1) translucent white ("onion skin") with colorless cell walls, no plastids; (2) opaque white, with colorless cell walls, colorless plastids; (3) deficiency-brown-midrib, similar in detail to tissues homozygous for the bm1 gene; (4) pink colored tissues with colorless cell walls, colorless plastids; (5) blotched chlorophyll pattern. The following types of compound mutant sectorials have been found: (1) pink, deficiency-bm, viable in sunlight; (2) pink, deficiency-bm, dries in sunlight; (3) opaque white, deficiency-bm; (4) blotch, dries in sunlight; (5) blotch, deficiency-bm, dries in sunlight. The compound mutant sectorials are far more frequent than the simple mutant sectorials. The summation of the characters exhibited by these sectorials is equal to the characters present in tissues homozygous for the full extent of the deficiency in Def1, with the exception that the tissues with the total deficiency are incapable of growing at

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3 This is an estimate of the number of chromomeres. The chromomeres in maize appear to be compound and may show more or less in a particular region depending upon the state of contraction or the degree of stretching in a particular preparation.
a rate which will result in a large sector, whereas the simple mutant sectorials outlined above more nearly approximate the normal growth rate.

The theory is proposed that the simple mutant effects are due to losses of one particular region from the ring chromosome, each mutant effect being related to one particular region. The compound mutant effects are then produced by losses of several adjacent regions within the ring chromosome. On this theory, a linear arrangement of the mutant effects can be referred to the chromosome in the following order: pink, deficiency-\textit{bm}, dries in sunlight and blotch. Translucent white must be removed from deficiency-\textit{bm} and opaque white close to it. The presence of a ring chromosome in the cells of a sectorial has been determined cytologically when the sectorial included a part of the tassel. If an altered ring chromosome was present in the mutant sectorials, streaks of tissue homozygous deficient for the full extent of the deficiency in Def1 should appear within these sectors through loss of this altered ring chromosome. This is especially easy to confirm in sectorials of deficiency-\textit{bm} and blotch.

In double-deficient plants with two ring chromosomes, the same types of sectorials are encountered. Here, however, all of the sectors are not solid; many of them are variegated. The explanation is similar to variegated patterns of \textit{Bm1} and \textit{bm1} in plants which have two normal chromosomes \textit{V} carrying \textit{bm1} and two ring chromosomes carrying \textit{Bm1} and to the patterns of homozygous deficient tissues in double-deficient plants with two ring chromosomes. One ring chromosome has suffered a deletion. Production of homozygous deficient tissues showing the character of the deletion can occur only after loss of the second normal ring chromosome. Thus the sectorial is a variegated of normal and mutant tissues. Should the altered ring chromosome be lost in a somatic mitosis many generations later than that which produced the alteration, the tissues resulting after this loss will appear as normal one-ring double-deficient tissue inserted into variegated tissue since only the one normal ring chromosome is left in this tissue. These variegated sectors, mosaics of two types of patterns, are frequently encountered in the two-ring double-deficient plants.

If the theory that the mutant sectorials are due to losses of specific regions within the ring chromosome is correct, plants homozygous for Def2 R2 should show mutant sectorials similar to those exhibited by the double-deficient plants plus a number of types not exhibited by these latter plants since the extent of the deficiency in Def2 includes the same four chromomerers plus five more. This has been fully realized. Simple and compound mutants of pink, deficiency-\textit{bm}, blotch, and opaque white plus an additional number of chlorophyll and growth types have been found.

Should a change occur in the ring chromosome, it should remain un-
altered for most of the cell generations and thus should be capable of being passed from one generation to the next, provided the original alteration occurred in tissues which give rise to the gametes. A female gamete containing a deficient rod chromosome plus an altered ring chromosome united with a male gamete with the deficient rod chromosome and a normal ring chromosome would give rise to plants which are complete mosaics of normal and changed tissues. The changed tissues should be of the same type in an individual plant. In some cases there should be many female gametes in the original plant with this same altered ring chromosome if the alteration occurred early in the ontogeny of the ear. Thus there should be many individuals in a culture resulting from the outlined cross each exhibiting the same type of variegated pattern involving the identical mutant characters. This has been realized in one culture. These plants are useful for cytological determinations of the nature of the modification in the ring chromosome but a detailed account of this will appear in a later paper. It is not the purpose of this paper to consider all the evidence concerning changed rings and their mutant effects. It is too extensive. It is necessary to mention it, however, since a comprehension of the mitotic behavior of ring chromosomes would lead one to anticipate the presence of such changed rings with phenotypic effects.

DISCUSSION

The presence of ring-shaped chromosomes and a suggestion as to their inconstant behavior in somatic tissues was first published by Nawashin (1930; see also, 1936) for a single plant of Crepis tectorum. Since this time, ring-shaped chromosomes have been found or produced in Drosophila (L. V. Morgan 1933; Sturtevant and Beadle 1936; Sidorov, Sokolov and Trofimov 1936; Schultz and Catcheside 1937), Trillium (Huskins and Hunter 1935), Locusta (White 1935), Pisum (Atabekowa 1936), Tradescantia (Husted 1936), Tulipa (Upcott 1937) and Nicotiana (Claussen, unpublished). In none of these cases has an intensive cytological study been made to determine the mitotic behavior of the ring chromosomes. In Zea a number of ring-shaped chromosomes have been found (McClintock 1932, 1933, and unpublished; Rhoades and McClintock 1935; Creighton, unpublished; Cameron, unpublished). In all cases studied, the mitotic behavior of these ring chromosomes has been similar. Deletions of sectors from the ring, duplication of sectors, additions in numbers of ring chromosomes of varying constitutions and total loss of the ring chromosome from the nuclei have been observed. In Drosophila, phenotypic effects which could be definitely ascribed to alterations in constitution of the ring-shaped X chromosome have not been described. The chances of detecting such an altered ring chromosome would depend
on the rate at which aberrant anaphase figures would be formed. As pointed out in section II, the rate at which alterations occur in ring chromosomes in maize depends upon the length of the chromonema composing the ring. Since the primary cause of double-sized and interlocking rings, the first step in the production of altered ring chromosomes, has not been determined, it is difficult to argue that the behavior found in Crepis, Nicotiana (R. E. Clausen, unpublished) and Zea would likewise be found in Drosophila.

Husted (1936) reported double-sized, continuous ring-shaped chromosomes, in some of which the two chromonemata made a half turn around each other, and interlocked rings in the microspores of Tradescantia following irradiation. Such configurations should be expected if, before irradiation, the chromosomes were split and the two chromatids were relationally coiled about one another. Although the Tradescantia cases were not followed beyond the microspore stage, Husted attempted to explain the method by which such figures could be produced throughout the life of a plant. The presence of a continuous ring with two spindle fiber attachment regions or two interlocked sister ring chromatids at somatic anaphase, whatever the method by which it originally arose, would result in a chromatin bridge the strands of which would eventually break. Husted assumes that each chromosome is split at somatic anaphase. "A broken end of an anaphase chromatid (caused by breakage of continuous or interlocking rings) may unite as often with the broken end of its sister as with its other broken end. Ring chromosomes which are continually breaking might persist in this way. Whenever the two broken ends of one chromatid unite, however, and the two sister strands are not twisted, a 'disjunctonal' [two sister halves free to disjoin] ring-shaped chromosome would result. There would be a trend toward displacement of the 'continuous' and 'interlocked' types by 'disjunctonal' rings which separate without breaking unless relational coiling of chromatids is increased before each union of broken ends" (page 551). The disjunctional rings, once established, should remain free from further complications. Such a theory of the continuous appearance of double-sized and interlocked ring chromosomes throughout the life of a plant cannot account for the origin of such configurations in maize, although it may contribute to some of the cases. This arises from the following consideration. In plants homozygous for Def2 and R2, the two split halves of the ring chromosomes separate freely from one another at anaphase I in most of the sporocytes, that is, are "disjunctional," not continuous or interlocked. Thus, the ring chromosome in the majority of the spores has been derived from a ring chromosome whose two split halves have separated freely in the previous division. As seen in section VII, only those gametes which possess a ring chromosome
are functional. If Husted's theory of the continuous appearance of double-sized and interlocked rings were correct for maize, most of the individuals resulting from the cross of normal bm1 by homozygous Def2 R2 should be totally Bm1 through elimination of the cause of loss of the ring chromosomes in somatic mitoses. As shown on page 364, all plants resulting from the cross are variegated for Bm1 and bm1. The cause of the interlocked or double-sized rings arises anew and is dependent upon the length of the chromonema in the ring for its frequency. The primary cause of these configurations may be related to the occurrence of a crossover between the two split halves of a ring chromosome. The high frequency of normally disjoining ring chromosomes, even when the chromonema of the ring is long, leads one to conclude that the plane of splitting or method of reproduction of a new chromonema from an old, is definitely predetermined along a given plane and that trouble might arise only during or after the split had occurred at some point of tension or torsion in the chromosome, that is, a tension relieved by an interchange of segments at this point, resulting in a somatic crossing over between the two chromatids. Unless two crossovers occurred in a chromosome, only continuous, double-sized rings would result. As noted in section II, there was a high frequency of such continuous, double-sized rings as compared to other possible complicated configurations. The maize chromosomes are too small in somatic cells to give a clear picture of the configurations in each cell other than in those with simple continuous rings. On this theory, interlocked rings could arise (1) after two somatic crossovers in which the second crossover did not counteract the first, or (2) following a previous anaphase break and reunion of broken ends in which the twist in the chromonema was present before the union occurred. In this latter case, it is not necessary to assume that the chromosome is split before union of broken ends occurs as interlocked or continuous rings could arise depending upon the plane of splitting or reproduction of the chromonema assumed. On this hypothesis, the proportion of interlocked rings to continuous rings would be expected to be greater with large ring chromosomes than with small ring chromosomes. Likewise, sister nuclei in these plants should show aberrant ring configurations more frequently than sister nuclei in plants with small ring chromosomes.

Since no rod-shaped fragment chromosomes have been observed to arise from ring-shaped chromosomes through breakages in somatic anaphase and telophases, it has been assumed that union of broken ends must occur. In an effort to determine if two broken ends which enter a nucleus would unite, the following experiment was outlined. A plant was made heterozygous for two inversions on two different chromosomes. The inversions did not include the spindle fiber attachment regions and therefore, should give
bridges at anaphase I (or II) and free fragments after a crossover within the inverted segment (McClintock 1933; Müntzing 1934; Smith 1935; Richardson 1936; Darlington 1936; Upcott 1937; Sax 1937 and others). With normal crossing over, the size of the fragment is constant for any particular inversion. In the two inversions chosen, the size of the fragment produced by each was readily distinguishable. In many sporocytes of the plants with the inversions, two chromatin bridges with their respective fragments produced by a crossover in each of the two inversions, were present at anaphase I. In most cases, the bridges of chromatin had broken by late telophase and the broken ends had been drawn into the telophase nuclei. Thus broken ends from two chromosomes entered the same nucleus. If fusion of these broken ends occurred, the product of this fusion should be obvious in some of the second division figures in the cells of which two recognizable fragments were present. In maize, the second meiotic anaphase figures are oriented in one plane and thus can be observed together. However, the evidence for fusions was negative. It was then considered that the broken ends might be too far apart from one another, in many cases, to join together before the second meiotic mitosis. Therefore, cases of double-crossing over in plants heterozygous for a single inversion were investigated. When a four-strand double crossover occurs within the inverted region, a double bridge involving all four chromatids, and two free fragments of similar size are found in anaphase I. The strands composing these bridges break and the two broken ends, lying side by side, enter the same nucleus. If fusions of these broken ends occurs in each telophase I nucleus, each sister cell in anaphase II should show a chromosome involved in an anaphase bridge. The sister cells to be examined can be distinguished by the two fragments of recognizable dimensions. The evidence for fusions of broken ends entering the same nucleus was likewise mainly negative in this case. On the supposition that each chromatid might already be split in anaphase I and that fusions occurred between broken ends of the two split halves of a chromatid rather than between the broken ends of each chromatid, anaphase configurations in the microspores of these plants were examined. Such fusions should give rise to a chromatin bridge at anaphase of the first mitosis in the spore (see Sax 1937). Upon examination, a chromosome showing a bridge configuration was found in a number of spores. By a method which will be described in a separate publication, it was possible to show that the spores which have a bridge configuration likewise possess a chromosome which was broken during the meiotic mitoses. The examinations indicated, also, that such fusions probably always occur. Such evidence illustrates, directly, the tendency of broken ends to fuse. One would be tempted to use this information and transfer the process to the somatic chromosomes. How-
ever, the evidence at present indicates that one is not justified in doing so. Until the contradictory features of this evidence are completely analysed, the author is unwilling to interpret the ring chromosome behavior on this basis.

Viable homozygous deficiencies in Drosophila giving effects similar to "genes" known to be located in the region which has been made deficient, have been described by Muller (1935) for yellow and achaete, Ephrussi (1934), Stern (1935) and Demerec and Hoover (1936) for yellow, Sturtevant and Beadle (1936) for scute, Emmens (1937) for roughest–2, and Oliver (1937) for facet. Viable individuals homozygous deficient for a region possessing no known genes have been described by Demerec and Hoover. Homozygous deficiencies producing immediate or delayed effects in zygotes and embryos, which eventually result in death to the individual, have been described by Poulsom (1937). As far as the author is aware, homozygous deficiencies in plants which simulate a gene known to be located in the deleted segment, have been found only in the bm1 case described in this paper. Evidence that the known genes in the Drosophila cases might be due to deficiencies in the regions involved, has been given only for the gene roughest–2. Simulation of the known gene by a region deficient for its locus has been claimed for the other cases and applies likewise to the bm1 case in Zea. It is unprofitable at present to estimate to what extent homozygous deficiencies are responsible for known genic effects. That some of these may be related to position effects seems possible from the accumulating evidence in Drosophila. In how many of these cases the factor of a minute deficiency can be eliminated, remains to be decided, granting that the presence of a deficiency introduces the possibility of a position effect. In the case described in this paper, the two independent segments of chromosome V, the deficient rod and the unaltered ring fragment, produce effects, with regard to the Bm1 character, which are indistinguishable from that produced by a normal chromosome V carrying Bm1. The only evidence so far obtained of a "position effect" is derived from the appearance of plants homozygous for Def2 and R2 and from the lack of expected transmission through the pollen of gametes with Def1 R1 or Def1 R2. In neither case can the factor of a minute deficiency be eliminated, the deficiency affecting plant growth in the former case and pollen transmission in the latter case.

The method of producing phenotypic effects by alterations in ring chromosomes in plants with two deficient chromosomes plus a covering ring fragment, briefly described in section VIII, should prove useful in analysing in considerable detail the genetic composition of small sections of chromosomes. The effects produced have been ascribed to minute homozygous deficiencies rather than to position effects since homozygous
deficiencies must be produced by alterations in the ring chromosomes. If individual regions within the deficient segment produce particular effects independent of their neighbors, combinations of these effects should be produced by loss from the ring chromosome of two or more of these regions. Since the method by which the ring chromosomes become altered should delete adjacent segments from the ring chromosome, an orderly arrangement of compound effects should result. The order of the particular regions within the ring chromosome which have specific effects could be developed from analyses of the individual effects contributing to the compound effects. Since the results obtained so far substantially correspond to the requirements of this theory, the notion of a particulate nature of the composition of this region of the chromosome has been retained. The development of this method of analysing the composition of sections of chromosomes has just been started. It would be premature to draw rigid conclusions from the results so far obtained. Three deficiencies of chromosome V, each of which can be covered by a ring fragment, are available for this study. Two of these fall within the range of the third. Correlations of results from all three deficiencies should conform to a predicted pattern if the above theory is correct. Until the evidence from these studies has accumulated, no attempt will be made to force a particulate theory of the organization of the chromosome in contradistinction to a continuum theory. The former will be retained as a working hypothesis until the evidence definitely requires a modified view.

**SUMMARY**

1. Two cases of a deficiency adjacent to the spindle fiber attachment region in the short arm of chromosome V involving approximately \( \frac{1}{20} \) (Def1) and \( \frac{1}{7} \) (Def2) the length of the chromosome, respectively, were produced by X-ray treatment. The piece deleted in each case formed a small ring-shaped chromosome, R1 and R2, respectively. In each case a section of the spindle fiber attachment region was removed to the ring chromosome and a section was retained by the deficient rod chromosome. Since the deficient rods and compensating ring chromosomes possessed a functional section of the spindle fiber attachment region, each was capable of participating successfully in the mitotic process. The ring chromosome in each case carried the locus of \( Bm1 \) (allele of \( bm1 \), brown mid-rib, producing a brown color in the lignified cell walls). The rod chromosomes lacked the locus for \( Bm1 \).

2. Plants with two normal chromosomes V carrying \( bm1 \) (or one normal chromosome V carrying \( bm1 \) and a deficient chromosome V) plus either ring chromosome are variegated for \( Bm1 \) and \( bm1 \) through frequent losses of the ring chromosome from the somatic nuclei.
3. Somatic loss of ring-shaped chromosomes is described.
4. The abnormal mitotic behavior of large and small ring-shaped chromosomes is contrasted. Large ring-shaped chromosomes frequently change in size and chromatin constitution during somatic mitotic cycles. Small ring-shaped chromosomes are more frequently lost from nuclei during mitotic cycles although changes in size and chromatin content sometimes occur.
5. The frequency of aberrant mitotic configurations of the ring chromosomes, leading to loss or change in size, is related to the length of the chromonema composing the ring. The longer the chromonema the more frequent the aberrant configurations. With small ring-shaped chromosomes, whose aberrant mitotic configurations usually lead to loss of the ring chromosome from the nuclei, the extent of variegation (2 above) is proportional to the size of the ring chromosome.
6. Functional gametes with Def1, Def1 plus R1, and Def2 plus R2 were obtained. The functional capacities of these two deficiencies with various combinations of the ring chromosomes were tested. Some of these were functional, others were not.
7. Plants with Def1/Def2/R2 (R2 covers the deficiencies in the rod chromosomes) were a uniform mosaic of tissues heterozygous and homozygous for the full extent of the deficiency in Def1 (the smaller deficiency) through losses of the ring chromosome during somatic mitosis. The patterns of homozygous deficient tissues in plants with these two deficient rod chromosomes plus various combinations of ring chromosomes (R1, R2, two R2, R1 plus R2, two R1 plus one R2, two R2 plus one R1) have been compared and agree with expectancy on the basis of the cytological analysis of ring chromosome behavior in mitosis and the analysis of variegation (2 above) produced in plants with these same combinations of ring chromosomes.
8. The homozygous deficient tissues, lacking a locus for Bm1, have the phenotypic expression of bm1 in their cell walls.
9. Plants homozygous for Def2 R2 have 22 chromosomes in their zygotes. Although the chromosome number has been increased, there has been no increase in the genome. Losses of the R2 chromosomes during development produce cells homozygous deficient for the extent of the deficiency in Def2. These cells are abnormal in appearance and are short lived, degenerating before maturity of the surrounding cells.
10. In plants with two deficient chromosomes and one or more compensating ring chromosomes, somatic alteration in constitution of a ring chromosome is reflected in modified tissues having mutational characteristics. A number of repeatedly encountered, distinct types are briefly described. One type is indistinguishable from normal bm1.
RING CHROMOSOMES IN MAIZE

ACKNOWLEDGMENTS

Much of the investigation described in this paper was undertaken at Cornell University with the aid of a grant from the Rockefeller Foundation. The original material for this investigation was supplied by Dr. L. J. Stadler to whom the author is very grateful. To Dr. R. A. Emerson of Cornell University the author is particularly indebted for his generosity in placing the facilities of the Department of Plant Breeding at her disposal and for his continued encouragement.

LITERATURE CITED


1937 The production of maize plants mosaic for homozygous deficiencies: Simulation of the bm 1 phenotype through loss of the Bm 1 locus. (Abstract) Genetics 22: 200.


Oliver, C. F., 1937 Evidence indicating that facet in Drosophila is due to a deficiency. Amer. Nat. 71: 560–566.
Perspective: forthcoming

Citation: 43. *McClintock, Barbara. 1938c. The fusion of broken ends of sister half-chromatids following breakage at meiotic anaphase. Missouri Agricultural Experiment Station Research Bulletin 290: 1–48. ➁
*Reprinted in McClintock 1987

Online: http://digital.library.umsystem.edu/cgi/t/text/pageviewer-idx?c=agext;cc=agext;sid=d3c84d4350dd0409e5eb6b9ab057c8ec;q1=Bulletin%20290;rgn=full%20text;id-no=age000290;view=image;seq=1

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1 The symbol ➁ and the header ‘Citation’ indicate that this McClintock publication is not reprinted herein. However, in this case, an online URL is provided.
Perspective:¹

Citation: 44. *McClintock, Barbara. 1939. The behavior in successive nuclear divisions of a chromosome broken at meiosis. *Proceedings of the National Academy of Sciences* 25(8): 405–416. ²

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Online: [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1077932/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1077932/)

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¹ Perspective solicited.
Perspective: forthcoming

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Perspectives on Nobel Laureate
Barbara McClintock’s Publications (1926-1984):
A Companion Volume
edited by Lee B. Kass

Perspective:  

Reprint: 48. McClintock, Barbara. 1941b. The association of mutants with homozygous deficiencies in Zea mays. Genetics 26(5) 542–571. [Both the journal article and reprints are dated inaccurately as September 1940; only reprint cover page is dated accurately.]
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THE ASSOCIATION OF MUTANTS WITH HOMOZYGOUS DEFICIENCIES IN ZEA MAYS

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Reprinted from GENETICS 26: 542-571, September, 1941
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Received May 3, 1941

INTRODUCTION

The evidence to be presented in this paper supports the supposition that some recessive mutants in maize are caused by homozygous minute deficiencies. A method by which homozygous minute deficiencies may be produced in maize has been presented in a previous publication (McClintock 1938). It is related to the aberrant mitotic behavior of ring-shaped chromosomes. It has been shown both in maize and Nicotiana (Stino 1940; R. E. Clausen unpublished) that a chromosome in the form of a ring does not maintain itself unaltered through successive nuclear cycles. The details of this behavior have been presented in the two mentioned papers. They may be summarized briefly as follows: (1) Ring-shaped chromosomes may decrease in size by loss of segments of chromatin from the ring. (2) Ring chromosomes may increase in size by duplications of segments of chromatin composing the ring. (3) Ring chromosomes may be completely eliminated from sister telophase nuclei during a mitotic cycle. (4) The frequency of these occurrences depends upon the length of the chromonema composing the ring—the larger the ring chromosome, the more frequent the aberrant mitotic behavior. In maize, aberrant mitotic configurations producing alterations or elimination of the ring chromosomes may occur in 17 to 20 percent of all the division figures if the chromonema composing the ring is as long as that of the longest chromosome of the complement. If the ring chromosome is small—for example, composed of only four chromomereres—the aberrant mitotic configurations are very infrequent and may occur only once in every five or six hundred divisions. In all other nuclear divisions the ring chromosome behavior is normal; the two sister halves of the ring chromosome separate freely and pass to opposite poles of the spindle figure along with the rod chromosomes of the complement.

When aberrant configurations are produced by large ring-shaped chromosomes, the chromatin content of the ring chromosome usually is altered in each resulting sister telophase nucleus. Only rarely is the ring chromosome lost to one or both sister telophase nuclei. In contrast, aberrant mitotic configurations produced by small ring-shaped chromosomes usually result in total loss of the ring-shaped chromosome from both sister telophase nuclei. Only rarely do the telophase nuclei receive altered ring-shaped chromosomes.

Genetics 25: 542 September 1940
The method by which ring-shaped chromosomes are lost to nuclei or become altered has been described previously (McClintock 1938). It may be outlined briefly as follows. At some mitotic prophases the two sister halves of a divided ring chromosome form a continuous double-sized ring chromosome instead of two freely separating ring chromosomes (Prophase, fig. 1). It is not known whether this results from the method of reduplication of the chromatia composing the ring chromosome or from a somatic crossover between the two sister chromatids subsequent to this reduplica-

**Figure 1.**—Diagram illustrating a method by which a ring chromosome becomes altered in chromatia constitution. Upper left: a ring chromosome in a resting nucleus. The clear oval represents the centromere. The individual parts of the ring chromosome are designated by the numerals. Upper middle: A prophase configuration following a “crossover” between the two sister chromatids of the divided ring chromosome. A dicentric, double-sized ring chromosome is produced. Upper right: Appearance of the dicentric ring chromosome in the following anaphase. Breakage of the chromatia strands between the centromeres may occur at any position. Three possible positions, a, b, and c, respectively, are indicated by the dash lines. The resulting broken strands at late anaphase and the new ring chromosomes formed at telophase by fusions of broken ends of these strands are diagrammed below in the bracketed figures for the breaks a, b, and c, respectively.
tion. At early anaphase, the two centromeres which are present in this double-sized ring chromosome move toward opposite poles of the spindle figure (Anaphase, fig. 1). The subsequent behavior in the spindle figure depends on the size of the ring chromosome. The ring chromosomes used in the investigations to be described in this paper were small. Therefore, the following description will be confined to their behavior. With such small double-sized ring chromosomes, continued movement of the centromeres is usually suspended and the double-sized ring chromosome remains in the region of the equatorial plate during anaphase. It is consequently eliminated from both sister telophase nuclei. Very occasionally, however, the strands of chromatin between the two centromeres become broken, and a segment of the double-sized ring chromosome enters each telophase nucleus. Fusion occurs between the broken ends of the segment. Consequently, a newly organized ring-shaped chromosome is produced in each of these telophase nuclei. Illustrations of several types of ring chromosomes which may be produced following breakage of a double-sized, two-centromere ring chromosome are given in figure 1. It will be seen that ring chromosomes with duplicated segments or ring chromosomes with deficient segments may be produced by this process. In either case, the size of the segment may be minute or relatively large. Although aberrant mitotic configurations, which could result in loss or change in size of the ring-shaped chromosomes, are relatively infrequent, it has been found that the frequency of loss of the ring-shaped chromosome from both telophase nuclei is considerably greater than the frequency of breakage of the ring chromosome with inclusion of broken segments in each of the sister telophase nuclei. This relationship is of considerable importance in the study to be described.

A simple method of obtaining sectors of a plant or even whole plants which are homozygous deficient for a small segment of a chromosome may be outlined as follows. It is necessary to have a rod-shaped chromosome from which a relatively short segment has been deleted and a ring-shaped chromosome composed of the chromatin deleted from the rod-shaped chromosome. The deficient rod chromosome plus the compensating ring-shaped chromosome are thus equivalent in chromatin content to a single normal rod chromosome. (See Df-1 and R-1; Df-2 and R-2, fig. 2.) Union of two gametes, each with a complete genomic complement but possessing the deficient rod chromosome and its compensating ring chromosome (instead of the normal rod chromosome), could give rise to plants with two homologous deficient rod chromosomes and two homologous compensating ring chromosomes. However, because of their small size, the ring chromosomes would frequently be lost to telophase nuclei following the formation of aberrant mitotic configurations. Thus, in the development of such plants, some nuclei would contain only a single ring chromosome following
loss of one of the ring chromosomes. During subsequent multiplication of such cells, an aberrant configuration could eliminate the second ring chromosome. Thus, cells would be produced whose nuclei have no chromatin segment covering the deficiency in the rod chromosomes. These cells would be homozygous deficient for the full extent of the deficiency in the rod chromosomes. (For this preliminary presentation, it may be assumed that these cells are inviable or incapable of further multiplication.) Very occasionally, however, an aberrant mitotic configuration of a ring chromosome would result not in loss but in a changed composition of the ring chromosome as illustrated in figure 1. If, during development of the sporophytic tissues, one of the ring chromosomes becomes reduced in size by loss of a minute segment and subsequently, the second ring chromosome is lost to a cell at a mitotic anaphase, all the cells arising from this latter cell could be homozygous deficient for a minute segment—the segment which had previously been deleted from the remaining ring chromosome. When the loss of a minute amount of genic substance results in a change in the genotype which is neither cell lethal nor interferes greatly with the capacity of such cells to multiply, a sector of tissue could be formed with a visibly modified phenotype. Minute deletions of different segments of the ring chromosome could be produced following such aberrant mitoses. In one plant an aberrant mitosis might result in the deletion of one particular segment from the ring chromosome. In another plant, an aberrant mitosis might result in the deletion of an entirely different segment. Thus, tissues homozygous deficient for different segments within the limits of the deficiency in the rod-chromosomes could be produced. If, when homozygous, each minute deficiency results in a particular type of visible modification of the tissue, a number of different and distinguishable types of mutant sectorials should be produced in these plants, each of which should be associated with loss of a particular segment from the ring. Furthermore, sectors showing identical characters should arise independently in a number of different plants if sufficient numbers of plants are available for observation. This is because a double-sized dicentric ring chromosome (fig. 1) could be broken at the same position on a number of independent occasions resulting in the production of altered ring chromosomes from which the same segment has been deleted.

If two adjacent segments, each of which when homozygous deficient produces a mutant character, are simultaneously deleted from the ring chromosome during an aberrant mitosis, a compound mutant sectorial should be formed showing the characters caused by each deficiency—that is, comparable to a condition of homozygosity for two recessive mutants. If a mutant sector in a plant is included in the inflorescence, gametes could be formed containing the deficient rod chromosome plus the deficient ring
chromosome. Fusion of two such gametes could give rise to plants which are homozygous for the minute deficiency and thus homozygous for the phenotypic expression which it induces. In such a way, altered ring chromosomes may be isolated.

Table 1

The appearance of the tissues of plants with two deficient rod chromosomes and two compensating ring chromosomes, Ring 1 and Ring 2, columns one and two, respectively. N represents a ring chromosome whose chromatin completely covers the deficiency in the rod chromosomes. a, b, and c represent ring chromosomes with minute deficiencies; each deficiency, when homozygous, gives rise to the phenotypic character a, b, and c, respectively. a b and b c represent ring chromosomes with two such minute deficiencies which, when homozygous, give rise to the compound mutant a b and b c, respectively. The appearance of the tissues when both rings are present is shown in column 3. The + indicates normal, non-mutant tissues. In columns 4 and 5 the phenotypic appearance of the tissues following somatic loss of Ring 1 and Ring 2, respectively, is indicated.

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<th>RING 1</th>
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The meaning of simple and compound mutants produced by altered ring chromosomes is important for an understanding of the logic of this paper. Therefore, a table has been prepared (table 1) to illustrate this meaning. Assume three adjacent segments in the ring chromosome each of which when homozygous deficient results in the characters a, b, and c, respectively. If, in individual plants, altered ring chromosomes are isolated with the simple deficiency mutants a, b, and c, respectively, and the compound mutants a b and b c, respectively, combinations of each of these altered rings with a normal ring chromosome or with any other altered ring chromosome may be made and should give predictable results. These are shown in the table along with the characters of the tissues which would be formed following mitotic loss of one or the other of the ring chromosomes, respectively.
HOMOZYGOUS DEFICIENCIES IN MAIZE

It is the purpose of this paper to describe the types of simple and compound mutants which arise in plants possessing two homologous deficient rod-shaped chromosomes and one or more compensating ring-shaped chromosomes and to summarize the evidence which indicates that they are caused by homozygous minute deficiencies.

TWO CASES OF DEFICIENT ROD CHROMOSOMES PLUS COMPENSATING RING CHROMOSOMES

Two cases of deficient rod chromosomes with compensating ring chromosomes were available for this study. (For a complete description of their origin and behavior, see McClintock 1938). In both cases, segments of the short arm of chromosome 5 adjacent to the centromere were involved. The two cases are illustrated in figure 2. In the diagram, the numbers 1 to 9 in the normal chromosome 5 (first line in diagram) represent the positions of

![Diagram of chromosome positions](image)

Figure 2.—The upper figure is a diagram of a normal chromosome 5. The clear oval represents the centromere. The region adjacent to the centromere in the short arm is designated by the numerals 1 to 9. The middle figure represents a rod-shaped chromosome 5 deficient for the segments 1 to 4 (the Df-1 rod-chromosome). The dotted line represents the extent of the deficiency. To the right of this deficient rod chromosome, a ring chromosome is diagrammed possessing the segment which has been deleted from the rod chromosome (R-1). In a similar manner, the Df-2 rod chromosome and its compensating ring chromosome have been diagrammed.

The first nine chromomerers adjacent to the centromere. The rod chromosome, Df-1, is deficient for the four chromomerers adjacent to the centromere. Its compensating ring chromosome (R-1) possesses these four chromomerers (four chromomere ring below arrow, fig. E, Plate 1). The Df-2 rod chromosome (fig. 2) is deficient for these same four chromomerers plus the next five chromomerers. The R-2 ring chromosome possesses these nine chromomerers (fig. A, Plate 1; lower arrow, fig. B and G 1, Plate 1). It should be noted that the R-1 ring chromosome does not cover the deficiency in the Df-2 chromosome but the R-2 ring chromosome does cover the deficiency in the Df-1 chromosome. The transmission through gametes of the two deficient rod chromosomes without compensating ring chromosomes or with one or more ring chromosomes is given in table 2.

The types of plants most used in this investigation and the types of tissues which result from loss of the ring chromosome during development in these plants are summarized in table 3. In all cases, the tissues are normal when a single ring chromosome is present (either R-1 or R-2) covering the deficiency in the rod chromosomes. Tissues homozygous deficient for
Table 2

Transmissions through gametes of the deficient rod chromosomes without ring chromosomes or with various ring chromosomes. + represents transmission; - represents non-transmission.

<table>
<thead>
<tr>
<th></th>
<th>THROUGH ♂</th>
<th>THROUGH ♀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df-1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Df-1 plus R-1</td>
<td>+</td>
<td>+*</td>
</tr>
<tr>
<td>Df-1 plus R-2</td>
<td>+</td>
<td>+*</td>
</tr>
<tr>
<td>Df-1 plus R-1 plus R-2</td>
<td>+</td>
<td>+*</td>
</tr>
<tr>
<td>Df-2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Df-2 plus R-1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Df-2 plus R-2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Df-2 plus R-1 plus R-2</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* In a previous publication (McClintock 1938) it was shown that Df-1 was not transmitted through the pollen even when the deficiency was covered by a ring chromosome. Since this publication, a strain was obtained which will transmit the Df-1 chromosome through the pollen when a covering ring chromosome is present.

Chromomeres 1 to 9 are completely inviable; tissues homozygous deficient for chromomeres 5 to 9 are likewise inviable. In contrast, the tissues which are homozygous deficient for chromomeres 1 to 4 are viable, but the growth capacity of these cells is very poor. Only very minute sectors of such homozygous deficient tissues are found. (See McClintock 1938, text fig. 31-38 and this paper, a, fig. 3.) The cells in these sectors are small. The lignified walls of cells with a full genomic complement are white, but the walls of cells homozygous deficient for chromomeres 1 to 4 are brown in color. There are no well developed plastids in these cells; therefore, the tissues of the leaf and stalk which are homozygous deficient for chromomeres 1 to 4 are not green and contrast strikingly with non-deficient tissues which are green. On exposure to direct sunlight, these cells soon die, and the

Figure 3.—a. Photograph of a part of a leaf of a Df-1/Df-2 plant with ring chromosomes R-1 and R-2. A sector showing the character of the blotch mutant is evident slightly to the right of the letter a. The wide band to the left of this letter is the mid-rib. The continuous parallel fine lines are the veins of the leaf. The shorter, narrower lines between these veins are the sectors of tissue homozygous deficient for regions 1 to 4 of chromosome 5 which arise following somatic loss of both the R-1 and the R-2 ring chromosomes. b. Photograph of part of a leaf of a Df-1/Df-2 plant with two ring chromosomes, a normal R-2 ring and the brown-blotch-dries I ring. Two sectors showing the blotch character are visible to the right and left of the mid-rib, respectively. The photograph was taken before the tissue had commenced to disintegrate and dry. c. Similar to b. A wide sector showing the mutant character blotch-dries is present at each edge of the leaf. The disintegration and drying process has commenced at the outer edge of each sector. d. Leaves from plants of the constitution Df-1/Df-2 plus one R-2 ring chromosome. Note the solid (non-variegated) sector of mutant tissue (pink) in each leaf.

Figure 4.—Appearance of a young Df-2/Df-2 plant with two R-2 ring chromosomes, a normal R-2 ring and the brown-pink I ring. The plant is variegated for the compound mutant character brown and pink (white sectors in the photograph). These sectors arise following somatic loss of the normal R-2 ring.
HOMOZYGOUS DEFICIENCIES IN MAIZE

TABLE 3
Viability of tissues following somatic loss of ring chromosomes.
+ = normal. − = very poor growth. o, inviable.

<table>
<thead>
<tr>
<th>CHROMOSOME CONSTITUTION OF PLANTS</th>
<th>APPEARANCE OF TISSUES FOLLOWING SOMATIC LOSS OF RING CHROMOSOMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEFICIENT ROD-CHROMOSOME</td>
<td>RING CHROMOSOMES</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Df-1/Df-1</td>
<td>1 R-1</td>
</tr>
<tr>
<td>Df-1/Df-1</td>
<td>2 R-1</td>
</tr>
<tr>
<td>Df-1/Df-2</td>
<td>1 R-2</td>
</tr>
<tr>
<td>Df-1/Df-2</td>
<td>1 R-1 plus 1 R-2</td>
</tr>
<tr>
<td>Df-1/Df-2</td>
<td>2 R-2</td>
</tr>
<tr>
<td>Df-2/Df-2</td>
<td>2 R-2</td>
</tr>
<tr>
<td>Df-2/Df-2</td>
<td>1 R-1 plus 2 R-2</td>
</tr>
</tbody>
</table>

DESCRIPTION OF FIGURES FOR THE PLATE

All photographs are of pachytene configurations in microsporocytes. All magnifications are approximately 1100× except D 2, E 2, and G 2, which are approximately 2750×.

FIGURE A.—Synaptic association of two R-2 ring chromosomes.

FIGURE B.—The lower arrow points to a collapsed normal R-2 ring. The upper arrow points to a collapsed brown-pink II ring chromosome which is approximately double the size of the normal R-2 ring.

FIGURE C.—Two collapsed ring chromosomes associated at their centromeres. The arrow points to the associated centromeres. The normal R-2 ring is below the arrow. Above is the reduced ring chromosome which produces the brown I phenotype.

FIGURE D 1.—Two collapsed ring chromosomes associated at their centromeres. The arrow points to the associated centromeres. The normal four chromomere R-1 ring (above) is slightly out of focus. The ring below the arrow is a reduced R-2 ring composed of seven chromatids. It gives rise to the brown-pink IV phenotype. D 2. Enlargement of the same.

FIGURE E 1.—Two ring chromosomes associated at their centromeres. The arrow points to the associated centromeres. The normal, four chromomere R-1 ring lies below the arrow. An R-2 ring reduced to three chromatids lies above the arrow. This latter ring gives rise to the brown-blotch-dries II phenotype. E 2. Enlargement of the same.

FIGURES F and H.—The arrows point to the collapsed ring chromosomes in two sister cells of a Df-1/Df-2 plant with one R-2 ring chromosome. All surrounding cells possessed a normal R-2 ring. In one of these sister cells, fig. F, the R-2 ring chromosome was enlarged. In the other cell, fig. H, the R-2 ring chromosome was reduced to two chromatids. In chromatin constitution the two rings are equivalent to two normal R-2 ring chromosomes.

FIGURE G 1.—A collapsed normal R-2 ring chromosome (lower arrow) and an R-2 ring chromosome reduced to two chromatids (upper arrow). This latter ring chromosome gives rise to the compound mutant brown-light green-poor growth. G 2. Enlargement of the same showing this latter reduced ring chromosome.

FIGURE I.—The arrow points to the collapsed double-sized R-2 ring chromosome which gives rise to the brown-pink II phenotype.

FIGURE J.—Pachytene configuration in a plant with three ring chromosome. A collapsed normal R-2 ring lies to the left. A normal R-1 ring lies immediately above it. The arrow points to the reduced R-2 ring chromosome which produces the brown-light green-poor growth phenotype.
tissue disintegrates and dries. Because cells and tissues completely deficient for these four chromomerars not only are viable but also show phenotypic modifications, it is expected that tissues deficient for segments within this region would not only be viable, but could have much better growth rates and might show specific characters.

Studies of somatic mitosis have shown that the larger of the two ring chromosomes (R-2) produces a double-sized, dicentric ring chromosome (Prophase, fig. 1) approximately once in every hundred nuclear divisions. All of the other nuclear divisions are completely normal; the ring chromosome divides normally, the two free sister halves pass to opposite poles along with the rod chromosomes. In nearly all cases, when a double-sized ring chromosome is formed at prophase, it remains in the region of the equatorial plate during anaphase and telophase and is thus completely eliminated from the sister telophase nuclei. Very occasionally, however, a double-sized dicentric R-2 ring chromosome becomes broken at anaphase, and a segment of the broken double-sized ring chromosome enters each sister telophase nucleus. In both telophase nuclei fusion of broken ends occurs, reestablishing a ring-shaped chromosome, but the content of the ring-shaped chromosome is usually altered. Similar studies of the smaller ring chromosome (R-1) have shown that aberrant anaphase behavior occurs approximately once in every 600 nuclear divisions. In this case, due to its small size, the ring chromosome is nearly always lost to the sister telophase nuclei. Only rarely have altered R-1 rings been detected. The alterations in the chromatin content of the ring chromosomes to be described in this paper involve the R-2 ring.

DETECTION OF ALTERED RING-SHAPED CHROMOSOMES BY MEANS OF MUTANT SECTORS IN THE DF-1/DF-1, DF-1/DF-2 AND DF-2/DF-2 PLANTS

As stated in the previous section, alterations of the R-2 ring are considerably more frequent than alterations of the R-1 ring. Consequently, plants with one or two R-2 rings have shown the greatest number of detectable alterations. The plants most suitable for this study have had the following constitutions: Df-1/D-2 plus one R-2 ring; Df-1/Df-2 plus two R-2 rings; Df-2/Df-2 plus two R-2 rings.

In plants of the constitution Df-1/Df-2 plus one R-2 ring, a solid sector of modified tissue should appear following an alteration in the ring chromosome which produces a visible change in the character of the tissues. The size of the sector would depend upon when the altered ring chromosome arises in the development of the plant and the subsequent rate of growth of the cells possessing this ring. If the alteration occurred in a single nuclear division early in the development of the plant, a wide mutant sector could
result. If it occurred in a nuclear division late in development, only a small mutant sector would be produced. In Df-1/Df-2 plants with two ring chromosomes (either one R-1 plus one R-2 or two R-2) or in Df-2/Df-2 plus two R-2 rings, both solid sectors and sectors which are variegated for the mutant characters should be produced. The solid sectors should arise in the tissues of the plant which have only a single ring chromosome—that is, subsequent to a mitotic loss of one of the ring chromosomes. An alteration in the remaining ring chromosome could give a solid sector with a modified appearance. Variegated mutant sectors should arise when two rings are initially present in the tissue, one of which has become altered in an earlier mitotic division. When the two rings are present, a deficiency in the altered ring chromosome would be covered by the presence of this segment in the normal ring chromosome. No character caused by the deficiency in the altered ring chromosome could appear until after the normal ring chromosome has become lost in a later nuclear division. All the cells which arise from a cell which has lost the normal ring chromosome would be able to show the mutant character associated with the deficiency in the altered ring chromosome. Since loss of the normal ring chromosome should occur independently in a number of cells, a sector which is variegated for the mutant character should be produced. The appearance of a solid sector in a plant with a single ring chromosome is illustrated in a and d of figure 3; the appearance of variegated sectors in two-ring plants is illustrated in b and c of figure 3 and in figure 4.

If a particular mutant character is associated with loss of a particular region from the ring chromosome—that is, if the character is produced by a homozygous minute deficiency—only a restricted number of mutant characters should appear in the Df-1/Df-1 and Df-1/Df-2 plants. The segment within which homozygous deficiencies may be detected is only four chromosomes long (fig. 2). If this theory is correct, the same mutant characters which appear in the Df-1/Df-1 and Df-1/Df-2 plants should likewise appear in the Df-2/Df-2 plants because homozygous deficiencies within regions 1 to 4 (chromomeres 1 to 4, fig. 2) may be produced in all three types of plants. However, other mutants which are not found in the Df-1/Df-1 and Df-1/Df-2 plants should be present in the Df-2/Df-2 plants. These characters should be related to homozygous deficiencies within the region 5 to 9 (chromomeres 5 to 9, fig. 2). Such mutant characters should not appear in the Df-1/Df-1 or Df-1/Df-2 plants because the Df-1 chromosome possesses chromomeres 5 to 9, and thus no homozygous deficiencies can be produced within this region following aberrations of the ring chromosome. These anticipations have been completely fulfilled. Since the altered ring chromosomes may be isolated following the formation of gametes with a deficient rod chromosome and an altered ring chromosome,
it has been possible to conduct tests which show that a certain group of characters is associated with region 1 to 4 and another group of characters with region 5 to 9.

The frequency of mutant sectors is greater in the Df-1/Df-2 plants with one or two R-2 rings than in the Df-2/Df-2 plus two R-2 rings. The evidence, which is conclusive but which cannot be considered here because of limitations in space, indicates that this is due to changes in the R-2 ring chromosomes which have deleted rather large segments from the ring. In a Df-2/Df-2 plant, the cells with such large homozygous deficiencies are either inviable or produce tissues with such poor growth rates that they are not suitable for precise character studies. However, removal of a large segment from the R-2 ring in a Df-1/Df-2 plant may result in quite viable tissues with readily identifiable mutant characters. As an example, removal of region 4 to 8 in the R-2 ring in a Df-2/Df-2 plant would result in cells homozygous deficient for this region. These cells are inviable. In a Df-1/Df-2 plant, only region 4 could be homozygous deficient because the segment 5 to 8 covering the deficiency in the ring chromosome is present in the Df-1 rod chromosome.

Considering the number of mitoses taking place in the development of a plant, the occurrence of an abnormal mitosis which results in an altered ring chromosome is extremely rare. Once an altered ring chromosome has been isolated, it can be maintained unchanged through as many plant generations as desired by avoiding those plants in which this ring chromosome has again become altered. There are relatively few such plants.

**Mutant Characters Associated with Region 1 to 4 of Chromosome 5**

As stated previously, tissues which are homozygous deficient for the segments 1 to 4 of chromosome 5—the full extent of the deficiency in the Df-1 rod chromosome—are capable of multiplication but at a very slow rate. Consequently, only minute visible sectors are produced following complete loss of the ring chromosomes in Df-1/Df-1 or Df-1/Df-2 plants. The mutant characters shown by these homozygous deficient tissues are: brown cell walls, colorless plastids, very poor growth capacity, and disintegration and drying of the tissue when exposed to direct sunlight. It is to be expected that deficiencies for minute segments within this region should be viable. They may possess very much better growth capacities and could show mutant characters. Thus, relatively wide sectors showing mutant characters associated with a minute homozygous deficiency within region 1 to 4 might be produced in these plants. In the Df-1/Df-1 or Df-1/Df-2 plants, all the mutant characters observed as sectorials should be related to changes within region 1 to 4 of chromosome 5. In the Df-2/Df-2 plants, these same types of mutant characters should appear
plus additional mutant characters which are not found in the former types of plants. These latter mutants should be related to changes within region 5 to 9.

The various types of mutant characters having a clearly recognizable phenotypic expression which have appeared as sectorials in all three types of plants—that is, those mutants associated with region 1 to 4 of chromosome 5—have been divided into three groups: (1) Those which show a single recognizable character in the mutant sector (simple mutants); the tissue may have a normal growth rate. (2) Those which exhibit two or more of these characters in a single sector (compound mutants); the tissues may have a normal growth rate. (3) Sectors with distinct mutant characters. These sectors are always narrow—that is, associated with a reduced growth rate of the cells of the sector; many of these are recognizable as compound mutants.

The simple mutants to be described involve some obvious color change. Although other mutants would be expected and are probably present, their positive detection as sectorials in these plants is both difficult and uncertain. Therefore no attempt was made to isolate mutants which did not produce an unmistakable character change which could express itself as a sector in a plant. The color mutants belong to the unmistakable class.

**Group 1. Simple mutants which may have a normal growth rate**

**Brown cell walls**

The lignified cell walls are brown in color. This character is similar in all details to the character produced by the recessive mutant brown mid-rib (symbol, bm) previously located within the region 1 to 4 of a normal rod chromosome 5. Henceforth the character will be designated as brown. The normal R-1 and R-2 rings are known to carry the dominant allele of bm (Bm colorless cell walls). (For a complete description of the bm phenotype and proof of the presence of bm in the normal ring chromosomes, see McClinock 1938.) Three different ring chromosomes producing the brown phenotype have been isolated. Two appear to be normal in size and could not be distinguished from normal R-2 rings. Either ring will produce the brown character in Df-1/Df-1, Df-1/Df-2 or Df-2/Df-2 plants. The third brown ring is reduced to six chromomeres (upper collapsed ring, fig. c, Plate 1). When this ring is present, brown will appear only in Df-1/Df-1 or Df-1/Df-2 plants. In Df-2/Df-2 plants the cells which have only this ring are inviable. In this case, the three chromomere deficiency in the ring chromosome probably includes a segment of region 5 to 9 which could express itself as a homozygous deficiency only in the Df-2/Df-2 plants. As explained previously, in the Df-1/Df-1 or Df-1/Df-2 plants, the cells with only this reduced ring chromosome might possess only a minute homo-
zygous deficiency and thus be quite viable. This would explain the difference in viability of the cells possessing only this ring in the three types of plants.

Pink

Chlorophyll is absent although plastids are present. In the stalk, the number of layers of cells from the outer circle of bundles to the epidermis frequently is greater in the pink sectors than in the non-modified parts of the plant. Consequently, the pink sector produces a protuberance. On the stalk and in the upper leaves of the growing plant, the color is an intense salmon pink. As the leaves mature, the pink color gradually fades to white. Two ring chromosomes giving rise to the pink phenotype have been isolated, but the constitution of only one of these rings has been investigated cytologically. It is an R-2 ring with no clearly observable change in chromatin constitution. It produces the pink phenotype in either a Df-1/Df-1, a Df-1/Df-2 or a Df-2/Df-2 plant.

Blotch

The chlorophyll pattern is finely speckled (a, fig. 3). No ring chromosome producing a blotch character associated with a good growth capacity has been isolated so far, although a large number of such sectorials have been observed in both Df-1/Df-2 and Df-2/Df-2 plants. Several ring chromosomes producing blotch but associated with poor growth capacities have been isolated. None of these has been investigated extensively.

Blotch-dries

This character is similar to blotch although the chlorophyll color usually is less intense. In the young leaf, the character is first detected as a light green sector. The blotch pattern of the chlorophyll is acquired as the leaf matures. After a short time, the cells in the sector which have been exposed to direct sunlight begin to disintegrate. Finally, the whole sector so exposed becomes a mass of dead, dried tissue (see b and c, fig. 3). Two ring chromosomes producing blotch-dries have been isolated recently. Neither has been sufficiently investigated as yet.

**Group 2. Compound mutants which may have normal growth rates**

Brown-pink

Many sectors showing the combined characters of brown and pink have been observed in all three types of plants. Four different ring chromosomes producing this compound character have been isolated. One is an obviously reduced R-2 ring chromosome composed of seven chromomeres (lower collapsed ring, fig. D, Plate 1). Two are slightly reduced R-2 rings, and one is an enlarged R-2 ring, approximately double the size of the normal R-2 ring (collapsed ring, upper arrow, fig. B and fig I, Plate 1). The
brown-pink character produced by the seven-chromomere ring appears only in the Df-1/Df-1 or Df-1/Df-2 plants. In the Df-2/Df-2 plants, the cells with only this ring chromosome are inviable. The three ring chromosomes will produce the brown-pink character when present in either a Df-1/Df-1, a Df-1/Df-2, or in a Df-2/Df-2 plant.

Brown-pink-dries

At the initial stage, the sectors showing this compound mutant character are strictly comparable to the compound mutant brown-pink. However, as in blotch-dries, on exposure to sunlight, the cells die and the tissue dries. These sectors appear in both Df-1/Df-2 and Df-2/Df-2 plants. Several rings causing this compound mutant character have been isolated, but none has been investigated fully.

Brown-blotch-dries

The sectors exhibiting this compound mutant are identical in appearance and behavior to the blotch-dries sectors described above. In addition, however, the lignified cell walls are brown. Two ring chromosomes producing this compound mutant have been isolated. One is a slightly enlarged R-2 ring. The other is a much reduced ring chromosome composed of only three chromomeres (ring above arrow, fig. E, Plate 1). When the former ring chromosome is present, the character will appear in either a Df-1/Df-2 or a Df-2/Df-2 plant. When the latter ring is present, the compound mutant character will appear only in a Df-1/Df-1 or a Df-1/Df-2 plant. In the Df-2/Df-2 plants, the cells which possess only this ring chromosome are inviable—probably because of the six chromomere deficiency.

Group 3. Compound mutants whose growth rates are considerably reduced

Brown-pink-dries-poor growth

The sectors showing this group of characters are similar in appearance to the compound mutant brown-pink-dries of group 2. However, the growth rate of the mutant tissues is always considerably reduced. Thus, only narrow sectors are formed. Three rings producing this compound mutation have been isolated, but only one has been sufficiently studied. It is a slightly reduced R-2 ring. The character which this ring produces is exactly the same in the Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants. Thus, the phenotypic modification which this ring chromosome induces is probably restricted to a segment within region 1 to 4 of chromosome 5.

Light green-poor growth

The chlorophyll color in these sectors is a light green, but the growth rate of the cells is so reduced that sectors are not wider than 3 or 4 mm.
Only one ring chromosome producing this character has been isolated. It possesses only two chromomeres. Thus, the character associated with this ring appears only in the Df-1/Df-1 and Df-1/Df-2 plants. It does not appear in the Df-2/Df-2 plants due to the extensive deficiency producing inviability in the cells possessing only this ring.

Brown-light green-poor growth

Sectors showing this character are similar in appearance to those described above. In addition, the lignified cell walls are brown. Only one ring chromosome giving rise to this compound mutant has been isolated. It possesses two chromomeres (upper arrow, fig. G 1 and enlargement, fig. G 2, Plate 1). Due to the extensive deficiency in this ring chromosome, the characters associated with this ring appear only in Df-1/Df-1 or Df-1/Df-2 plants.

Many sectors exhibiting very poor growth capacities associated with a blotched chlorophyll pattern or with colorless tissues, either with or without brown, have been observed in the Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants. No attempt has been made to isolate the rings responsible for such characters because of the impracticability of work with characters having such poor growth rates.

MUTANT CHARACTERS ASSOCIATED WITH REGION
5 TO 9 OF CHROMOSOME 5

The mutant characters which may be associated with alterations in the R-2 chromosome within region 5 to 9 are those which do not appear in the Df-1/Df-1 or Df-1/Df-2 plants but appear only in the Df-2/Df-2 plants. It is difficult to obtain sufficient seed for an extensive investigation of the types of mutants appearing in this latter type of plant. Only one to several kernels are likely to occur on an ear of a Df-2/Df-2 plant, since the Df-2 chromosome is not transmitted through the female gametophyte without the compensating R-2 ring chromosome. During meiosis, the R-2 rings are so frequently eliminated that few megaspores are formed possessing this ring, and thus few seed may develop. Among the several thousands of plants which have been examined, a number of mutant sectorials have been observed. Many of these are related to changes in region 1 to 4 of the R-2 ring chromosome. Only a few clear-cut mutants have been observed which may be related to changes within region 5 to 9. In these plants, many mutant sectorials are composed of tissues which grow too poorly to be useful for character studies. No attempt has been made to isolate the changed rings responsible for the mutations with very poor growth rates. When a whole plant or half of a plant was variegated for such a character, a cytological study was made to determine the types of ring chromosomes which
were present. In all examined cases, one of the ring chromosomes was
decidedly reduced. Following loss of the normal ring chromosome to some
cells during development, a relatively large homozygous deficiency had to
be present. If these cells reproduce at a very reduced rate, only very
narrow sectors of homozygous deficient tissue could be formed. The pres-
ence of a reduced ring in all examined plants showing variegated sectors
with considerably reduced growth rates leads one to suspect that the cells
of these sectors possess only the reduced ring chromosomes—that is, they
are homozygous deficient for the segment which had been deleted from the
ring chromosome.

Of the mutants which possess a good growth capacity, only three have
been found which may be ascribed to region 5 to 9; and up to the present
time, only two of these have been proven to be in this region. These mu-
tants are:

**Pale green**

The chlorophyll color in the leaves and the stalk is a pale green. The pale
green character does not develop in the very young plants but becomes
very striking in the older plants. The growth rate of this tissue is usually
quite normal. Only one pale green ring has been isolated. No obvious
change in the chromatin constitution of this R-2 ring was observed.

**Striate**

The sectors showing this character are composed of streaks of yellow-
green tissues. Only one ring chromosome has been isolated which produces
this character. This R-2 ring is slightly reduced. A gamete with the Df-2
rod chromosome and the R-2 striate ring chromosome is transmitted
through the female gametophyte but not through the pollen. The location
of the character within region 5 to 9 has been determined by use of a third
deficiency rod chromosome (Df-3) possessing a small deficiency located
within the limits of region 5 to 9. Striate appears when the striate ring
chromosome is present in Df-2/Df-3 plants.

**White**

Sectors with a chalk white color have been observed in the Df-2/Df-2
plants but ring chromosomes producing white have not been isolated as
yet. Since similar sectors have not been observed in many thousands of
Df-1/Df-2 plants, it is assumed, although not proven, that this character
is related to an alteration within region 5 to 9.

The pale green ring is the only one of this series which has been exten-
sively investigated. It is not related to striate, since the pale green charac-
ter does not appear in Df-2/Df-3 plants, whereas striate does appear in
these plants. The following tests have shown that the pale green character
is related to region 5 to 9 and not to region 1 to 4 of chromosome 5. Following isolation of the pale green ring, plants were obtained with the constitution Df-1/Df-2 plus the pale green ring. These plants were indistinguishable from those of a similar constitution but having a normal R-2 ring. Thus, pale green does not appear in a Df-1 background. The functional male gametes produced by these plants possess the Df-2 rod chromosome plus the pale green ring chromosome. When pollen of such plants was placed on silks of plants of the constitution Df-2/Df-2 plus two normal R-2 ring chromosomes, all the resulting individuals (Df-2/Df-2 plus one normal R-2 ring plus the pale green R-2 ring) were variegated for pale green. The variegation arose as a consequence of the mitotic losses of the normal R-2 ring chromosome contributed by the female parent, thus giving rise to tissues containing only the pale green ring chromosome. It is in these tissues that the pale green character appears.

**CYTOLOGICAL OBSERVATIONS OF THE RING-SHAPED CHROMOSOMES WHICH GIVE RISE TO THE MUTANT CHARACTERS**

A pachytene configuration showing the association of two normal R-2 ring chromosomes is shown in figure A, Plate 1. When a single R-2 ring chromosome is present or when two R-2 ring chromosomes which have failed to associate at the meiotic prophase are present, the ring chromosome is collapsed (lower arrow, fig. B and G, Plate 1; ring below arrow, fig. C, Plate 1). This results from non-homologous associations of the chromatin forming the ring (McClintock 1933, 1938).

Following the formation of a double-sized dicentric ring chromosome in a mitotic prophase and its breakage in the following anaphase, an enlarged ring chromosome may be formed in one telophase nucleus and a reduced ring chromosome in the sister telophase nucleus (see fig. 1). This situation is illustrated by the constitution of the ring chromosome in two sister cells (arrows fig. F and H, Plate 1). These two sister cells are in the prophase of meiosis; therefore the event which resulted in the formation of these two altered ring chromosomes occurred at the last premeiotic anaphase. In individual cases, R-2 rings have been observed with various degrees of reduction in chromatin constitution. These range from a barely observable deficiency in the ring chromosome to deficiencies so large that the ring chromosome was composed of but a single large chromomere. Likewise, in individual cases, R-2 ring chromosomes have been observed with duplicated segments varying in extent from a very small duplication to one which produced a ring chromosome eight times the size of the normal R-2 ring.

Cytological observations of the composition of the ring chromosomes which produce visible mutations are summarized in table 4. As stated pre-
viously in the description of the mutant characters, several ring chromosomes have been isolated which produce the same mutant character (simple mutant) or the same group of mutant characters (compound mutant). Although the character which each of these ring chromosomes produces appears to be the same, the event which is responsible for the character which the ring chromosome produces occurred independently in each case in a single Df-1/Df-1, Df-1/Df-2, or Df-2/Df-2 plant. The numerals I, II, and III, etc., have been used to distinguish ring chromosomes producing the same character or the same group of characters which have originated independently of one another. For example, brown I was isolated from a sector of a Df-1/Df-2 plant, brown II from a sector of another Df-1/Df-2 plant, and brown III from a Df-2/Df-2 plant. Where a photographic illustration of a particular altered ring chromosome is reproduced in this paper, the figure reference is indicated in the table. Four of the fifteen ring chromosomes showed no observable change in the constitution of the R-2 ring. All four ring chromosomes are associated with simple mutants (brown II, brown III, pink, pale green). In nine cases, the ring chromosome was reduced. In two cases, the ring chromosome was enlarged (brown-pink II, brown-blotch-dries I). The brown-pink II ring, when first observed, was approximately double the size of the normal R-2 ring (upper arrow fig. B and fig. 1, Plate 1). The brown-blotch-dries I ring is only slightly enlarged. In both of these cases, the alteration in the ring chromosome, which is responsible for the character the ring chromosome produces, could have occurred subsequent to a previous alteration which had enlarged the R-2 ring chromosome. It is likewise conceivable that the enlargement of the ring chromosome could have occurred following the alteration which produces the character but before isolation of the ring. It is known that the enlargement itself is not the factor responsible for the appearance of the character which such an enlarged ring chromosome produces. This is clearly indicated by the enlarged brown-pink II ring. This ring, when first isolated, was approximately double the size of a normal R-2 ring chromosome. Because of the increased length of the chromonema of this ring chromosome, aberrant mitoses involving this ring chromosome occur with increased frequency. Furthermore, the added length of the chromonema of this ring chromosome brings it into the range where aberrant mitoses may frequently lead to recoverable alterations in the ring chromosome. Because of its size, it was expected that this ring chromosome would not maintain itself but would tend to become reduced to the size where aberrant mitoses would lead most frequently to elimination of the ring chromosome rather than alterations in its chromatin content. This proved to be true. Several strains have been isolated in which this ring chromosome is only slightly larger than the normal R-2 ring chromosome. At this reduced size, the
ring chromosome tends to remain stable because aberrant mitoses now tend to eliminate the ring rather than alter its chromatin composition. It should be emphasized, however, that the characters produced by the brown-pink II ring following its reduction in size from a double-sized ring to one only slightly larger than a normal R-2 ring have not been altered by the reduction. It is assumed that even in its enlarged state, the brown-pink II ring chromosome possessed a minute deficiency which was responsible for the compound mutant character it produced. No duplications of the remaining segments of the ring chromosome or subsequent deletions of these duplicated segments should alter the expression of the character, for the minute deficiency would still be present regardless of the presence or absence of these duplicated segments.

**Table 4**

Chromatin constitution of the ring chromosomes which produce mutant characters.

1. No observed change in constitution of the R-2 ring.
   brown II; brown II; pink; pale green.
2. Slightly reduced R-2 ring: Loss of approximately one chromomere.
   brown-pink I; brown-pink II; striate.
3. Obviously reduced R-2 ring: Loss of two or three chromomeres.
   brown-pink IV (fig. D, Plate 1); brown-pink-dries poor growth; brown I (fig. C, Plate 1).
4. Very reduced ring: Loss of six or seven chromomeres.
   brown-blotch-dries II (fig. E, Plate 1); light green-poor growth; brown-light green-poor growth (fig. G, Plate 1).
5. Enlarged ring: Larger than a normal R-2 ring.
   brown-pink II, approximately double the size of a normal R-2 ring (fig. B and I, Plate 1);
   brown-blotch-dries I, approximately $\frac{1}{2}$ larger than the normal R-2 ring.

In 11 of the 15 cases it has been established that the ring chromosome has undergone an alteration. It would require considerable space to indicate the methods used which allow one to be certain that the ring chromosome being observed in a particular plant is the ring chromosome responsible for the character ascribed to it. The methods will be considered in a separate report.

**TRANSMISSIONS OF ALTERED RING CHROMOSOMES THROUGH THE MALE AND FEMALE GAMETES**

Tests have been made of the transmissions of the various altered ring chromosomes through the female gametophyte with the Df-1 or Df-2 rod chromosomes and through the pollen with the Df-2 rod chromosome. These results are summarized in table 5. Transmissions through the pollen of the various altered ring chromosomes with the Df-1 rod chromosome have not been included in the table, since a male-transmissible strain of Df-1 has only recently been obtained, and the tests have not been completed. The cytological determination of the type of ring chromosome associated with
each particular mutant has been included in column 2 of this table, since it allows the tests to be more readily interpreted. Since many of these altered ring chromosomes are readily transmitted through the pollen, even in competition with gametes carrying normal ring chromosomes, it has been possible to obtain various combinations of the ring chromosomes in Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants. These will be described in the following section.

Table 5

Gametic transmissions of the altered ring chromosomes with the Df-1 or Df-2 rod chromosomes. Transmissions of the altered ring chromosomes with the Df-1 rod chromosome through the pollen have not been included in the table because these tests have not been completed. + represents transmission. 0 represents no transmission. − indicates the test has not been completed.

<table>
<thead>
<tr>
<th>RING CHROMOSOME</th>
<th>CHROMATIN CONSTITUTION OF ALTERED R-2 RING</th>
<th>TRANSMISSIONS THROUGH Ψ</th>
<th>TRANSMISSIONS THROUGH Ψ'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Df-1</td>
<td>Df-2</td>
</tr>
<tr>
<td>brown I</td>
<td>Reduced to six chromomeres</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>brown II</td>
<td>No apparent reduction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>brown III</td>
<td>No apparent reduction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pink</td>
<td>No apparent reduction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pale green</td>
<td>No apparent reduction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>striate</td>
<td>Slightly reduced</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>brown-pink I</td>
<td>Slightly reduced</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>brown-pink II</td>
<td>Enlarged</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>brown-pink III</td>
<td>Slightly reduced</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>brown-pink IV</td>
<td>Reduced to seven chromomeres</td>
<td>+</td>
<td>o</td>
</tr>
<tr>
<td>brown-pink-dries-poor growth</td>
<td>Slightly reduced</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>brown-blotch-dries I</td>
<td>Slightly enlarged</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>brown-blotch-dries II</td>
<td>Reduced to three chromomeres</td>
<td>+</td>
<td>o</td>
</tr>
<tr>
<td>light green-poor growth</td>
<td>Reduced to two chromomeres</td>
<td>+</td>
<td>o</td>
</tr>
<tr>
<td>brown-light green-poor growth</td>
<td>Reduced to two chromomeres</td>
<td>+</td>
<td>o</td>
</tr>
</tbody>
</table>

THE CHARACTERS PRODUCED IN PLANTS FOLLOWING COMBINATIONS OF ALTERED RING CHROMOSOMES: PROOF THAT THE COMPOUND MUTANTS ARE COMPOSED OF TWO OR MORE OF THE SIMPLE MUTANTS

It is obvious from the description given earlier in this paper that the characters exhibited by the compound mutants bear a relation to the simple mutants. The compound mutants appear to be composed of a combination of two or more of the observed simple mutants. It was also obvious in this study that the sectorials showing compound mutant characters occurred far more frequently than those showing a simple mutant character. Sectorials showing a simple mutant character were relatively rare. The method by which ring chromosomes become reduced in size at a mitotic anaphase would suggest that adjacent blocks of chromatin should fre-
quently be deleted from the ring chromosome following an aberrant mitosis which results in an alteration in the ring chromosome. If each of the simple mutants is produced following loss of a particular minute segment from the ring chromosome, then the compound mutants should be produced following loss of two or more of these segments. It would be expected that breakage of the double-sized ring at a mitotic anaphase would delete a block of chromatid more frequently than a very minute segment. Thus, compound mutants should appear far more frequently than simple mutants. With this interpretation, one should be able to deduce the order of the various segments in the ring chromosome which produce a particular character when homozygous deficient, merely by observing the characters which are present in the compound mutants. For illustrative purposes, one may use the characters pink, brown, and blotch dries. The most frequent types of compound mutations involving these characters are: brown-pink, brown-pink-dries, and brown-blotch-dries. Since brown may be with either pink or blotch dries, the suggested order is (1) pink followed by (2) brown followed by (3) blotch dries. Loss of the first two adjacent segments would give rise to the compound mutant brown-pink; loss of the second and third adjacent segments would give rise to the compound mutant brown-blotch dries; loss of all three adjacent segments would give rise to the compound mutant brown-pink-dries.

Proof that the compound mutants are composed of two or more of the simple mutants may be obtained readily. It is known that all of the mutant types behave as recessives. It is only necessary to combine two altered rings each of which gives a character or a group of characters in a Df-1/Df-2 or a Df-2/Df-2 plant and observe the characters of the tissues which possess the two altered ring chromosomes. The method is essentially that which is diagrammed in table 1, and the results are in complete agreement with it.

Various combinations of the altered ring chromosomes have been made. Those which involve the mutants brown, pink, brown-pink, brown-pink-dries, brown-blotch-dries are given in table 6. The appearance of the cells and tissues possessing both ring chromosomes are given in column 2. In column 3, the character of the tissues which possess only one or the other of the altered ring chromosomes (following mitotic loss of one or the other ring chromosome, respectively) has been indicated. Where more than one similar combination was made, the particular altered ring chromosome (that is, I, II, or III) is given below the character in each specific combination. It made no difference which ring chromosome producing a given mutant type was used. In every tested case the results were the same. Combination 1 of table 6 (X plus Normal) gives the characters resulting from the combination of any one altered ring chromosome, X, with a normal R-2 ring chromosome. In all of the 15 isolated cases the normal R-2 ring chro-
mosome suppressed the character produced by the altered ring chromosome when both were present in the same cell. However, the presence of the altered chromosome in these plants was made obvious by the variegation which appeared (fig. 4). This variegation resulted from the mitotic losses of the normal R-2 ring chromosome. The character produced by the altered ring chromosome could then be expressed.

**Table 6**

*Phenotypic characters in Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants with various combinations of ring chromosomes together with the type of variegation which is observed in each of these plants following somatic loss of one or the other ring chromosome, respectively. + indicates normal, non-mutant tissue.*

<table>
<thead>
<tr>
<th>COMBINATIONS OF RING CHROMOSOMES</th>
<th>CHARACTER PRODUCED WHEN BOTH RING CHROMOSOMES ARE PRESENT</th>
<th>TYPES OF VARIEGATION: CHANGE IN CHARACTER OF TISSUE FOLLOWING SOMATIC LOSS OF ONE RING CHROMOSOME</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. brown II plus brown III</td>
<td>Plant totally brown</td>
<td>Loss of brown II ring: No change in tissue character.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss of brown III ring: No change in tissue character.</td>
</tr>
<tr>
<td>3. brown II plus pink</td>
<td>+</td>
<td>Loss of brown II ring: pink tissue.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss of pink ring: brown tissue.</td>
</tr>
<tr>
<td>4. brown plus brown-pink</td>
<td>Plant totally brown</td>
<td>Loss of brown ring: brown-pink tissue.</td>
</tr>
<tr>
<td>II * I</td>
<td></td>
<td>Loss of brown-pink ring: No change in tissue character.</td>
</tr>
<tr>
<td>III * I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II * II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III * II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. brown plus brown-blotch-dries</td>
<td>Plant totally brown</td>
<td>Loss of brown ring: brown-blotch-dries tissue.</td>
</tr>
<tr>
<td>II * I</td>
<td></td>
<td>Loss of brown-blotch-dries ring: No change in tissue character.</td>
</tr>
<tr>
<td>III * I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. brown I plus brown-pink-dries II</td>
<td>Plant totally brown</td>
<td>Loss of brown ring: brown-pink-dries tissue.</td>
</tr>
<tr>
<td>II * I</td>
<td></td>
<td>Loss of brown-pink-dries ring: No change in tissue character.</td>
</tr>
<tr>
<td>7. pink plus brown-pink I</td>
<td>pink†</td>
<td>Loss of pink ring: brown-pink tissue.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss of brown-pink ring: No change in tissue character.</td>
</tr>
<tr>
<td>8. pink plus brown-blotch-dries I</td>
<td>+</td>
<td>Loss of pink ring: brown-blotch-dries tissue.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss of brown-blotch-dries ring: pink tissue.</td>
</tr>
<tr>
<td>I * II</td>
<td></td>
<td>Loss of brown-blotch-dries ring: brown-pink tissue.</td>
</tr>
</tbody>
</table>

* X represents any altered ring chromosome producing a mutant character.
† Due to lack of chlorophyll, plants that are totally pink do not survive beyond the seedling stage. The test was made by combining these two ring chromosomes with a normal ring chromosome. The character produced by the combination (column 2) and the characters indicated in column 3 were obtained from sectors of these plants which had lost the normal ring chromosome.

Combination 2 in table 6 indicates that the simple mutant brown produced by the brown II ring chromosome is identical with the simple mutant brown produced by the brown III ring chromosome. Plants with these two ring chromosomes are totally brown. No detectible alteration
occurs in the tissues following loss of either ring chromosome. Combination 3 indicates that the simple mutant brown \( II \) and the simple mutant pink are completely independent of one another, for the pink character is suppressed in the presence of the brown \( II \) ring chromosome, whereas the brown \( II \) character is suppressed in the presence of the pink producing ring chromosome. However, the plant is variegated for both brown and pink following mitotic losses of the pink ring or the brown ring, respectively. There were no mutant sectors with the combined character brown-pink. Combination 4 indicates that the brown in the compound mutants brown-pink \( I \) and brown-pink \( II \) are identical with the brown character produced by either the brown \( II \) or the brown \( III \) ring chromosome, for these plants are totally brown although variegated for pink, following mitotic losses of the brown \( II \) or the brown \( III \) ring chromosome, respectively. Since the character brown \( II \) is identical with brown \( III \), the brown character in the compound mutant brown-pink \( I \) must be identical with the brown character in the compound mutant brown-pink \( II \). Similarly, as combination 5 indicates, the brown character of the compound mutants brown-blotch-dries \( I \) and brown-blotch-dries \( II \) is identical with the simple mutant character produced by either the brown \( II \) or brown \( III \) ring chromosomes. The plants are totally brown but variegated for blotch-dries following mitotic losses of the brown \( II \) or brown \( III \) ring chromosomes, respectively. Combination 6 indicates that the simple mutant brown produced by the reduced brown \( I \) ring chromosome is identical with the brown produced by the ring chromosome giving the compound mutant brown-pink-dries \( II \). (This latter compound mutant has not been mentioned previously in this paper.) The homology of the simple mutant pink with the pink produced by the ring chromosome giving the compound mutant brown-pink \( I \) is shown by combination 7. However, the pink ring covers the brown part of the compound mutant produced by the altered ring chromosome giving brown-pink \( I \). Combination 8 shows that the simple mutant pink and the compound mutant brown-blotch-dries \( I \) have no overlapping effects. The pink ring suppresses the brown-blotch-dries \( I \) character. Likewise, the brown-blotch-dries \( I \) ring chromosome suppresses the pink character produced by the pink ring. The combination of the compound mutants brown-pink \( I \) with either brown-blotch-dries \( I \) or \( II \) indicates that the brown character is identical in both compound mutants, for the plants are totally brown. However, the pink character produced by the brown-pink \( I \) ring is suppressed by the brown-blotch-dries rings, and the blotch-dries character produced by the latter ring chromosomes is suppressed by the brown-pink \( I \) ring chromosome.

From the combinations given in table 6, it may be seen that the brown mutant produced by an altered ring chromosome is the same whether it
appears as a simple mutant or in combination with other mutants. A similar conclusion may be drawn regarding the pink mutant, although there is less experimental evidence for this on the basis of the combinations given in table 6. It is quite obvious from these combinations that brown, pink, and blotch-dries are independent mutants and that the compound mutants result from combinations of these simple mutants. As stated earlier one may deduce the order of the segments in the R-2 ring chromosome which are responsible for the mutant effects. This order, as stated previously, was: pink followed by brown followed by blotch-dries. The experimental evidence given in table 6 supports this interpretation.

From these tests it may be concluded that the compound mutants are produced by combinations of two (or more) of the observed simple mutants and that the mutants giving the same phenotypic appearances, although arising independently of one another, are identical.

To discuss adequately the characters which are produced in plants carrying the deficient rod chromosomes following combinations of the various altered ring chromosomes would require considerable space and cannot be undertaken within the limits of this paper. However, the results of combining an altered ring chromosome with normal, non-deficient rod chromosomes 5 should be mentioned. If a plant possesses a normal rod chromosome 5 carrying $Bm$ and likewise any one of the various altered ring chromosomes, the characters which these ring chromosomes would produce if deficient chromosomes 5 were present now do not appear. The plants are normal. However, when the ring chromosome is returned to plants carrying the deficient rod chromosomes in a successive plant generation, the character produced by the ring chromosome again appears. The characters produced by the ring chromosomes are thus completely recessive to “dominant alleles” which are present in segment 1 to 9 of the normal, non-deficient rod chromosome 5. This is not true, however, if a brown producing ring is combined with normal rod chromosomes 5 carrying the recessive mutant $bm$. All the ring chromosomes which give rise to the brown character (simple or compound mutants) in plants with the deficient rod chromosomes have been placed in plants possessing normal rod chromosomes 5 carrying $bm$. In every case, the plants are totally brown. In these plants, only the brown character appears. The pink, pink-dries, blotch-dries, or light green characters are suppressed by this rod chromosome 5. From these combinations it is strikingly evident that the brown character produced by the altered ring chromosomes is homologous to the known mutant $bm$ previously located within region 1 to 4 of a normal chromosome 5 (McClintock 1938). A normal chromosome 5 carrying any one of the other mutants produced by the altered ring chromosomes has not been isolated as yet.
REVIEWS OF THE EVIDENCE WHICH INDICATES THAT THE CHARACTERS
PRODUCED BY THE ALTERED RING CHROMOSOMES ARE CAUSED
BY HOMOZYGOUS DEFICIENCIES

Throughout this paper it has been assumed that the mutant characters are caused by homozygous minute deficiencies. It has been determined by many observations that ring chromosomes may increase in size by duplications and repeat duplications of segments composing the ring, or they may become reduced in size through loss of segments from the ring. It has also been determined that whole plants or sections of a plant which possess a ring chromosome with duplicated segments are not obviously modified in phenotypic appearance. Thus, the ring chromosomes with deficiencies are the ones which would be expected to produce an alteration in the phenotypic appearance of the tissues in the plants with the deficient rod chromosomes. Deficient ring chromosomes must be produced in some cells of some of these plants. It could be objected that such homozygous deficiencies might be expected to be cell lethal, and therefore the character produced by the altered ring chromosome could be caused by some other process than deficiency. However, this has proved not to be true. It is known that cells and tissues homozygous for the four chromomere deficiency of the Df-1 rod chromosome are viable. It is also known that these cells and tissues are decidedly modified. The lignified cell walls are brown. This brown is strictly comparable in time of development in the cell wall, in color, and in behavior to light to that produced by the known mutant bm which had previously been located within the limits of region 1 to 4 of a normal chromosome 5. (The character of the lignified walls produced when the altered ring chromosomes brown I, II, and III, brown-pink I, II, etc., are present is likewise strictly comparable to the character produced by the mutant bm.) No chlorophyll develops in the cells homozygous for the four chromomere deficiency. Therefore, with regard to chlorophyll, these tissues are colorless. Furthermore, on exposure to sunlight, these cells die and the tissue dries. These tissues have a very reduced growth capacity. It is known that the characters brown, pink, and blotch-dries must be related to changes within the region 1 to 4 of chromosome 5—that is, within the limits of the deficiency in the Df-1 chromosome. It has also been shown that the two very reduced rings (light green-poor growth and brown-light green-poor growth) produce tissues with a decidedly reduced growth capacity. Since the latter two ring chromosomes obviously do not have enough chromatin to cover the deficiency in the Df-1 chromosome, the reduced growth could be ascribed to the presence of the two chromomere deficiency in each case. If we add together the characters produced by the changed rings—that is, brown plus pink (no
HOMOZYGOUS DEFICIENCIES IN MAIZE

chlorophyll) plus blotch-dries plus poor growth—we arrive at the compound character brown-colorless-dries-poor growth, which is exactly the compound character exhibited by the tissues which are homozygous deficient for the four chromomeres deleted from the Df-1 chromosome. This four chromomere deficiency expresses itself as a compound mutant just as brown-pink, brown-pink-dries, and brown-blotch-dries express themselves as compound mutants. Evidence for the compound nature of these mutants has been given in this paper (see table 6). It would be difficult to arrive at a more simple explanation of all these facts than that the characters are produced as the result of homozygous deficiencies, each individual character being produced by a relatively minute deficiency of a specific locus. This will explain both the simple and the compound mutants: the simple mutants resulting from loss of one specific locus, the compound mutants resulting from loss of two or more specific loci. It will also account for the recessive expression of these characters, for it is known that plants with one normal chromosome 5 and either the Df-1 or the Df-2 chromosome 5—that is, hemizygous for regions 1 to 4 or 1 to 9, respectively—are phenotypically normal.

Notwithstanding the cytological observations of reduction in size of many of the ring chromosomes giving mutation effects, genic mutation might be suggested as an alternative to homozygous deficiencies as the cause of the mutant characters. If so, two or more genic mutations would have to occur simultaneously in closely related loci in many instances to account for the considerably greater frequency of occurrence of the compound mutations. Furthermore, the rate of such mutations in the ring chromosome would have to be considerably greater than the rate of mutation in the same region of a normal rod-shaped chromosome 5. Our knowledge of “genic” mutations would not lead us to anticipate such behavior, for we know of no cases where supposedly genic mutations behave in exactly this manner.

Likewise, one might argue that the alterations in the ring chromosomes producing mutant effects are due to changes in the relative positions of the genes in the chromatin of the ring. One must then explain what should be expected of the reduced ring chromosomes, for they are being produced in these plants following aberrant mitoses which alter the chromatin content of the ring. Again, on this basis, one would have to explain away the cytologically obvious deficiencies in nine of the 15 isolated ring chromosomes which produce mutant effects. Since tissues homozygous deficient for chromomeres 1 to 4 of chromosome 5 are known to produce a compound mutant effect which equals the sum of the simple mutant effects located within this region, it would be hazardous to consider that smaller deficiencies within this region would produce no phenotypic effect. One might
object that not all the ring chromosomes giving mutant effects were detectibly reduced in chromatin content, for in four of them no deficiency was detected and in two cases the ring chromosome was enlarged. In all four cases where no deficiency in the ring chromosome was detected, simple mutants were produced (brown II, brown III, pink, pale green). If, in each case, the phenotypic expression is due to a minute deficiency, it is probable that detection of a single minute deficiency at the meiotic prophase in maize would be extremely difficult. In the cases of the two enlarged ring chromosomes it has been shown (table 6) that the characters produced by these two ring chromosomes, brown-pink II and brown-blotch-dries I, are identical with the characters produced by the reduced rings brown-pink I and brown-blotch-dries II, respectively. The enlargement does not mean that there is no deficiency in these ring chromosomes, for an enlargement may have occurred in an aberrant mitosis subsequent to an aberrant mitosis which deleted a segment from the ring chromosome. Following this deletion, no further duplication of the remaining chromatin could restore the original loss. Neither should subsequent deletions of duplicated segments cause any change in the expression of the character produced by such a ring chromosome. It should be emphasized that this proved to be true in the case of the enlarged brown-pink II ring.

The above considerations are the basis of the inferential evidence that the plant characters associated with altered ring-shaped chromosomes are produced as a consequence of homozygous deficiencies. In a previous section, the chromatin constitutions of the altered ring chromosomes have been considered briefly. It was pointed out that the brown-blotch-dries II ring, the light-green-poor growth ring, and the brown-light green-poor growth ring were all smaller than the normal four chromomere R-1 ring. In the Df-I/Df-I or Df-I/Df-II plants, the cells which contain only one such ring must be homozygous deficient for some chromatin within the limits of the deficiency in the Df-I chromosome. All three of these reduced ring chromosomes may be distinguished from one another by differences in their chromomere constitution. Therefore, they do not have exactly the same deficiencies. Furthermore, each of these three rings produces, respectively, a particular and distinguishable type of compound mutant character. It would be difficult to escape the conclusion that the obviously different total deficiencies in these three rings are the cause of the obviously different total group of mutant characters that each of these three ring chromosomes produces. If one did attempt to account for these observations on some other bases, it would be necessary to assume that each deficiency in these three cases was without a distinct phenotypic effect—which hardly seems probable, considering the very striking compound phenotypic effect that a total deficiency of all four chromomeres produces.
HOMOZYGOUS DEFICIENCIES IN MAIZE

On the basis of the above inferential and observational evidence, it is concluded that the characters exhibited in tissues containing an altered ring chromosome are caused by homozygous deficiencies, each character being caused by a relatively minute but specific deficiency, and that the compound mutant characters are caused by the removal of two or more such segments from the genomic complement.

CONCLUSION

This paper presents only a summary of the evidence which has led to the conclusion that some phenotypic characters in maize may be produced by homozygous minute deficiencies. A complete account of all the evidence obtained from altered ring chromosomes—their origin, their gametic transmissions both male and female, their stability, the variation in phenotypic expression of the various mutants when combined with different genotypes, the cytological analysis of the chromatin constitutions of the various altered ring chromosomes, the appearance of plants with various combinations of three or more altered ring chromosomes, the rate of production of altered ring chromosomes, evidence showing that the mutants do not revert to normal, and other phenomena related to the behavior of these ring chromosomes—could not be included in this paper due to limitation of space. The evidence of particular interest will be reported in separate publications. It is hoped, however, that sufficient evidence has been included to indicate the nature of the method of attacking this problem and to indicate the type of evidence obtained. Again, due to limitation of space, an adequate discussion of the relation of homozygous deficiencies giving mutant effects in maize to similar phenomena in other organisms cannot be undertaken here. It should be pointed out, however, that the number of analyzed cases is very limited and is best represented by the yellow mutant in Drosophila (Ephrussi 1934; Stern 1935; Muller 1935; Demerec 1934, 1936; Demerec and Hoover 1936; Kaliss 1939).

In conclusion, the author wishes to point out the possible usefulness of the evidence presented in this paper in formulating a prediction as to the nature, the location, and the crossover values to be expected of mutants not as yet obtained in the normal complement of maize. The ring mutants pink, brown, blotch-dries, and pale green are readily transmitted through the pollen, although the relative efficiency in competition with grains carrying an unmodified complement could not be determined. It is possible, however, that through natural causes or by X-ray or ultra-violet radiation, these mutants would be produced in a normal rod-chromosome 5. Pale green and brown should produce viable plants, but pink and blotch-dries would result in plants which die in the seedling stages. A mutant (brown mid-rib, bm, Jorgenson 1931), allelic and indistinguishable from the
brown produced by the ring chromosome mutants, has been isolated. Although it may not be concluded from observational evidence that the \textit{bm} mutant in the normal rod-chromosome 5 is caused by a homozygous minute deficiency, it may be stated, however, that a deficiency of the locus of the dominant allele of \textit{bm} will reproduce in all details the phenotypic expression of \textit{bm}. Pink, blotch-dries, and pale green remain to be isolated. Following isolation, pink and blotch-dries should prove to be very closely linked to the known mutant \textit{bm}, whereas pale green should show a small amount of crossing-over.

**SUMMARY**

It is the purpose of this paper to show that viable mutants in maize may be produced by homozygous minute deficiencies. The aberrant mitotic behavior of ring-shaped chromosomes has been the method of obtaining a large number of such deficiencies. These deficiencies are located within the limits of a relatively small segment of the genomic complement composed of the proximal 9 chromomereres of the short arm of chromosome 5.

Mutants arise following changes in the chromatin constitution of the ring-shaped chromosomes. These changes are produced following aberrant behavior of the ring-shaped chromosome in some of the mitotic divisions. The types of mutants which are produced by the altered ring-shaped chromosomes are simple, composed of a single recognizable character or compound, composed of two or more of the characters recognizable as simple mutants. In a number of cases it has been possible to isolate the altered ring-shaped chromosomes which produce the simple or compound mutants.

Through appropriate tests, it has been proven that the compound mutants are the products of two or more of the simple mutants. One group of mutants has been located within the limits of the proximal 4 chromomereres of the short arm of chromosome 5. The second group of mutants has been located within the limits of the next five chromomereres.

Evidence is presented which leads to the conclusion that each mutant character, whether appearing as a simple mutant or in combination with other mutants, is produced by a homozygous minute deficiency, each mutant character being associated with loss of a particular minute segment. The simple mutants are associated with loss of one such segment; the compound mutants are associated with loss of two or more such segments.

**LITERATURE CITED**


HOMOZYGOUS DEFICIENCIES IN MAIZE


Perspective:¹


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SPONTANEOUS ALTERATIONS IN CHROMOSOME SIZE AND FORM IN ZEA MAYS

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Reprinted from COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY 9:72-80, 1941
SPONTANEOUS ALTERATIONS IN CHROMOSOME SIZE AND FORM IN ZEA MAYS

BARBARA McCLINTOCK

Spontaneous aberrations in maize leading to changes in size and form of the chromosomes have not been investigated from the point of view of determining, systematically, the frequency and positions of breakages and reunions of broken ends of the chromosomes of the complement, as has been done in Tradescantia (Giles, 1940), in Allium (Nichols, 1941) and in other forms (Darlington and Upcott, 1941). Nevertheless, through studies of various problems not directed toward this goal, much has been learned of the process underlying the origin of changes in size and form of the chromosomes of maize which are not conditioned by the usual methods of inducing aberrations, such as X-radiation, ultraviolet radiation, high temperatures and aging.

In the early cytological studies of maize, it became clear that spontaneous aberrations were occurring to give rise to various types of altered chromosomes. In many cases, the time of occurrence or the conditions which gave rise to the alteration were not known. These aberrations were first observed in various plants of particular strains which were under cytogenetic investigation. These aberrations included reciprocal translocations, inversions, deficiencies, ring-chromosomes, a duplication, fragments, and a secondary trisome. Through further studies, it became apparent that chromosome modifications were occurring in individual plants under investigation. A single plant of a culture may show one of the various types of aberrations mentioned above. Although, in some cases, it could not be determined whether all of the cells of the plant possessed the aberration, in other cases it was determined that the plant was sectorial for the modification. In these latter cases, it was obvious that the modification occurred during the development of the individual plant. The factors responsible for these spontaneous aberrations were not apparent in any of these cases. However, there are types of chromosomal aberrations which are induced by known factors or are correlated with known conditions. These will be considered under appropriate headings in the following discussion.

Spontaneous Chromosome Aberrations Under Genic Control

There are two well investigated cases which indicate that the rate of spontaneous chromosome aberration in maize may be controlled by the genic composition of the nucleus. The first case is strikingly illustrated by the sticky gene studied by Beadle (1932, 1937). Sticky is a recessive mutant located in chromosome 4 which causes a tremendous increase in the rate of spontaneous chromosome aberration in all types of tissues. At the first meiotic anaphase in homozygous plants, the chromosomes appear adhered to one another. This sticking together of the chromosomes of the complement suggested the designation sticky for this mutant. As a consequence of this sticking, many of the chromosomes are ruptured during the meiotic anaphase. In the mitotic divisions, numerous types of chromosomal aberrations were observed in plants homozygous for the sticky mutant. The continued production of spontaneous aberrations during development causes plants homozygous for sticky to be stunted in growth and to possess numerous streaks of tissues with altered phenotypes. The endosperm tissues are likewise a mosaic of various types of aberrant cells. The extremely high rate of spontaneous chromosome aberrations, both in the sporophytic and endosperm tissues, undoubtedly is the cause of the observed phenotypic alterations of the cells and tissues, for various grades of chromosomal unbalance must be present in these cells. It is likewise of particular interest to note that the sticky mutant is responsible for a marked increase in the rate of spontaneous mutation.

The second case of spontaneous chromosome alteration under genic control has been studied by Jones (1937, 1940). In some of his strains of maize, the endosperm tissues give unmistakable evidence of a high rate of spontaneous chromosome aberration. Unlike the sticky mutant, the high rate of chromosome aberration appears to be confined to the endosperm tissues alone. The genetic evidence indicates that reciprocal translocations are occurring between non-homologous chromosomes and that chromosomes with unstable broken ends are likewise produced. Other types of chromosomal aberrations could not be detected genetically. Cytological observations of the endosperm tissues of these plants have produced direct evidence of a high rate of spontaneous chromosome aberration (Clark and Copeland, 1940).

It is needless to say that any systematic study of spontaneous chromosome aberration in maize must be considered with reference to the genic composition of the plants under investigation.

Chromosome Alterations Induced by Crossing Over Between Homologous Segments of Chromosomes

The normal process of crossing-over may be responsible for the production of chromosomes with
altered sizes and forms. When individual plants are heterozygous for an inversion, a duplication or some rearrangement in the linear organization of the chromosome, predictable types of chromosomal alterations may follow crossing-over between homologous segments of chromosomes. Although structural heterozygosity greatly increases the rate of production of altered chromosomes following crossing-over, it will be shown that chromosome aberrations likewise may be induced within a normal complement following the regular process of crossing-over. Several examples illustrating the part that crossing-over plays in the production of chromosomal aberrations will be considered.

Fig. 1. Diagrammatic representation of the method by which a chromosome with a broken end gives rise to chromosomes with altered sizes and constitutions. The diagram at the top of the figure represents a chromosome with a broken end. The centromere is represented by the clear oval. The organization of the arm with the broken end is represented by A, B and C, A being adjacent to the broken end. Following reduplication of the chromosome, the two sister chromatids are fused at the position of previous breakage (Prophase, second diagram from top). The centromeres pass to opposite poles in the succeeding anaphase. This produces a bridge configuration (Anaphase, third diagram from top). If breakage of this bridge configuration occurs at the position of the arrow, a broken chromosome will enter each telophase nucleus (Telophase, right and left, fourth diagram from top). The broken chromosome to the left possesses a duplicated segment, that to the right is deficient for a terminal segment. Continuation of this breakage-fusion-bridge cycle in succeeding nuclear divisions may result in the production of chromosomes with various duplications, deficiencies or duplications plus deficiencies as illustrated in the diagrams below each of these telophase chromosomes. (From McClintock 1941a, through the courtesy of Genetics.)

Plants heterozygous for an inversion which does not include the centromere will give rise to altered chromosomes following a crossover within the inverted segment. It is well known that this results in the production of a dicentric chromatid and an acentric fragment. Passage of the two centromeres of the dicentric chromatid toward opposite poles in the meiotic anaphase spindle produces a chromatid bridge configuration. Rupture of this bridge occurs either before or following the formation of the cell plate. The position of rupture varies. It may be adjacent to one centromere or at any position between the two centromeres. In all cases, a ruptured chromatid possesses a deficiency because the dicentric chromatid itself is deficient for a segment carried by the acentric fragment. If the position of breakage in the bridge configuration is non-median, the broken chromatid entering one nucleus will be more deficient than the broken chromatid entering the sister nucleus. The latter broken chromatid will possess a duplication besides a deficiency. Several such inversions have been investigated in maize (McClintock 1933, 1938b). In several cases, a deficient broken chromosome, presumably derived from crossing over within an inverted segment, has been recovered in the following plant generation.

The breakage of a chromosome at a meiotic anaphase is the starting point in the production of chromosomes with various modifications of size and chromatin constitution. The direct cause of these modifications is related to the subsequent behavior of the broken end. When a chromatid is broken at a
the chromosomes, fusion occurs between the two sister chromatids at the position of the last breakage and a bridge configuration is produced in the succeeding anaphase which is followed by rupture and the inclusion of a chromosome with a broken end in each sister telophase nucleus. If this process continued, each succeeding mitosis would possess an anaphase bridge configuration because each preceding telophase nucleus had received a chromosome with a broken end. The continuation of this breakage-fusion-bridge cycle should produce chromosomes with various deficiencies, duplications and reduplications of segments following non-medial rupture of the bridge configurations in successive anaphases. This subsequent behavior is illustrated in Figure 1. Thus, the production of a dicentric chromatid following crossing-over at a meiotic prophase may initiate a breakage-fusion-bridge cycle. It has been demonstrated that this cycle will continue in all subsequent gametophytic and endosperm mitoses following its origin at a meiotic anaphase (McClintock 1939, 1941a). However, this cycle will cease whenever such a broken chromosome is delivered to the zygote. The broken end heals. This healing is permanent for no further fusions and breakages will occur in the sporophytic mitoses or in any tissues of succeeding plant generations. Because this cycle occurs in the gametophytic divisions (two in the male, three in the female) preceding the formation of the zygote, a wide range of newly organized chromosomes with stable broken ends could be recovered in the sporophytic tissues. These could possess deficiencies of various lengths, duplications of various lengths, deficiencies plus duplications or simple or multiple duplicated segments if the original broken chromosome possessed at least a complete complement of genes of the chromosome.

Although plants heterozygous for an inversion which does not include the centromere produce chromosomes with broken ends at meiotic anaphases following crossing-over within the inverted segment, these inversions usually may not be used to recover chromosomes with modified constitutions because all of these broken chromatids are deficient for a segment of chromatin. In most cases, the deficiency in the genomic complement of the spore receiving a broken chromatid is sufficient to hinder the functioning of the gametophyte arising from it. Consequently, other structural modifications have been used which will produce at meiosis a chromatid with a broken end but with no deficiency of genes of this chromosome. A spore nucleus receiving such a broken chromosome has no deficiency in its genomic complement. A functional gametophyte could be produced from such a spore. Two alterations in the structural composition of chromosome 9 in maize have been used for this study. One is a moderately complex rearrangement of segments composing the chromosome 9 (Fig. 2; a, normal chromosome 9; b, rearranged chromosome 9). In the second case, the chromosome 9 possessed a duplica-

tion of the short arm as shown in Figure 3. In each of these two cases, a dicentric chromatid is produced following a crossover between the normal chromosome 9 and the modified chromosome 9 (d, fig. 2; e, fig. 3). If breakage of the dicentric chromatid occurred at or to the left of the arrow in the diagrams, the broken chromatid to the right would possess at least a complete set of genes for this chromosome. The breakage-fusion-bridge cycle which occurs in the subsequent gametophytic divisions could produce chromosomes with various constitutions by the method illustrated in Figure 1. Thus, chromosomes 9 with the various modifications described above could be delivered to the zygote. A wide range of structurally modified chromosomes 9 have been recovered in the progeny of individuals heterozygous for these two modifications (McClintock, 1941a).

It may be seen that the breakage-fusion-bridge cycle is a particularly favorable means of obtaining
chromosomes with altered sizes and chromatin constitutions. It is not understood why this cycle is confined to the gametophytic and endosperm tissues in the generation immediately following the meiotic origin of the broken end nor why it ceases in the sporophytic tissues and never reappears. The recovered broken chromosome is as permanent in its morphology as any normal chromosome of the complement. The behavior of a chromosome initially broken in the sporophytic tissues is not the same and will be discussed later.

In several dissimilar cases the normal process of crossing-over has been held responsible for alterations in the structural composition of the chromosome. Two of these will be mentioned (McClincock, unpublished). The first case involves the nucleolus chromosome. The appearance of the nucleolus chromosome at prophase is diagrammed in Figure 4. The nucleolus organizer, a deep staining body adjacent to the nucleolus, is responsible for the organization of the nucleolus at telophase (McClincock, 1934). During this process, the segment of chromatid from the organizer to the end of the short arm (the satellite) is removed from the main body of the chromosome by growth of the nucleolus. Although removed some distance from the organizer, it is attached to it by a thread running through the nucleolus substance. If at a meiotic prophase, a chiasma forms between the centromere and the nucleolus organizer and if terminalization of this chiasma proceeds toward the end of the arm of the chromosome, will the terminalizing chiasma stop at the organizer or will it pass through the nucleolus substance to reach the end of the arm? It apparently cannot pass through the nucleolus. The terminalization process either stops at the nucleolus organizer, or, if the force is great enough, the chromatids involved are ruptured at the position of attachment of the organizer to the nucleolus. Following this rupture, fusion occurs at the position of breakage between the nucleolus and the two chromatids involved. Consequently, a dicentric chromosome is formed which results in a bridge configuration at a meiotic anaphase. It will be noted that the segment between the centromere and the

**Fig. 3.** The synaptic association of a normal chromosome 9 (with a large terminal knob) and a chromosome 9 with a duplication of the short arm in the inverted order (no knob present). The clear oval represents the centromere. A crossover as indicated, following the association in a, will produce the dicentric chromosome shown in c. Likewise, a crossover as indicated, following the association shown in b, will produce the dicentric chromosome shown in c. This dicentric chromosome is equivalent to two chromosomes 9 fused at the ends of their short arms. This dicentric chromosome produces a bridge configuration at a meiotic anaphase. If the break occurs at or to the left of the arrow, the broken chromatid to the right will possess at least a full complement of genes of this chromosome. (From McClincock 1941a, through the courtesy of Genetics.)

**Fig. 4 and 5.**

**FIG. 4 (above):** Diagrammatic illustration of the normal nucleolus chromosome in maize. The large circle represents the nucleolus. The small, clear oval (4) represents the centromere. The large, deep-staining body (3) attached to the nucleolus represents the nucleolus organizer. The satellite is represented by 1. Because of the growth of the nucleolus at telophase, the satellite is removed from the nucleolus organizer but remains attached to it by a thread (2) which is in or on the nucleolus itself. This condition is maintained from telophase to the following late prophase. If, in a normal plant, a chiasma forms between the nucleolus organizer and the centromere (between 3 and 4) and if terminalization of this chiasma proceeds toward the end of the arm, obstruction occurs when the chiasma reaches the nucleolus. The nucleolus organizers may be ripped from the nucleolus resulting in breakage of the chromatids at this position. Fusion 2-by-2 then occurs between the broken chromatids at the position of rupture. This produces a dicentric chromatid composed of two chromosomes 6 fused at the distal part of their nucleolus organizers. Breakage of this dicentric chromosome at various positions between the centromeres during the following meiotic anaphases produces chromosomes with various modifications in size and chromatin content.

**FIG. 5 (below):** The nucleolus chromosome in a plant homozygous for a translocation between chromosome 6 and chromosome 5. The description of this chromosome is similar to that given in the legend of Figure 5. The translocation occurred adjacent to the centromere on the short arm of a normal chromosome 6 and toward the end of the long arm of a normal chromosome 5. The centromere of the resulting nucleolus chromosome is located a considerable distance from the nucleolus organizer. Chiasma formation between region 3 (the nucleolus organizer) and region 4 (the centromere) is very frequent in plants homozygous for this translocation. Consequently, the chromatids are frequently ruptured at the attachment of the nucleolus organizer to the nucleolus during terminalization of these chiasmata. Dicentric chromatids are produced following 2-by-2 fusions of ruptured nucleolus organizers.
nucleolus organizer where an effective chiasma could be formed is relatively short in the normal nucleolus chromosome. Relatively few bridge configurations following this process appear at meiotic anaphases. The true nature of these bridge configurations was clearly revealed during a study of meiosis in plants homozygous for a translocation which placed the centromere at a considerable distance from the nucleolus organizer. This translocation chromosome is diagrammed in Figure 5. Chiasma formation is very frequent in the long segment between the centromere and the nucleolus organizer. Many bridge configurations arising from fusions of ruptured nucleolus organizers were observed at meiotic anaphases in these plants. Rapture of the anaphase bridge configurations at various positions between the two centromeres gives rise to chromosomes with variously modified constitutions. As expected, they include various degrees of duplication or deficiency. Their constitutions may be observed readily in the prophase of the following spore divisions. Thus, if chiasmata are the result of crossing-over, as the combined evidence suggests, the normal process of crossing-over may be a factor in the origin of modified chromosomes even when no structural rearrangements are present.

The second case of alteration in the constitution of chromosomes for which crossing-over is held responsible is again related to chiasmata. In several cultures of maize, it has been observed that both the terminal and the interstitial chiasma in all of the chromosomes of the complement are released or unraveled only with considerable difficulty at the first meiotic anaphase. Consequently, as the disjoining centromeres of the bivalent chromosomes pass toward opposite poles, the chromatin between the centromeres and the chiasma is drawn out into a very fine thread. Frequently the tension becomes great enough to rupture these threads before the chiasma has unraveled and a broken, deficient chromosome enters the telophase nucleus.

From the evidence reviewed, it may be seen that the normal process of crossing-over is a means by which chromosomes with altered constitutions are produced.

ALTERNATIONS IN SIZE, FORM AND CONSTITUTION OF CHROMOSOMES FOLLOWING NON-HOMOLOGOUS OR ILLEGITIMATE CROSSING-OVER

Synaptic associations involving non-homologous parts of chromosomes are regularly present in maize when the chromosome complement is heterozygous for some structural rearrangement or when an unbalanced chromosome complement is present. The nature of this association has been extensively investigated (Burnham, 1932, McClintock, 1932, 1933). The synaptic behavior of the univalent chromosome in monosomic plants or of the extra chromosome in trisomic plants may be used as an example. The non-homologous synaptic associations of the univalent at the meiotic prophase are variable but one of the most frequent types of associations is diagrammed in Figure 6. This 2-by-2 association is completely non-homologous. Breakage of the chromatid threads and reunions of the broken ends following an illegitimate crossover at $x - x'$ could give rise to a chromosome with an inverted segment or to a deficient rod-shaped chromosome and anacentric ring-shaped chromosome depending upon the origin of modified chromosomes even when no structural rearrangements are present.

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**FIG. 6. Diagram representing the non-homologous synaptic association of a univalent chromosome. The clear oval represents the centromere. The chromosome is folded upon itself at the mid-region. The association is completely non-homologous. If an illegitimate crossover occurs at $x - x'$ there are two possible consequences depending upon the resulting 2-by-2 fusions of broken ends. These broken ends are designated 1, 2, 3 and 4 in b. The fusion of alternate broken ends, 1 with 4 and 2 with 3, will result in a chromosome with an inverted segment. Fusions of opposite broken ends, 1 with 3 and 2 with 4, will give rise to a deficient rod-shaped chromosome and anacentric ring-shaped chromosome. If the breaks occurred at $y - y'$ in a, fusions of alternate broken ends will give rise to a chromosome with an inversion. Fusions of opposite broken ends will give rise to a deficient ring-shaped chromosome possessing the centromere and a deficient, acentric rod-shaped chromosome.**

or opposite, respectively (b, fig. 6). If the illegitimate crossover occurred at $y - y'$, fusions of diagonal broken ends would produce an inversion while opposite fusions of broken ends would produce a deficient rod-shaped chromosome without a centromere and a ring-shaped chromosome possessing the centromere. With respect to opposite fusions, the deficient rod chromosome could be recovered from the $x - x'$ "crossover" whereas a deficient ring-shaped chromosome could be recovered from the $y - y'$ "crossover." It is interesting to note that just these types of modified chromosomes have appeared in the progeny of trisomic plants. Although no effort has been made to obtain the frequency of these events, the interpretation of their origin as a consequence of illegitimate crossing-over between synapsed non-homologous segments of chromosomes is strengthened by the types of individuals which are occasionally produced in the progeny of plants heterozygous for reciprocal translocations. Heterozygous translocations frequently exhibit extensive non-homologous associations at meiotic prophase. Secondary translocations involving the same
two chromosomes have been recovered from such heterozygous plants. From knowledge of the types of non-homologous synaptic configurations which were known to be present in the parent plant, these secondary translocations may readily be derived on the hypothesis of illegitimate crossing-over.

Haploid plants are characterized by very extensive non-homologous associations. This process may be initiated in some parts of the complement by homologous attractions of unidentified duplicated segments but much of the observed 2-by-2 synaptic association is definitely non-homologous. At the first meiotic anaphase, several of the chromosomes may be associated, 2-by-2, by what appears to be a chiasma. Fragments of various sizes may likewise be present. Both the chromosome associations and the fragments may well arise as the consequence of illegitimate crossing-over between associated non-homologous segments of chromosomes although legitimate crossing-over between homologously associated duplicated segments has not been excluded. As yet, we do not know whether such duplicated segments are present in the complement of maize. A more detailed study of the types of chromosome associations and aberrations at meiosis in the haploid plants or a study of the chromosome complements of the progeny of haploid plants could distinguish between legitimate and illegitimate crossing-over. The legitimate crossovers would be expected to give the same chromosomal rearrangement on a number of independent occasions. On the other hand, illegitimate crossing-over following non-homologous associations would not be expected to occur at the same position on a number of independent occasions. The progeny of haploid plants has not been extensively investigated but it is expected that chromosomes with altered constitutions would appear.

**New Types of Chromosomes Arising from the Aberrant Behavior of a Telocentric Chromosome**

It has been suspected for some time, on good observational evidence, that true telocentric chromosomes—that is, chromosomes with strictly terminal centromeres—normally are not present in the chromosome complements of organisms. From these observations, one could conclude that some aberrant behavior of telocentric chromosomes must result in their elimination from the complement, or that they become modified in such a way that a true telocentric condition no longer exists. In a recent study by Rhoades (1940) the behavior of a strictly telocentric chromosome has been investigated. The suspicion, based on deductive evidence, that telocentric chromosomes are unstable has been confirmed by this investigation. This telocentric chromosome investigated by Rhoades was discovered in a single plant in the progeny of an individual trisomic for chromosome 5. It was composed of a complete short arm of chromosome 5 with the proximal end terminating in the centromere. No chromat in extended beyond the centromere. Both genetic and cytophotometric evidence indicates that the mitotic behavior of the telocentric chromosome is normal in the majority of mitoses. In some mitoses, however, its behavior must be aberrant. Although these aberrant mitoses have not been observed directly, they may be inferred from the genetic behavior and the types of altered chromosomes which are derived from the telocentric chromosome. Plants were obtained with two normal chromosomes 5, each carrying the recessive mutant bm (brown mid-rib, located in the short-arm adjacent to the centromere), and a telocentric chromosome carrying the dominant allele, Bm. Variegation for Bm and bm appeared in some of these plants. It was concluded that this variegation was related to aberrant behavior of the telocentric chromosome which either eliminated the telocentric chromosome from some nuclei or eliminated the Bm locus from this chromosome. In several cases, a bm sector extended into the tassel. This allowed a cytological determination to be made of the chromosome complement of such a sector. In one such case, the observations showed that the telocentric chromosome had been completely eliminated from the nuclei of the sector. In four other such cases, the original telocentric chromosome had undergone considerable modification. In two of these cases, the telocentric chromosome had been modified and reduced to a small fragment with a subterminal centromere. In a third case, a telocentric chromosome was present but its size was only one-half that of the parental telocentric chromosome. In the fourth case, a minute fragment was present composed of only two or three chromomeres and a terminal centromere. Although the observational evidence is insufficient to indicate the methods of origin of these modifications of the original telocentric chromosome, it does indicate that telocentric chromosomes are unstable. They may be eliminated totally from the nuclei or they may produce variants with decidedly altered constitutions.

One recurring type of modification suggests the nature of one type of instability of the telocentric chromosome. When a plant containing this telocentric chromosome in addition to the normal complement is crossed by or onto normal plants, three types of plants are expected in the progeny. These are (1) normal diploids, (2) plants trisomic for chromosome 5 and (3) plants carrying the telocentric chromosome in addition to the normal chromosome complement. Such plants appear in their expected proportions when the female parent carried the telocentric chromosome. Pollen grains carrying either an extra chromosome 5 or the telocentric chromosome rarely function in competition with grains carrying a normal chromosome complement. However, an unexpected type of plant appeared in approximately the same relative proportions in the progeny of these reciprocal crosses. These plants possessed an extra chromosome. This extra chro-
mosome was composed of two short arms of chromosome 5 joined by a single median centromere—a true isochromosome. It is known that pollen grains carrying such an isochromosome in addition to the normal complement do not function in competition with normal grains. However, in the case mentioned, sperm nuclei carrying isochromosomes were delivered to egg nuclei.

It is necessary to explain first the origin of this isochromosome and secondly, how a pollen grain may deliver such a chromosome to the egg nucleus. If one assumed that normal reduplication of the chromatin of the telocentric chromosome occurred in some of the mitoses in the plant carrying the telocentric chromosome, which was accompanied by some form of misdivision of the centromere of this chromosome either in the prophase or in the subsequent spindle figure, an isochromosome in addition to the normal complement could enter one telophase nucleus. Under these circumstances, only the normal chromosome complement could enter the sister telophase nucleus. If this occurred during the division of the microspore nucleus, a generative nucleus carrying an isochromosome and a tube nucleus carrying only the normal complement could be produced. It is assumed that the functioning of a pollen grain is controlled by the constitution of its tube nucleus. Thus, a pollen grain could deliver an isochromosome to the egg nucleus if its tube nucleus possessed a normal chromosome complement and its sperm nuclei carried, in addition, an isochromosome. Such misdivision of the centromere of the telocentric chromosome may be one of the factors responsible for the Bm—bm variegation mentioned above.

The evidence reviewed indicates that the telocentric condition is another factor leading to the production of a wide range of spontaneous chromosome alterations.

The Relation of Chromosome Form to Constancy of Chromatin Constitution

In the previous sections it was pointed out that permanency of the constitution of a chromosome will not be maintained through successive nuclear cycles if the chromosome possesses an unstable broken end or if it possesses a strictly terminal centromere. Extensive and varied modification in the size and genic content of chromosomes arise from these two conditions. There is a third condition which leads to extensive modification of the composition of a chromosome. If a chromosome has the form of a ring rather than a rod, it does not maintain itself unaltered through successive nuclear cycles. Although its form does not change, its chromatin composition is continuously subject to alteration. The ring chromosomes may become enlarged by duplication and reduplication of segments comprising the ring or they may decrease in size by deletions of segments from the ring. Alteration after alteration will occur if the ring form of the chromosome is maintained. A plant possessing a ring-shaped chromosome may be a complete mosaic of altered ring-shaped chromosomes. In some cells and tissues the ring chromosome may be deficient for segments of various lengths. In other cells and tissues, the ring chromosome may possess duplications or reduplications of segments. In still other cells and tissues, the ring chromosome may possess both deficient and duplicated segments. The size of the ring chromosome is no indication of its genic content.

Observations of somatic mitoses have indicated the method by which alterations in the chromatin constitution of the ring-shaped chromosome arise (McClintock, 1932b, 1938a, 1941b). It is related to the mitotic cycle. At some mitotic prophases, the two sister halves of a divided ring-shape chromosome form a continuous, double-sized, dicentric ring chromosome instead of two freely separating, monocentric ring chromosomes (Prophase, fig. 7). This condition could arise subsequent to reduplication of the chromonema of the ring chromosome if a somatic crossover occurred between the two sister chromatids, or the reduplication process itself could lead to this condition. At early anaphase, the two centromeres of the double-sized, dicentric ring chromosome move toward opposite poles of the spindle figure (Anaphase, fig. 7). Tension on the chromatin strands between the two centromeres in late anaphase or early telophase causes them to rupture. The position of rupture is variable. Three such possible positions are indicated by the dash lines a, b, and c, respectively (Anaphase, fig. 7). The subsequent behavior of the broken strands is illustrated in the bracketed figures (lower row, fig. 7) for each of these breakages. Segments of the broken double-sized ring chromosome enter each telophase nucleus but the chromatin composition of the segment in the sister telophase nuclei may differ considerably. In each telophase nucleus, fusion occurs between the broken ends of the segment thus reestablishing the ring form of the chromosome but not necessarily its original chromatin and genic composition (Telophases, fig. 7). It may be seen that ring chromosomes with duplicated segments of ring chromosomes with deficient segments may be produced by this process. Repetition of this process in a later mitosis could give rise to ring chromosomes with reduplicated or multiple segments of the original ring chromosome, to ring chromosomes with still greater deficiencies or to ring chromosomes with deficiencies plus duplicated segments. The frequency of occurrence of these aberrant mitoses depends on the length of the chromonema of the ring chromosome—the longer the chromonema the more frequent the aberrant mitoses. The aberrant mitotic configurations may occur in twenty percent of all mitoses if the chromonema composing the ring is as long as the longest chromosome of the normal complement. If the ring chromosome is only one-tenth of this size, an aberrant mitosis may occur in only one percent of the mitoses. If the ring chromosome
is only one twenty-fifth of the size of the longest chromosome, an aberrant mitosis may occur in only 0.2 percent of the mitoses. In all other nuclear divisions, the behavior of the ring chromosomes is normal; the two sister halves of the ring chromosome separate freely at anaphase along with the rod chromosomes of the complement.

segments of the dicentric ring chromosome might likewise heal and become stable.

**Conclusions**

Permanency of chromosome form and constitution through successive nuclear cycles is a basic postulate of genetic theory. This postulate is well

![Diagram](image)

**Fig. 7. Diagram illustrating a method by which a ring chromosome becomes altered in chromatin constitution.** Upper left: A ring chromosome in a resting nucleus. The clear oval represents the centromere. The individual parts of the ring chromosome are designated by the numerals. Upper middle: A prophase configuration following a "crossover" between the two sister chromatids of the divided ring chromosome. A dicentric, double-sized ring chromosome is produced. Upper right: Appearance of the dicentric ring chromosome in the following anaphase. Breakage of the chromatin strands between the centromeres may occur at any position. Three possible positions a, b and c, respectively, are indicated by the dash lines. The resulting broken strands at late anaphase and the new ring chromosomes formed at telophase by fusions of broken ends of these strands are diagrammed below in the bracketed figures for the breaks a, b and c, respectively. (From McClintock 1941b, through the courtesy of *Genetics*.)

It should be emphasized that fusion of broken ends apparently always follows the breakage of a double-sized, dicentric ring chromosome during an aberrant mitosis in the sporophytic tissues. Although extensively looked for, no cases have been found where the broken ends had failed to unite. Since it has been proved (McClintock, 1941a) that a single broken end which is unstable in the gametophytic tissues may heal and become permanently stable in the following zygote or early sporophyte, it is expected that under certain conditions which at present are not known, the broken ends of the

founded on extensive observational evidence in a wide range of organisms. Knowledge of conditions which will produce changes in this constancy has been of utmost importance in recent years. X-rays, ultraviolet radiation, heat, aging, etc., have been the usual agents producing these desired conditions. It has been known for a long time that other conditions may lead to changes in the form and constitution of chromosomes. When the previous history is not known, the observed changes would naturally fall under the heading of spontaneous aberrations because the conditions responsible for their
occurrence were not apparent. In this discussion, I have attempted to indicate the extent of our knowledge in maize of the conditions which are responsible for such “spontaneous” aberrations. We do know that they may occur (1) under genic control, (2) following legitimate crossing-over, (3) following illegitimate crossing-over, (4) during or following reduplication of the chromonema of the chromosome, (5) as the consequence of the instability of broken ends of chromosomes, (6) during terminalization of chiasmata and (7) as the result of the aberrant behavior of a strictly terminal centromere.

Studies of spontaneous aberrations in maize have contributed and should continue to contribute to our knowledge of the behavior of chromosomes in general. A few such contributions may be summarized. We know from the study of ring-shaped chromosomes that the reduplication process of a chromosome usually occurs along a single plane. The possibility that some sister strand crossover chromatids may be present at meiosis is likewise suggested by these studies for the double-sized dicentric ring chromosomes represent some form of sister chromatid exchange. Although the method of origin of these exchanges is not known, they may be the result of some process which is shared by all chromosomes. If so, the frequency of sister-strand crossover chromatids at meiosis should be directly proportional to the length of the chromosome. However, the process which gives rise to these strands need not be related to the normal process of crossing-over. We are considerably better informed about the stability of broken ends of chromosomes through studies of the types of modified chromosomes which arise following mechanical rupture of chromosomes at meiosis, and following mechanical rupture of dicentric ring-shaped chromosomes at a somatic mitosis. With regard to this, we know that following mechanical rupture of two adjacent chromatids at a late meiotic prophase, fusion may occur between these two chromatids at the position of breakage. However, if these two adjacent chromatids are broken at the first meiotic anaphase, fusions will now occur at the position of breakage between the two sister halves of each of these chromatids. Likewise, if a single chromatid is ruptured at a meiotic anaphase, fusion will occur at the position of breakage between the two sister halves of this chromatid and thus initiate the breakage-fusion-bridge cycle which characterizes the behavior of this chromosome in subsequent gametophytic and endosperm tissues. We know also that fusions may occur between two broken ends of a single chromosome if this single chromosome has suffered mechanical rupture at two points during anaphase of a mitotic division in the sporophytic tissues. From these studies we have determined that a recently broken end of a chromosome is unstable in certain tissues and under certain conditions but may become completely and permanently stable under other conditions. Because of these observations, we know why ring-shaped chromosomes and telocentric chromosomes cannot maintain themselves in nature and thus why they are not frequently encountered. Through the study of the composition of altered chromosomes arising from haploids we may be able to detect the presence of possible duplicated segments in the normal complement of maize which at present are undetected but suspected from genetic evidence. Even the concepts of chiasmata formation and terminalization may be illuminated through the studies of specific chromosome alterations.

It is obvious that the changes in size, form and constitution of chromosomes have made it possible to detect and study some of the processes underlying chromosome behavior in general.

REFERENCES

1937, Cytologia, Fujit Jubilee Vol.43-56.
1940, Amer. J. Bot. 27:149-155.
1938a, Genetics 23:315-376.
1941a, Genetics 26:234-282.
1941b, Genetics 26:542-571.
Nichols, C., 1941, Genetics 26: 89-100.
Perspective: forthcoming


Online: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1078518/
Perspective:¹

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Perspectives on Nobel Laureate Barbara McClintock’s Publications (1926-1984):
A Companion Volume
edited by Lee B. Kass

Perspective:¹


¹ Perspective solicited.
During the past few years, a number of terminal deficiencies of the short arm of chromosome 9 have been isolated. Each deficiency arose as the consequence of a meiotic breakage of the short arm of chromosome 9 following crossing over in plants heterozygous for a chromosome 9 with a duplication of the short arm or for a structural rearrangement of the segments of chromosome 9. In each case, the extent of the deficiency was determined at pachytene in the F1 plants which had received a normal chromosome 9 from one parent and a recently broken (deficient) chromosome from the other parent. Tests showed that deficiencies which ranged from minute to one-third of the distal segment of the short arm were all female transmissible. Those which extended into the first distinct chromosome were transmissible through the pollen. None of the longer terminal deficiencies were male transmissible. Because of the male and female transmission of the very short terminal deficiencies, plants which were heterozygous for these deficiencies were self-pollinated to determine if viable endosperms and embryos could be obtained which were homozygous for these deficiencies. In these F1 plants, the normal chromosome carried $c$ and the deficient chromosome carried $C$. The $C$ mutant is located in the short arm within the 5th or 6th chromosome from the distal end. In these F1 plants, 30 individuals were classified as having received a broken chromosome 9 which was deficient for only the knob. Self-pollinations of these heterozygous deficient plants gave typical ratios of 3 $C$ to 1 $c$. The endosperms and embryos in both classes of kernels were normal. Plants arising from both the $C$ and $c$ kernels were likewise normal in appearance. Cytological
examination of some of these F₂ plants showed the presence of the two
deficient chromosomes 9. It may be concluded that a homozygous deficiency
of the knob does not obviously alter the appearance and functioning of
any tissues.

Seven of the original F₁ plants were classified as having a
chromosome 9 which was deficient for the knob and the adjacent segment
of thin chromatin which joins the knob with the first distinct chromomere.
Self-pollinations of these plants likewise gave typical ratios of 3  recessive to 1  dominant. The endosperms and embryos were normal in appearance. In all
7 cases, the seedlings arising from these kernels segregated in the ratio
of 3 green to 1 pale-yellow. The pale-yellow seedlings are normal in
morphology but die following exhaustion of food supplies in the kernels.
Linkage of the pale-yellow phenotype with G, carried by the deficient
chromosome, was obvious in each case. Through genic and cytological
means, it was possible to determine in each case that the recessive pale-
yellow phenotype is produced as a consequence of the homogygous deficiency.
Intercrosses between plants heterozygous for these 7 pale-yellow mutants
showed that all 7 were either identical or allelic. The recessive
mutant yg2 is known to be located close to the end of the short arm of
chromosome 9. Combinations of a chromosome 9 carrying yg2 with any of the
7 deficient chromosomes 9 produced only normal green seedlings and plants.
It may be concluded that the deficiencies which produce the pale-yellow
phenotype are not long enough to include the yg2 locus.

In six F₁ plants, the broken chromosome 9 was classified as being
deficient for a terminal segment which extended into and included a part
of the first distinct chromomere. These deficiencies were slightly longer
than those which produced the pale-yellow phenotype. Following self-
pollinations of these plants, normal F₂ ratios of 3 recessive to 1  dominant appeared in
four of the six cases and a slight reduction of the recessive class in two of these
cases. When these kernels were germinated, white seedlings segregated in
ratios expected from a recessive mutant. In all cases, linkage of the
white seedling mutants with G was obvious. It was possible to determine
for each case that the white seedling phenotype resulted when these
seedlings were homozygous for the deficient chromosomes 9. Intercrosses
of heterozygous deficient plants of all 6 cultures were made to determine
the allelic relations of the white seedling mutants. White seedlings
segregated in the F₁ following all 15 combinations, indicating that the
white seedling mutants were allelic if not identical. Intercrosses between
plants heterozygous for the 7 pale-yellow producing deficiencies and the
6 white producing deficiencies gave rise to the typical pale-yellow phenotype
in one-fourth of the progeny of all 42 crosses. It was determined that the
pale-yellow phenotype arose following combinations of the two deficient
chromosomes in a zygote. Thus, the deficiency mutants pale-yellow and
white are allelic. Pale-yellow is dominant over white. This would be
expected because the residual homogygous deficiency following combinations
of the two deficient chromosomes is only that which would produce the
pale-yellow phenotype.

Plants heterozygous for the 6 white seedling producing deficiencies
were crossed by plants homozygous for yg2. In the progeny of all 6 crosses,
a ratio of 1 green plant to 1 yellow-green plant appeared. Appropriate tests
showed that the yellow-green plants were those which had received the
deficient chromosome 9 from the heterozygous parent. Therefore, it may be concluded that the white mutants are allelic to \( yg^2 \), with \( yg^2 \) dominant over white. This would be expected if the terminal deficiencies causing the white seedling mutants included the locus of \( yg^2 \). From the point-of-view of genetic analysis, the pale-yellow and white seedling mutants are comparable in all ways to other known recessive mutants in maize. The allelic expressions of pale-yellow and white and \( yg^2 \) and white, and the non-allelic expression of pale-yellow and \( yg^2 \) would be difficult to interpret following a purely genetic analysis. Those results are readily interpretable when the cytological conditions are known. The phenotypic expression following combinations of any two of the three mutants may be considered a reflection of the residual effects of overlapping deficiencies.

The mutants pale-yellow and white are repeatedly produced following the meiotic breakage of chromosome 9. Among 2577 such recently broken chromosomes 9 which were tested, 55 gave rise to the pale-yellow phenotype and 33 to the white phenotype. In contrast to most mutation inducing agents, the chromosomal breakage mechanism is a "mutation" inducing process which "induces" the same mutant time and again.

Barbara McClintock
Perspective:¹

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THE RELATION OF HOMOZYGOUS DEFICIENCIES TO MUTATIONS AND ALLELIC SERIES IN MAIZE

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Reprinted from Genetics: 29: 478-502, September, 1944
THE RELATION OF HOMOZYGOUS DEFICIENCIES TO MUTATIONS AND ALLELIC SERIES IN MAIZE

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Received February 8, 1944

IN PREVIOUS investigations (McCLINTOCK 1938, 1941b), the author has presented evidence that homozygous minute deficiencies of specific regions of chromosomes in maize are responsible for the appearance of readily recognizable modified phenotypes. These phenotypes resemble recessive mutations in their expression. One of them exactly simulated and was allelic to a known recessive mutant (bmr). The evidence obtained from these investigations strongly suggests that one type of mutation process in maize is induced by loss of a minute segment of a chromosome which, when homozygous, produces a distinct phenotypic expression. The present investigation both supports and elaborates this contention in the following way. A particular minute segment was lost from the tip of the short arm of chromosome 9. In plants that were heterozygous for the deficient chromosome, the gametophytes and gametes possessing the deficient chromosome were completely functional. Upon self-pollination of such plants, normal appearing kernels were obtained that were homozygous for the deficiency. The homozygous deficient seedlings arising from these kernels were specifically modified in their phenotypic expression. They were pale-yellow. The pale-yellow mutant, although caused by a homozygous deficiency, is comparable in its genetic behavior to any typical recessive mutant. Its mendelian ratio, its “locus” in the chromosome and its linkage with other known mutants in the chromosome are strictly orthodox. If the presence of the deficiency were not known, the mutation would receive the same consideration as a “gene mutation.” This relationship between deficiency and mutation may be elaborated further. This same segment plus an additional adjacent segment was removed from the chromosome. Even when this longer deficiency is present, the male and female gametophytes and gametes are viable and functional. Thus, individuals homozygous for this deficiency may be obtained. These individuals, in turn, show a phenotypic modification (white seedling) distinguishable from that produced by the shorter deficiency. When the two deficient chromosomes are combined in a zygote, the resulting seedling shows the pale-yellow phenotype associated with the shorter of the two deficiencies. In other words, the two mutants are allelic. The mutant produced by the shorter deficiency is dominant over the mutant produced by the longer deficiency. This might be expected, for the individuals possessing these two deficient chromosomes are homozygous deficient for only the shorter of the two deficiencies, that is the deficiency which produces the pale-yellow phenotype. Combinations of these two deficiency mutants with the previously known recessive mutant yg2 (yellow-green plants; mutant located near the tip of the short arm of chromosome 9) have shown that yg2 is allelic to and dominant
over the mutant produced by the longer deficiency (the white seedling mutant) but is non-allelic to the mutant produced by the shorter deficiency (the pale-yellow mutant). It is the purpose of this paper to show that the pale-yellow and white mutants are caused by specific homozygous deficiencies and to clarify the seemingly anomalous allelic relationships.

The method which produces these specific deficiencies is relatively simple. Thus, similar deficiencies and consequently similar mutations may be independently and repeatedly obtained.

THE METHOD OF OBTAINING TERMINAL DEFICIENCIES OF THE SHORT ARM OF CHROMOSOME 9

The deficiency mutants pale-yellow and white seedlings are associated with losses of terminal segments of the short arm of chromosome 9. The method by which terminal deficiencies arise has been described elsewhere (McClintock 1941a). It may be summarized briefly. Plants which possess a normal chromosome 9 and either a special rearrangement of segments of chromosome 9 or a chromosome 9 with a duplication of the short arm, can produce a dicentric chromatid at a meiotic prophase following specific types of crossing over between the two chromosomes 9. This dicentric chromatid produces a bridge configuration at one of the meiotic mitoses. Following breakage of this bridge, a chromosome 9 with a broken end will enter a spore nucleus. Depending upon where the break occurred within the bridge configuration, this chromosome 9 will be normal in chromatin constitution or will possess either a duplication or a deficiency of the short arm. All spores, except those with the very longest deficiencies, continue to develop. Because the two sister halves of the meiotically broken chromatid are fused at the position of previous breakage, a chromatin bridge is produced at the first spore anaphase as the two centromeres of the dicentric chromatid pass to opposite poles in the spindle figure. This bridge, in turn, will be broken and a newly broken chromatid will enter each telophase nucleus. The original meiotically broken chromosome 9 will continue this breakage-fusion-bridge cycle in the successive gametophytic divisions and also in the successive endosperm divisions whenever such a recently broken chromosome is introduced into the primary endosperm nucleus. However, when a chromosome 9 with such a recently broken end is introduced into the zygote by one of the gametes, the breakage-fusion-bridge cycle usually ceases in the young embryo. The broken end permanently heals and no longer participates in any fusions. The subsequent mitotic behavior of the chromosome 9 with a broken end is similar to that of any normal chromosome. In relatively infrequent cases, the chromosome with the broken end may continue the breakage-fusion-bridge cycle in the young embryo and even into the later developing sporophytic tissues.

When a zygote receives a normal chromosome 9 from one gamete and a recently broken chromosome 9 from the second gamete, the plants arising from these zygotes will have one normal chromosome 9 and, most frequently, one chromosome 9 with a broken but permanently healed end. The chromatin
constitution of this latter chromosome may be one of various types. This is because of its previous history of having been broken at meiosis and then having undergone the breakage-fusion-bridge cycle during the mitoses from meiosis to embryo formation. This chromosome may be normal in constitution or it may possess a duplication or a complex reduplication of segments of the short arm; or, terminal deficiencies or deficiencies plus duplications of segments may be the consequence of this behavior. Kernels which possess such a recently broken chromosome 9 may be identified by genetic means (for details, see McClintock 1941a). The chromatin constitution of the broken chromosome 9 in a plant arising from such a kernel can be determined by examination of pachytene configurations in this plant. The chromatin constitution of the broken chromosome 9 has been determined in over 500 plants which have arisen from such kernels. These plants were classified according to the observed modification in the constitution of the short arm of the broken chromosome 9. In all cases, the broken chromosome 9 contributed by one parent carried a dominant genetic marker in the short arm (C, aleurone color) whereas the normal chromosome 9 contributed by the other parent carried the recessive allele (c, colorless aleurone). The factor C is located in a normal chromosome 9 approximately one-third the distance from the end of the short arm. Although in a plant with unmodified chromosomes 9, more than 20 percent crossing over may occur between the locus of C and the end of the arm, a disturbed ratio of C to c could be expected following self-pollination of those plants which possessed a broken chromosome 9 with a decided modification of the short arm, such as a duplication or a deficiency. This is because the gametophytes with the modified chromosome carrying C might fail to function or might be reduced in functional capacity. Although all plants were self-pollinated to obtain this preliminary information on gametophytic functioning, our attention will be confined to (1) those that were classified as having received a broken chromosome 9 which is approximately normal, and (2) those which received a broken chromosome 9 with a deficiency. The extent of the deficiencies ranged from minute to extensive. All plants classified by pachytene studies as having a broken chromosome 9 with approximately a normal chromatin constitution gave normal ratios for aleurone color following self-pollination. Those classified as having received a chromosome 9 deficient for approximately one to six terminal chromomeres gave aleurone ratios suggesting that transmission of the deficient chromosome through the pollen did not occur, although transmissions through the female gametophyte were either normal or nearly so. (Six terminal chromomeres represent approximately the distal third of the short arm.) This was verified for each deficiency in later and more exacting tests. The majority of those plants classified as having a broken chromosome 9 with a very short terminal deficiency gave little or no evidence from the aleurone ratios of lack of functioning of either eggs or pollen grains carrying the deficient chromosome. It was presumed, therefore, that endosperms and embryos were being formed which were homozygous deficient for small terminal segments of the short arm of chromosome 9. From the
morphological appearance of endosperms or embryos, no distinction could be made, in many cases, between those kernels which were either normal or heterozygous for the deficiency and those which were homozygous for the deficiency.

To determine whether embryos with these homozygous deficiencies would germinate and produce viable seedlings, kernels from these self-pollinated ears were sown. For comparison, kernels from the self-pollinated ears of 30 plants classified as having a newly broken chromosome 9 with no deficiency were sown. All the seedlings arising from this latter group appeared normal. The plants arising from these seedlings were likewise normal in appearance. Some of these plants were examined at pachytene for their chromosome 9 constitutions. Two broken chromosomes 9 were present in some of these plants, indicating that no obvious phenotypic effects were being produced in plants that were homozygous for these broken chromosomes 9. In contrast, a segregation for seedling types occurred in the progeny of self-pollinated plants heterozygous for some of the small deficiencies. The modified seedlings in any one culture were either all pale-yellow or all white and in each culture, the ratio of these seedlings to the normal seedlings suggested a simple recessive mutation. In each case, linkage of the modified seedling type with the dominant aleurone factor C was clearly evident, indicating that the seedling character was associated with the deficient chromosome 9. This evidence suggested that the mutant seedlings, pale-yellow and white, might be produced when the chromosome complement was deficient for a small terminal segment of the short arm of chromosome 9. The following two sections of this paper will elaborate the methods used to verify this association. Seven independent cultures segregating pale-yellow seedlings and six independent cultures segregating white seedlings were selected for intensive study.

THE PALE-YELLOW SEEDLING MUTANTS

The pale-yellow seedlings in all seven cultures were very much alike in appearance. The seedlings appear to be normal in morphological characters and in growth rates. Although chlorophyll is present in the coleoptile, which is light green in color, the leaves show only a light yellowish color. These seedlings die following depletion of essential nutritive reserves in the kernels. The seven independently arising pale-yellow mutants will be referred to as pyd 1 to 7, respectively. (This symbolization implies a pale-yellow phenotype, produced by a deficiency.)

From a purely genetic standpoint, the pyd mutants may be treated as any other recessive mutant in maize. Typical ratios of 3 normal green seedlings to 1 pale-yellow seedling appear in the selfed progeny of plants heterozygous for any one of the pyd mutants (table 1). Linkage with C is shown in table 2. Linkage with yg2 (yellow-green plants), which is known to be located near the end of the short arm of chromosome 9 (Creighton 1934, McClintock 1941a), must be very close; tests of 115 chromosomes derived from plants which carried Pyd yg2 in one chromosome and pyd Yg2 in the homologous chromosome gave
The numbers of green and pale-yellow seedlings which appeared in the progenies of self-pollinated plants heterozygous for a deficient chromosome 9.

<table>
<thead>
<tr>
<th>Source of the Deficient Chromosome</th>
<th>Green Seedlings</th>
<th>Pale-Yellow Seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td>pyd1</td>
<td>1411</td>
<td>445</td>
</tr>
<tr>
<td>pyd2</td>
<td>3404</td>
<td>1148</td>
</tr>
<tr>
<td>pyd3</td>
<td>1214</td>
<td>408</td>
</tr>
<tr>
<td>pyd4</td>
<td>747</td>
<td>253</td>
</tr>
<tr>
<td>pyd5</td>
<td>1505</td>
<td>545</td>
</tr>
<tr>
<td>pyd6</td>
<td>1114</td>
<td>400</td>
</tr>
<tr>
<td>pyd7</td>
<td>1910</td>
<td>636</td>
</tr>
</tbody>
</table>

No chromosomes with Pyd Yg2 or pyd yg2. The reported amount of crossing over between yg2 and C is approximately 19 percent (Emerson, Beadle and Fraser 1935). Estimates from the F2 ratios of table 2 show that the amount of crossing over between pyd and C is similar to that between yg2 and C, although considerable variation between the pyd cultures is obvious. This variation is not considered significant since wide variations occurred between individual progenies within each pyd culture. On purely genetic evidence alone, the pyd mutants would be located near the tip of the short arm of chromosome 9.

**Table 2**

F2 progenies showing linkage of the pale-yellow phenotype with C.

Constitution of the F1: pyd C/Pyd c

<table>
<thead>
<tr>
<th>pyd Culture</th>
<th>C Kernels</th>
<th>E Kernels</th>
<th>% Recombination</th>
</tr>
</thead>
</table>
|             | Kernel    | Seedlings | Number | % Germ. | Pale-Yellow | Number | % Germ. | Pale-Yellow |%
|             | Planted   |           | Planted | % Germ. |            | Planted | % Germ. |            |        |
| pyd1        | 1151      | 96.1      | 361     | 96.1    | 338        | 9       | 16       |            |        |
| pyd2        | 1261      | 99.9      | 376     | 98.2    | 363        | 7       | 14       |            |        |
| pyd3        | 287       | 98.2      | 101     | 73.2    | 73         | 1       | 11       |            |        |
| pyd4        | 719       | 96.9      | 228     | 86.4    | 195        | 2       | 11       |            |        |
| pyd5        | 776       | 95.8      | 284     | 94.7    | 260        | 9       | 18       |            |        |
| pyd6        | 1114      | 96.1      | 361     | 96.1    | 338        | 9       | 16       |            |        |
| pyd7        | 1910      | 95.8      | 284     | 94.7    | 260        | 9       | 18       |            |        |

* The mutant sh (shrunken endosperm) closely linked with c was segregating in some of these cultures. Lowered germination rates are often encountered when the kernels are homozygous sh.

Pachytene examinations of plants heterozygous for any one of the pyd mutants were likewise heterozygous for their chromosome 9 constitutions. In all cases, a normal chromosome 9 and a chromosome 9 with a deficiency of a minute terminal segment of the short arm were present. The short arm of a
normal chromosome 9 usually terminates in a knob composed of heterochromatin. This knob is joined to the first distinct chromomere by a relatively thin strand of stainable chromatin (see diagram a, fig. 2). The knob and this thin strand of chromatin are missing in the deficient chromosome of all plants heterozygous for a pyd mutant (see diagram c, fig. 2). It has been determined that plants which are homozygous deficient for only the knob are quite normal in appearance. Thus, the effective chromatin loss, associated with a mutation to pyd, is presumably confined to the segment which joins the knob and the first distinct chromomere (or to a particular minute region within this segment; see the Discussion). For each case, the exact extent of the deficiency could not be stated with certainty. The segments being examined are too small for such microscopic resolution. Whether a specific pyd deficiency includes a minute segment of the first terminal chromomere or whether a minute proximal segment of the strand joining this chromomere with the knob is present could not be determined. However, in all pyd cultures, there is no question of the presence of a terminal deficiency which includes most of the strand joining the first chromomere with the knob.

If the homozygous deficiency were responsible for the pale-yellow character, the surviving green seedlings in the progeny of a selfed heterozygous plant would be either homozygous for normal chromosomes 9 or heterozygous for the deficient chromosome 9. Cytological examination of the chromosome 9 constitutions of the surviving green plants showed only these two types. In turn,

**Table 3**

*The cytologically determined chromosome 9 constitutions of the green plants of the various pyd cultures together with the results of tests for the presence or absence of the pyd mutants in each plant* 

<table>
<thead>
<tr>
<th>pyd CULTURE</th>
<th>TWO NORMAL CHROMOSOMES 9</th>
<th>ONE NORMAL AND ONE DEFICIENT CHROMOSOME 9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NUMBER OF PLANTS EXAMINED</td>
<td>SEGREGATED</td>
</tr>
<tr>
<td>pyd1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>pyd2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>pyd3</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>pyd4</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>pyd5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>pyd6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>pyd7</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>34</td>
<td>0</td>
</tr>
</tbody>
</table>

* Presence of the pyd mutant detected by one or more of the following methods: selfing, crosses to plants heterozygous for long terminal deficiencies of the short arm of chromosome 9, sib-crosses and intercrosses to various plants heterozygous for pyd or wd mutants (see text for elaboration of these methods).
if these latter plants are appropriately tested for the presence of the *pyd* mutant in one of their chromosomes 9, only the plants heterozygous for the deficiency should give rise to pale-yellow seedlings whereas those having two normal chromosomes 9 should not segregate any pale-yellow seedlings. This was found to be true in all subsequent progenies of cultures carrying the pale-yellow mutants. This evidence is summarized in table 3. Among the 151 plants cytologically examined, the 34 which possessed two normal chromosomes 9 gave rise only to normal green seedlings whereas all of the 117 plants which possessed a normal and a deficient chromosome 9 segregated pale-yellow seedlings.

To obtain further evidence for the association of the pale-yellow phenotype with a homozygous deficient condition, pollen from heterozygous deficient plants of each of the seven *pyd* cultures was placed upon silks of plants heterozygous for only female transmissible terminal deficiencies of the short arm of chromosome 9. These terminal deficiencies ranged in length from one chromomere to six chromomeress. If *pyd* were associated with a minute, terminal, male and female transmissible deficiency, pale-yellow seedlings should appear in the progeny of all such crosses following zygotic combinations of the two deficient chromosomes 9. In no case would the deficient chromosome contributed by the female parent cover the deficiency in the chromosome contributed by the male parent. This proved to be true (table 4).

The similarity in phenotypic appearance and location in the chromosome, as shown by linkage relations with *yg*2 and *C*, of all seven independently arising

### Table 4

Phenotypic appearance of plants with the short terminal deficiency of the *pyd* and *wd* cultures and a longer terminal deficiency; *py* represents pale-yellow seedlings, *w* represents white seedlings

<table>
<thead>
<tr>
<th>SOURCE OF DEFICIENT CHROMOSOME FROM ♂ PARENT</th>
<th>APPROXIMATE EXTENT OF TERMINAL DEFICIENCY OF THE SHORT ARM OF THE CHROMOSOME 9 CONTRIBUTED BY THE FEMALE PARENT</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>pyd</em>1</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>pyd</em>2</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
</tr>
<tr>
<td><em>pyd</em>3</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
</tr>
<tr>
<td><em>pyd</em>4</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
</tr>
<tr>
<td><em>pyd</em>5</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
</tr>
<tr>
<td><em>pyd</em>6</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
</tr>
<tr>
<td><em>pyd</em>7</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
<td>py</td>
</tr>
<tr>
<td><em>wd</em>1</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td><em>wd</em>2</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td><em>wd</em>3</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td><em>wd</em>4</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td><em>wd</em>5</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td><em>wd</em>6</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
</tr>
</tbody>
</table>
pyd mutants, together with a similar extent of deficiency in the chromosome 9 associated with each mutant, suggested that all seven pyd mutants were the expression of one and the same causal condition. If this were so, then combinations of any two of the seven pyd mutants should produce the pale-yellow phenotype. Intercrosses between heterozygous deficient plants of all seven cultures were made. Pale-yellow seedlings segregated in the expected ratios in the

![Diagram](image)

**Fig. 1.—**The phenotypic appearance of seedlings following combinations of all seven pyd mutants (upper triangle), of all six wd mutants (triangle to lower right) and of all seven pyd mutants with all six wd mutants (central rectangle). The symbols py and w in the small squares represent pale-yellow and white seedling phenotypes, respectively.

progeny of all 21 possible combinations (fig. 1). For economy of space, the ratios of green to pale-yellow seedlings in the progeny of the 21 combinations have not been included in tabular form. However, all gave typical 3:1 ratios. These crosses established the iso-allelic if not identical nature of all seven pyd mutants. (Iso-alleles are defined by Stern and Schaeffer (1943) as alleles indistinguishable except by special tests.)
THE WHITE SEEDLING MUTANTS

The six white seedling mutants are readily distinguishable from the pale-yellow mutants. The coleoptile in some cultures is very slightly tinged with yellow color whereas in other cultures it is chalk-white. The leaves are either chalk-white or slightly tinged with a very faint yellow color. Although the general morphological form of these white seedlings appears to be normal, they are always smaller than their sister green seedlings of the same age. The six white seedling mutants will be referred to as \textit{wd1} to \textit{wd6}, respectively. This symbolization refers to the white phenotype produced by a deficiency.

The plants which segregate white seedlings in the six \textit{wd} cultures are heterozygous for a terminal deficiency of the short arm of chromosome 9. These deficiencies are longer than those associated with the \textit{pyd} mutants. They include not only the knob and the chromatin thread connecting the knob with the first distinct chromomere, as in the \textit{pyd} mutants, but in addition a part of the first distinct chromomere is missing (see diagram d, fig. 2). In each case, it was not possible to determine the exact amount of terminal chromatin that was missing. However, the best preparations indicate that the deficiencies which cause the \textit{wd} mutants extend to about the middle of the first chromomere.

The white seedlings in the progeny of self-pollinated heterozygous deficient plants die following depletion of essential nutritive reserves in the kernels. Cytological examination at pachytene of the chromatin constitution of the chromosomes 9 were confined, therefore, to the surviving green plants. Like

### Table 5

<table>
<thead>
<tr>
<th>CHROMOSOME 9 CONSTITUTION OF TESTED PLANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CULTURE</strong></td>
</tr>
<tr>
<td>\textit{wd1}</td>
</tr>
<tr>
<td>\textit{wd2}</td>
</tr>
<tr>
<td>\textit{wd3}</td>
</tr>
<tr>
<td>\textit{wd4}</td>
</tr>
<tr>
<td>\textit{wd5}</td>
</tr>
<tr>
<td>\textit{wd6}</td>
</tr>
<tr>
<td>Totals</td>
</tr>
</tbody>
</table>

* See footnote, table 3.
the *pyd* mutant cultures, only plants which were homozygous for normal chromosomes 9 or heterozygous for the deficient chromosome 9 were found. These plants, in turn, were tested for segregations of white seedlings in their progeny. None of the 36 examined plants with two normal chromosomes 9 gave rise to white seedlings, whereas all of the 93 examined plants which were heterozygous for the deficiency gave rise to white seedlings (table 5). This is to be expected if the white seedling phenotype is caused by the homozygous deficiency.

It was emphasized that the deficiencies associated with the pale-yellow phenotypes gave none of the usual genetic evidences of the presence of a deficiency. Except for the changed chlorophyll condition, all other examined tissues, homozygous for the deficiency, appeared to be normal. In contrast, the homozygous deficiency associated with the white seedling condition reflects the presence of a deficiency in several ways. In the first place, the transmission of the deficient chromosome through the pollen in competition with pollen carrying a normal chromosome 9, is reduced in two of the six *wd* mutants (*wd2* and *wd6*). Indications of this were apparent from the ratios of *C* to *c* obtained following self-pollinations of the heterozygous deficient plants (*C* carried by the deficient chromosome; *c* carried by the normal chromosome) and following backcrosses of these plants to normal plants homozygous for *c* (table 6, I and II). The aleurone ratios obtained from similar crosses involving the other four *wd* cultures did not suggest such selective reduction in pollen func-

**Table 6**

1. Ratios of *C* to *c* following self-pollination of plants heterozygous for the deficient chromosomes 9 of the white-seedling cultures. *C* carried by the deficient chromosome, *c* carried by the normal chromosome.

2. Ratios of *C* to *c* obtained when the pollen of plants in I was placed upon silks of normal plants homozygous for *c*.

<table>
<thead>
<tr>
<th><em>wd</em> culture</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C</em></td>
<td><em>c</em></td>
</tr>
<tr>
<td><em>wd1</em></td>
<td>4296</td>
<td>1416</td>
</tr>
<tr>
<td><em>wd2</em></td>
<td>745</td>
<td>371</td>
</tr>
<tr>
<td><em>wd3</em></td>
<td>925</td>
<td>288</td>
</tr>
<tr>
<td><em>wd4</em></td>
<td>1761</td>
<td>588</td>
</tr>
<tr>
<td><em>wd5</em></td>
<td>387</td>
<td>276</td>
</tr>
<tr>
<td><em>wd6</em></td>
<td>2545</td>
<td>1146</td>
</tr>
</tbody>
</table>

- More exact tests of the gametophytic transmissions (see page 491 and table 10) have shown that the transmission of the deficient chromosome through the female gametophyte is normal for all six white seedling-producing deficiencies and is normal through the pollen for *wd1*, 3, 4 and 5. However, in competition with normal pollen, the functioning of pollen carrying the deficient chromosome is reduced in the *wd2* and *wd6* cultures. The percentage reduction is approximately the same in each case. The pollen utilized had equal
numbers of normal and deficient grains. However, only one deficient pollen grain effected fertilization for every two normal grains. Although white seedlings appear when all six deficiencies are homozygous, it is to be expected from the method of origin that all six of these independently arising deficiencies need not be exactly alike in the extent of the deficiency (see Discussion). However, they all include the segment of chromatin which, when homozygous deficient, is responsible for the chlorophyll abnormality.

Examination of the kernels derived from self-pollinations of plants that are heterozygous for these deficiencies revealed another character which is consistent with a homozygous deficient condition. In all six cultures, some of the embryos had died during various stages of embryonic development. These embryos were shrunken and discolored and did not germinate. Kernels with such dead embryos could readily be classified. There was no consistency in the proportion of kernels with defective embryos among the self-pollinated ears.

**Table 7**

*Segregation of defective embryos among the C and c kernels derived from self-pollination of plants heterozygous for the deficient chromosomes 9 of the white seedling cultures. C carried by the deficient chromosome, c carried by the normal chromosome.*

| wd culture | C Kernels | | | c Kernels |
|------------|-----------|----------|-----------|
|            | Normal Embryos | Defective Embryos | Normal Embryos | Defective Embryos |
| wd1        | 3553       | 743       | 1395      | 21          |
| wd2        | 710        | 35        | 367       | 4           |
| wd3        | 893        | 32        | 286       | 2           |
| wd4        | 1617       | 144       | 562       | 26*         |
| wd5        | 784        | 53        | 275       | 1           |
| wd6        | 1245       | 300       | 1140      | 6           |

* Twenty-five of these kernels came from two of the six ears counted. Their cause is probably not related to the deficiency in chromosome 9.

within any white seedling culture. On some ears, no such embryos were present whereas on other ears they ranged from a few to approximately 25 percent of the embryos. Linkage of this defective embryo character with the mutant C, carried by the deficient chromosome 9, was obvious in all cases, suggesting that the cause of the defective embryo was associated with the deficiency (table 7).

In the progeny of self-pollinated heterozygous deficient plants, the typical F2 ratio of 3 normal green seedlings to one white seedling is not always present. Sometimes there is a deficiency of the white seedling class. This would be expected if the homozygous deficiency causes death of some but not all of the developing embryos. Only those that survive during embryogenesis could produce white seedlings. Lack of effective germination of some apparently living embryos which are homozygous deficient probably takes place for germination.
rates were definitely reduced in some of these F₂ cultures. Also, within the \( w d₂ \) and \( w d₆ \) cultures, the reduced functioning of the pollen grains carrying the deficient chromosome 9 would tend to lower the percentage of homozygous deficient embryos and thus the proportion of white seedlings in the progeny. This latter factor, which reduces the expected proportion of white-seedlings in the F₂ progenies, is relatively constant whereas the former two factors are highly variable among the individual F₂ cultures.

Since wide variations in the proportion of normal to white seedlings occurred among the individual progeny tests within each white seedling culture, a composite table of these ratios for each of the white seedling cultures does not reveal the association of the reduction in the proportion of white seedlings with any one of the three mentioned causes. In a particular progeny, none, one, two or, in the \( w d₂ \) and \( w d₆ \) cultures, all three factors responsible for the reduc-

### Table 8

\( F₂ \) progenies showing linkage of the \( w d \) mutants with C. Constitution of F₁: Deficient chromosome 9 with C/normal chromosome 9 with c.

<table>
<thead>
<tr>
<th>( w d ) CULTURE</th>
<th>GOOD EMBRYOS</th>
<th>DEFECTIVE EMBRYOS (NO GERMINATION)</th>
<th>TOTAL SEEDLINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( C ) KERNS</td>
<td>( c ) KERNS</td>
<td>( C ) KERNS</td>
</tr>
<tr>
<td></td>
<td>PLANTED</td>
<td>GERMINATED</td>
<td>SEEDLINGS</td>
</tr>
<tr>
<td>( w d₁ )</td>
<td>1631</td>
<td>89.5</td>
<td>1734</td>
</tr>
<tr>
<td>( w d₂ )</td>
<td>420</td>
<td>86.4</td>
<td>303</td>
</tr>
<tr>
<td>( w d₃ )</td>
<td>893</td>
<td>94.0</td>
<td>575</td>
</tr>
<tr>
<td>( w d₄ )</td>
<td>798</td>
<td>91.1</td>
<td>490</td>
</tr>
<tr>
<td>( w d₅ )</td>
<td>784</td>
<td>89.9</td>
<td>505</td>
</tr>
<tr>
<td>( w d₆ )</td>
<td>730</td>
<td>95.3</td>
<td>350</td>
</tr>
</tbody>
</table>

* See footnote, table 7.
† See footnote, table 2.

The association in the expected proportion of white seedlings may be active. This relationship is brought out in table 8 where the ratios for \( C \) and \( c \), the proportion of defective embryos in each class, and the germination rates are considered. To illustrate how the three factors operate individually, the progenies from three selected ears in which only one factor was effectively operating in each case are given in table 9. In this table, a fourth progeny is added in which none of these factors was effectively operating. In this latter case, the expected ratio of 3 normal green to 1 white seedling is apparent.

The association of the white seedling character and the defective embryo condition with a homozygous deficient state can be verified by combining the deficient chromosomes of the white seedling cultures with the various female transmissible deficient chromosomes 9 given in table 4. Plants heterozygous for the female transmissible deficiencies of table 4 were crossed by plants heterozygous for the deficiencies of the six white seedling cultures. Some defective
### Table 9

*F₂ progenies from three individual ears illustrating the three factors which materially reduce the expected proportion of white seedlings; together with the progeny from a fourth ear in which none of these factors was operating. Constitution of F₂: Deficient chromosome g with C/normal chromosome g with c.*

<table>
<thead>
<tr>
<th>FACTOR OPERATING</th>
<th>ALEURONE RATIO</th>
<th>GOOD EMBRYOS</th>
<th>DEFECTIVE EMBRYOS (NO GERMINATION)</th>
<th>TOTAL SEEDLINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C KERNELS</td>
<td>c KERNELS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>% SEEDLINGS</td>
<td>% SEEDLINGS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GERMINATED GREEN WHITE</td>
<td>GERMINATED GREEN WHITE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLANTED</td>
<td>PLANTED</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>ε</td>
<td>258 101</td>
<td>257 98.4 103 60</td>
<td>283 60</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced functioning of pollen with deficient chromosome (from wd culture)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defective embryos (from wd culture)</td>
<td>317 119</td>
<td>226 96.0 210 9</td>
<td>118 97.4 115 0</td>
<td>315 9</td>
</tr>
<tr>
<td>Poor germination (from wd culture)</td>
<td>333 113</td>
<td>312 98.7 200 108</td>
<td>111 97.3 106 2</td>
<td>306 110</td>
</tr>
<tr>
<td>No selective elimination (from wd culture)</td>
<td>314 111</td>
<td>312 98.7 200 108</td>
<td>111 97.3 106 2</td>
<td>306 110</td>
</tr>
</tbody>
</table>

embryos, showing linkage with C, appeared in many of these crosses. In all cases, white seedlings likewise appeared in the progeny (table 4). These results are comparable to the selfed progeny of heterozygous deficient plants within the various white seedling cultures. This could be expected because the gametic combination of the two deficient chromosomes g would give rise to an individual which is homozygous deficient for only the short terminal deficiency of the white seedling cultures. It seems clear, then, that both the defective embryo and white seedling character are the consequence of the particular homozygous deficient state.

As stated previously, yg₂ is known to be located close to the end of the short arm of chromosome g. Combinations of the deficient chromosomes g of the white seedling cultures with a normal chromosome g carrying yg₂ proved to be illuminating. It will be recalled that in the wd cultures the surviving green plants in the progeny of a self-pollinated heterozygous deficient plant are of two types: (1) those with two normal chromosomes g and (2) those with a normal and a deficient chromosome g. When 15 plants of the former type were crossed by plants homozygous for yg₂, the progeny from all 15 crosses gave only normal green plants. In contrast, when heterozygous deficient plants [(2) above] from all six wd cultures were crossed by plants homozygous for yg₂, normal green plants and yellow-green plants appeared in the F₁ progeny. The
ratios in each case (table 10) were those expected if the green plants resulted from the zygotic combination of the normal chromosome 9 from the heterozygous parent with the yg2 carrying chromosome and if the yellow-green phenotype resulted from the combination of the deficient chromosome with the yg2 carrying chromosome. Six of these yellow-green plants were examined at pachytene for their chromosome 9 constitutions. All possessed one normal chromosome 9 and the deficient chromosome 9 of the wd cultures. These and four other yellow-green plants not examined cytologically, were appropriately tested for the presence of the white seedling mutant. The progeny tests revealed the presence of the wd mutant in all ten cases. In turn, none of the 22

Table 10

<table>
<thead>
<tr>
<th>CULTURE</th>
<th>TRANSMISSIONS THROUGH THE ♀ GAMETOPHYTE</th>
<th>TRANSMISSIONS THROUGH THE ♂ GAMETOPHYTE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GREEN</td>
<td>YELLOW-GREEN</td>
</tr>
<tr>
<td>wd1</td>
<td>964</td>
<td>917</td>
</tr>
<tr>
<td>wd2</td>
<td>614</td>
<td>609</td>
</tr>
<tr>
<td>wd3</td>
<td>167</td>
<td>173</td>
</tr>
<tr>
<td>wd4</td>
<td>999</td>
<td>977</td>
</tr>
<tr>
<td>wd5</td>
<td>602</td>
<td>611</td>
</tr>
<tr>
<td>wd6</td>
<td>454</td>
<td>439</td>
</tr>
</tbody>
</table>

cytologically examined green plants possessed a deficient chromosome 9 and none of the green plants segregated white seedlings following appropriate tests. These results indicated that from the point of view of phenotypic expression, the wd mutants are allelic and recessive to yg2.

The yg2 factor was not present in either of the chromosomes 9 of the plants which gave rise to the broken chromosomes. Therefore, it cannot be concluded that yg2 was present in the original broken chromosome unless a mutation to yg2 occurred during the formation of the white seedling-producing deficiencies. As stated previously, the seven pyd mutants were not allelic to yg2. During the formation of these seven deficiencies responsible for pyd, no mutation to yellow-green occurred. The allelic relationships of yg2 and wd is best explained by considering that the deficiencies causing the wd mutants are long enough to include the Yg2 locus. The presence of the yellow-green plants in these crosses is, thus, the expression of a hemizygous condition, no complementary locus of yg2 being present in the deficient chromosomes 9 of the wd cultures.

The allelic relations of yg2 and the wd mutants allowed a convenient means of determining the transmissions of the deficient chromosomes through the
male and female gametophytes for each of the six deficiencies. To determine the transmissions through the female gametophyte, heterozygous deficient plants of the six \( wd \) cultures were pollinated by plants homozygous for \( yg2 \). To determine the transmissions through the male gametophytes (pollen grains), the reciprocal crosses were made. Because there are no viability factors connected with embryo development of the yellow-green phenotype, the ratio of green to yellow-green seedlings is a direct measure of the transmissions of the normal and the deficient chromosomes, respectively. The results are given in table 10. The deficient and the normal chromosomes are equally transmitted through the male and female gametophytes of cultures \( wd1, 3, 4 \) and 5. Equal transmissions occur through the female gametophytes of cultures \( wd2 \) and \( wd6 \) but the transmission of the deficient chromosome through the male gametophyte is definitely reduced. In both cases, approximately one-third instead of one-half of the progeny received the deficient chromosome. The allelic relationship of \( yg2 \) and \( wd \) also allowed a determination to be made of the amount of crossing over which occurs between the mutant \( C \) and the end of the short arm of the deficient chromosome. Reciprocal crosses were made between normal chromosome 9 plants homozygous for \( yg2 \) and \( c \) and heterozygous deficient plants carrying \( Yg2 \) and \( c \) in their normal chromosome 9 and \( C \) in their deficient chromosome 9. The non-crossover chromatids would give rise to (1) colorless kernels, green plants and (2) colored kernels, yellow-green plants. The crossover chromatids would give rise to (1) colorless kernels, yellow-green plants and (2) colored kernels, green plants. Within each white seedling culture, wide variations in crossover percentages were found among the individual progenies. Considerably less variation occurred when the male was the heterogametic parent; the average for all six cultures was 15.2 percent, which is close to the 19 percent previously reported for \( yg2 \) and \( C \).

The similar appearance of the white seedling phenotypes, the presence of defective embryos, the allelic relations with \( yg2 \) and the association with a terminal deficiency of all six white seedling mutants suggested that they might be either identical or iso-allelic. To determine this, intercrosses between heterozygous deficient plants of all six cultures were made. Both defective embryos and white seedlings showing linkage with \( C \) segregated in the \( F_1 \) progeny of all 15 combinations establishing, therefore, their iso-allelic if not identical nature (fig. 1).

The description so far given allows one to draw the following conclusions. The seedling mutant pale-yellow will appear whenever a plant is homozygous deficient for a small terminal segment composed of the knob and the chromatin strand joining the knob and the first distinct chromomere. All such mutants will be allelic if not identical. They will not be allelic to \( yg2 \) but will be very closely linked with this locus. A white seedling mutant will appear whenever a plant is homozygous deficient for this same segment plus a particular part of the adjacent chromomere. All of these white seedling mutants will be either identical or allelic. In contrast to the pale-yellow mutants, all the white seedling mutants will be allelic and recessive to the mutant \( yg2 \). Following these
conclusions, one should expect that the combination of the deficient chromosome which produces pale-yellow seedlings and the deficient chromosome which produces white seedlings would give rise to the pale-yellow phenotype for these seedlings would be homozygous deficient for only the segment associated with the pale-yellow phenotype. To determine this, intercrosses of heterozygous deficient plants of all seven pyd cultures with heterozygous deficient plants of all six wd cultures were made. In all 42 combinations, pale-yellow seedlings segregated in the expected ratios in the F1 progeny (fig. 1). Furthermore, there were no defective embryos regularly appearing in the progeny of these crosses. It is clear then, that the pale-yellow and white mutants are allelic and that the white mutants are recessive to the pale-yellow mutants.


A consistent hypothesis can be formulated to account for the appearance of the pale-yellow and white seedling mutants and their allelic relations with each other and with yg2. This hypothesis considers that the phenotypes pyd and wd are due to homozygous deficiencies, as elaborated in the previous sections. Likewise, it is possible that yg2 may be due to or simulated by a homozygous minute internal deficiency. Whether or not yg2 is due to a homozygous deficiency or a true "gene" mutation is immaterial, however, in the explanation of the allelic relations of these three mutants. To facilitate this interpretation, a diagram, figure 2, has been constructed. The short arm of a normal chromosome 9 carrying Yg2 and terminating in a knob is shown in a, figure 2; b, figure 2, represents a normal chromosome 9 carrying yg2. In a and b the arrow points to the locus of Yg2 and yg2 respectively. In c, a terminal segment is missing. This is the segment which, when homozygous deficient, is responsible for the pale-yellow mutant. It should be noted that this deficient segment does not include the Yg2 locus. In d, a longer terminal segment is missing. It is the segment which, when homozygous deficient, produces the white seedling mutants. It should be noted that this deficiency includes the locus of Yg2. Below and to the left of the diagram is given the phenotypes appearing when a plant is homozygous for any one of these chromosomes. To the right are given the phenotypes produced following combinations of any two of these chromosomes. The normal chromosome 9 with Yg2 (a, fig. 2) covers the recessive mutant yg2 of b, and the deficiencies of both chromosomes c and d. Thus, only green seedlings arise following combinations of this chromosome with any one of the other three. The combination of b plus c gives rise to a green seedling because the yg2 carrying chromosome covers the deficiency in chromosome c whereas the deficient chromosome c carries the dominant allele of yg2. The combination of c and d gives rise to a pale-yellow seedling because the residual homozygous deficiency is only that which produces the pale-yellow phenotype. In the combination b plus d, however, the seedling is yellow-green because the terminal deficiency in chromosome d is covered by chromosome b but chromosome d does not cover the yg2 locus with Yg2 because it is deficient for this locus.
Fig. 2.—a. Diagram of the chromatin organization of the end of the short arm of chromosome 9. The large hatched oval represents the terminal heterochromatic knob. This is followed by a thin chromatic segment which joins the first distinct chromomere with the knob. The small, solid ovals represent the two distal chromomeres. The arrow points to the locus of Yg2. b. Same as a, except that the chromosome carries the locus of yg2 (arrow). c. The end of the short arm of a chromosome 9 deficient for the knob and the segment which joins the knob with the distal chromomere. The locus of Yg2 is marked by the arrow. This deficiency, when homozygous, gives rise to the pale-yellow seedling phenotype. d. Slightly longer terminal deficiency than in c. The locus of Yg2 has been lost. This deficiency, when homozygous, gives rise to the white-seedling phenotype.

The mutants yg2, pyd, and wd give rise to peculiar allelic relationships which might be difficult to interpret were the cytology not known. With regard to dominance, there are two series of descending order: I, green→pyd→wd and II, green→yg2→wd. The white mutants are common to both series but the pyd mutants and yg2 are not allelic.

**The Rate of Production of the pyd and wd Mutants by Recently Broken Chromosomes 9**

It was stated in the introduction that the mutants pyd and wd appeared repeatedly in the progeny of plants which had received a newly broken chromosome 9. As described earlier, each of the seven pyd mutants and each of the six wd mutants described in this paper arose independently from a chromosome 9 which was first broken at a meiotic anaphase. Large numbers of functional male gametes containing recently broken chromosomes 9 may be obtained by
special methods (McCLINTOCK 1943). Because of the breakage-fusion-bridge cycle which these meiotically broken chromosomes undergo in the succeeding gametophytic mitoses, the gametes carrying recently broken chromosomes 9 have various modifications in the constitution of the short arm (see page 480). To obtain some estimate of the proportion of functional male gametes which introduce into the embryo the deficiencies responsible for pale-yellow or white seedlings, the following experiment was performed. The silks of plants that were heterozygous for the longer terminal deficiencies of table 4 (that is, deficient for four or six terminal chromomereres) were pollinated by plants that are producing meiotically broken chromosomes 9. The gametophytes produced by the female parent are of two types, those possessing a normal chromosome 9 and those possessing a long terminal deficiency of the short arm of chromosome 9. Whenever male gametes with recently broken chromosomes 9 are delivered by pollen tubes to these female gametophytes, kernels with morphologically normal endosperms will be produced when the female gametophyte possesses the normal chromosome 9. In contrast, aberrant endosperms will be produced when the female gametophyte possesses the deficient chromosome 9. This is due to the subsequent behavior of the broken chromosome 9 delivered to the endosperm by the male parent. It undergoes the breakage-fusion-bridge cycle (McCLINTOCK 1941a) during endosperm development. This process brings about deletions of segments of the short arm of this chromosome 9 in some cells during endosperm development. Since the chromosomes 9 delivered by the female parent are already deficient for a long terminal segment, the telophase nucleus which receives this newly broken chromosome with a terminal deficiency will be homozygous deficient for a segment of the short arm of chromosome 9. In these nuclei, the extent of the homozygous deficiency may range from minute to the full extent of the deficiency in the chromosomes 9 delivered by the female parent. All of these homozygous deficient cells are viable and capable of multiplication. Cells with the longer homozygous deficiencies produce sectors within the endosperm which are sufficiently aberrant to be readily recognizable (McCLINTOCK 1942). Thus, kernels receiving deficient chromosomes 9 from the female parent and a recently broken chromosome 9 from the male parent may be readily detected and selected from an ear. The embryos of these kernels will have the deficient chromosome delivered by the female parent and the newly broken chromosome delivered by the male parent with the exception of a few cases where hetero-fertilization may have occurred. The chromatid type of breakage-fusion-bridge cycle, which occurs in the gametophyte and endosperm tissues, usually does not occur in the sporophytic tissues. The broken end usually heals in the very young embryo and the broken chromosome is completely normal in its mitotic behavior from then on. If the healed broken chromosome 9 has at least a full genic complement of the short arm of chromosome 9, green seedlings should arise from the embryos of these kernels. If it has a short terminal deficiency either pale-yellow or white seedlings could appear because the cells would be homozygous deficient for the short terminal deficiency. If it has a terminal deficiency much beyond the ex-
tent of the \(wd\) mutants described in this paper, the embryos are expected to be inviable.

From the cross just described, 3287 seedlings were obtained from kernels classified as having received a deficient chromosome from the female parent and a newly broken chromosome from the male parent. Of these seedlings, 77 were pale-yellow and 48 were white. From these results it is concluded that among the viable zygotic combinations, one recently broken chromosome in every 26 had either a deficiency which produced \(pyd\) or a deficiency which produced \(wd\).

These results, together with those already presented for the seven \(pyd\) and six \(wd\) mutants described in the previous sections of this paper, illustrate the repeated occurrence of phenotypically and genetically similar mutants. The described chromosomal breakage mechanism is, then, a “mutation inducing” process which “induces” the same mutation time and again.

**DISCUSSION**

Evidence that some recessive mutations are the consequence of homozygous minute deficiencies has been accumulating in both Drosophila and maize. In Drosophila, the phenotypic characteristics of \(y\) (Ephrussi 1934; Stern 1935; Muller 1935; Demerec 1936; Demerec and Hoover 1936), \(sc\) (Sturtevant and Beadle 1936), \(ac\) (Muller 1935), \(rst^2\) (Emmens 1937; Prokopyeva-Belgovskaya 1939; Pashin 1941), \(w\) (Pashin 1938, 1941) and possibly \(fa\) (Oliver 1937, 1938) in the X chromosome may appear when the + locus of these mutants are missing from the chromosome, that is, when the organism is homozygous deficient, in each case, for a particular minute segment of chromosome. In maize, the appearance of white seedlings as the consequence of a homozygous deficiency of the tip of the short arm of chromosome 9 was first observed by Creighton (1937). This deficiency was internal in that only the proximal part of the knob was included in the deficiency. This deficiency was very minute and it probably included the same segment of chromatin that is responsible for the white seedling phenotypes described in this paper. This deficiency was male and female transmissible and produced white seedlings when homozygous. When combined with \(yg2\), the yellow-green phenotype appeared, indicating that the locus of \(Yg2\) had been included in the deficiency. The genetic behavior of this cytologically similar deficiency duplicated the behavior of the deficiencies causing the white seedlings described in this paper. However, because this stock has been lost, a test for identity could not be made.

A series of recessive mutants associated with homozygous minute deficiencies confined within the limits of a few chromomeres adjacent to the centromere of the short arm of chromosome 5 in maize has been reported previously (McClintock 1941a). One of these deficiencies resembled in all ways and was isoallelic if not identical to a previously known recessive mutant \((bmi)\) which has been located within this segment. It seems reasonable to conclude that one form of mutation is related to loss of a particular minute segment of
DEFICIENCIES AND MUTATIONS IN MAIZE

chromatin or to the inactivation of this particular minute segment. The same character could appear following either condition. However, reverse mutation would not be anticipated following loss of a locus, whereas such a reverse mutation might occur following inactivation of a locus. The \( y \) locus in the \( X \) chromosome of Drosophila may illustrate this distinction. Some mutations to \( y \) may be the consequence of a minute chromatin loss. Other mutations to \( y \) may be due to inactivations for reversions from \( y \) to \( y^+ \) have been reported (Johnston and Winchester 1934; Dubinin and Goldat 1936).

With so few analysed cases available, it is difficult to ascertain the role that homozygous minute deficiencies or inactivations play in the whole mutation process. It seems reasonable to believe that they may play a large part in maize. Within the confines of four chromomeremes adjacent to the centromere of the short arm of chromosome 5, six distinct non-allelic mutants, five of which were color mutants and one of which was a developmental mutant, were distinguished. All these mutants were associated with homozygous minute deficiencies. All were both male and female transmissible (McClintock 1941b and unpublished). Again, in this paper, mutants associated with minute losses of chromatin have been described. These, too, are both male and female transmissible. Since a color change was the factor which made most of these mutants readily recognizable, it is reasonable to conclude that other mutants, not associated with color changes, are being produced as the consequence of homozygous minute deficiencies. There is no reason to believe that the two chromosome regions in maize which have been selected for study are exceptional samples of the whole chromosomal complement. Their selection was merely a matter of chance because of structural abnormalities that had happened to these chromosomes. It was these structural abnormalities that furnished the means for a study of homozygous minute deficiencies.

In this paper, it has been stated that the recessive mutants pale-yellow and white were due to progressive losses of chromatin. The \( pyd \) mutants appeared when the chromatin between the knob and the first distinct chromomere was missing and the \( wd \) mutants appeared when this segment plus an adjacent segment of the first chromomere was missing. The author does not believe that this indicates that the phenotypes pale-yellow and white are due to cumulative effects of the losses described. It is possible that the pale-yellow phenotype is related to loss of a particular locus in the proximal region of the segment which is missing; and that the white phenotype represents the effect of this particular loss plus loss of another particular locus in the adjacent chromomere or loss of only a single locus in this chromomere. The fact that other chromatin is also missing in each case may have little or no relation to the phenotypic expressions of pale-yellow or white. A suggestion that the particular phenotypic characters pale-yellow and white may be due to losses of specific loci rather than cumulative effects of a series of loci, may be seen in the differences between \( wd1, 3, 4, 5 \) and \( wd2 and 6 \) in the transmission of the deficient chromosome through the pollen. The four white seedling mutants in the former case have normal transmissions of the deficient chromosomes whereas the latter
two white seedling mutants have a reduced transmission through the pollen. It is possible that a slightly longer deficiency is present in \( w d 2 \) and 6. However, when homozygous, this added deficiency does not affect the expression of the white seedling phenotype. The white seedling mutants are semi-dwarfed. This reduced growth rate may be due either to a cumulative effect of various homozygous deficient loci or to a specific locus which is not related to the locus whose absence is responsible for the chlorophyll abnormality. Similarly, death of some of the homozygous deficient embryos in the white seedling cultures may be a reflection of the same phenomenon. Internal deficiencies of specific loci within this segment are required to differentiate between these alternatives. Cytologically, it might be difficult to identify such minute internal deficiencies. In this study, it was only because the segments were terminal that it was possible to analyse the extent of the minute deficiencies with any reasonable degree of certainty. If these deficiencies had been internal, a positive conclusion might not have been obtained. This is because, following homologous association of a normal and a deficient chromosome, the chromomeres adjacent to the internal deficiency might frequently be stretched and distorted during the preparation of the sporocytes for microscopic observation. The sporocytes in pachytene are gently pressed to flatten the chromosomes. When no structural heterozygosity is present, homologous chromomeres remain together during this process. If, however, a small internal deficiency were present in one chromosome, the corresponding non-deficient segment in the homologous chromosome might be subject to tension while being flattened. This tension could result in distortion of the form of the chromomeres adjacent to the deficiency in the deficient chromosomes. This would cause difficulty in the determination of the extent of a very small internal deficiency. When the deficiency is terminal, the free ends of the synapsed chromosomes are not subject to this type of distortion so that small terminal deficiencies may be satisfactorily analysed.

In Drosophila, mutations may be associated with homozygous deficiencies, with duplications, with various "position effects," or dominant mutants may appear when various regions of the chromosome are hemizygous (the Minutes, Notchs, etc.). These mutants are not considered as having arisen solely from modifications of a specific locus—a "genic change." When a mutation arises which is not associated with a visible change in a chromosome, it is not possible with our present methods to know whether a minute deficiency or duplication is present, whether inactivation or a molecular change in a so-called gene has occurred, or whether structural alterations giving "position effects" have occurred. This applies to the majority of mutants that have been studied. From the accumulating evidence in maize and Drosophila, it is conceivable that many of these mutants are not caused by "genic changes," if this is construed to mean a molecular change in an isolated unit. It appears to the author that the interchangeable use of the terms "mutant" and "gene" should be avoided in order not to prejudice ones thinking of genic action.

Just as mutations are not always caused by "genic changes" at a specific
locus, so are alleles not always caused by "genic changes" at a specific locus. In this paper, pale-yellow and white behave as alleles and white and yellow-green behave as alleles but pale-yellow and yellow-green do not behave as alleles. The interpretation given in this paper adequately accounts for these allelic expressions. It is not necessary to invoke a "genic change." Whether or not allelic expressions for specific mutants will occur may depend upon the particular modification which gave rise to the mutation in each case. It is possible that the \textit{pyd} mutant is due to a loss of a specific locus and that \textit{wd} is due to loss of another nearby but independent locus, and also \textit{ygz} may be caused by loss of still another independent locus. If this is true, it should be possible to obtain a chromosome with only the \textit{Pyd} locus missing and also a chromosome with only the \textit{Wd} locus missing. No allelic expressions of \textit{pyd} and \textit{wd} or of \textit{ygz} and \textit{wd} would be anticipated following combinations of these chromosomes. It is only because the \textit{pyd} and \textit{wd} mutants described in this paper have relatively large segments of chromatin missing that we are certain to obtain residual deficiencies and thus allelic expressions following combinations of these deficient chromosomes. Thus, whether or not two or more mutants will show allelic relationships may depend upon the particular modification which gave rise to the mutant. Following some modifications, two mutants, \textit{a} and \textit{b}, may show allelic expressions. If, following another modification, the \textit{a} mutant phenotype arises again, this mutant may show an allelic expression with the original \textit{a} mutant but need not show an allelic expression with \textit{b}. Alleles in the Truncate series, the vestigial series and the facet-Notch series in Drosophila may illustrate such variations in allelic expressions.

In Drosophila, the allelic expression of the \textit{sc} (scute) series (for extensive literature citations see \textsc{Goldschmidt} 1938), resembles the allelic expressions of \textit{pyd}, \textit{wd} and \textit{ygz}. Overlapping and residual effects follow combinations of specific alleles. This similarity in allelic expression, however, does not presuppose a similarity in cause. The mutants \textit{ls}^a (spectacle) and \textit{ls}^b (glassy) behave as alleles but this allelic expression disappears following specific types of crossing over (\textsc{Oliver} 1940, 1941). Likewise, in Drosophila, the mutants \textit{S} (dominant star) and \textit{ast} (recessive asteroid), which are 0.02 crossover units apart behave as alleles when carried by opposite chromosomes but this allelic expression disappears when both mutants are carried by the same chromosome (\textsc{Lewis} 1941, 1942). Also, in Drosophila, a deficiency of a particular segment of a chromosome may give rise to a dominant (homozygous lethal) mutant which shows some resemblance to a recessive mutant whose locus is in the deficient segment. When the deficient chromosome and a chromosome with the recessive mutant are combined, the phenotypic expression may be exaggerated form of the recessive mutant. According to \textsc{Bridges} (\textsc{Morgan}, \textsc{Bridges} and \textsc{Schultz} 1938), these mutants are "pseudo-allelic." The term "pseudo-allelic" presupposes a knowledge of some special alteration which accompanies the expression of allelism. When this knowledge is not present no "pseudo" modifies the term "allelic." Various causal factors produce mutations and are responsible for allelic expressions. Mutants giving allelic expressions need not be "located"
at comparable positions in homologous chromosomes and they need not be inseparable by crossing-over. It has been the purpose of this paper to analyze one type of modification which gives rise to mutants that show allelic expressions.

The induction of mutations by various means (X-rays, neutrons, U.V. light, heat, age, moisture content of seeds, etc.) has occupied the attention of many geneticists and has proven highly effective. By none of these agents, however, has it been possible to control the particular mutation which will appear. To this list one can add the method described in this paper which involves the repeated occurrence of breaks that are confined within the limits of a single arm of a particular chromosome. Literally thousands of such newly arising broken chromosomes can be obtained with extraordinarily little effort. Since the chromosome arm involved is relatively short, there is a good chance that among a large number of such breakages, many will occur at approximately the same position. In other words, terminal deficiencies of approximately the same length could repeatedly be produced. It has been shown in this paper that the mutants \(pyd\) and \(wd\) are associated with such terminal deficiencies. Thus, the mutants \(pyd\) and \(wd\) should appear repeatedly in the progeny of plants receiving such newly broken chromosomes. That this is true, has been demonstrated. This broken chromosome method of mutation induction differs from the agents mentioned above in that it repeatedly produces the same mutants. In this respect, it simulates the behavior of the \(Dt\) mutant in maize which repeatedly induces mutations at a particular locus in another chromosome (Rhoades 1938). However, the mutation process in the two cases is altogether different.

**SUMMARY**

A number of individuals were obtained possessing a normal chromosome \(9\) and a chromosome \(9\) whose short arm was deficient for a terminal segment of chromatin. In each plant, the deficient chromosome was introduced by one parental gamete following breakage of the short arm of chromosome \(9\) in the previous meiotic mitosis of this parent. The extent of these deficiencies ranged from minute to the full short arm. The smaller terminal deficiencies were both male and female transmissible. Self-pollination of plants heterozygous for these smaller terminal deficiencies gave rise to kernels with homozygous deficient endosperms and embryos. In any one progeny, the seedlings arising from these kernels were either pale-yellow or white.

Seven of these cultures which segregated pale-yellow seedlings were selected for study. In each case, it was determined that the pale-yellow phenotype was produced when the seedlings were homozygous deficient for a minute terminal segment. The seven pale-yellow mutants were comparable in all ways to typical recessive mutants. All seven independently arising pale-yellow mutants were allelic.

Six cultures which segregated white seedlings were selected for study. In all six cases, it was shown that the white seedling phenotype appeared when the seedlings were homozygous for the deficiency. All six white seedling mutants
were allelic. The terminal deficiencies producing the white phenotype are slightly longer than those producing the pale-yellow phenotype.

Intercrosses of the seven pale-yellow mutants with the six white mutants showed that the two types of mutants were allelic. The pale-yellow mutants were dominant to the white mutants. This could be expected, for the individuals possessing a pale-yellow producing deficiency and a white producing deficiency are homozygous deficient for only the shorter of the two deficiencies, that is, the deficiency which produces pale-yellow.

The seven pale-yellow mutants and the six white mutants were combined with a previously isolated recessive mutant yellow-green 2 (yg2) known to be located near the end of the short arm of chromosome 9. The seven pale-yellow mutants were not allelic to yg2 but all six white mutants were allelic and recessive to yg2. The allelic expressions of pale-yellow and white, of white and yg2 and the nonallelic expression of pale-yellow and yg2 are readily interpretable if it is assumed that the longer deficiency, which produces the white phenotype, included the locus of Yg2 whereas the shorter deficiency, which produces pale-yellow, does not extend to this locus.

The method of origin of these terminal deficiencies in the short arm of chromosome 9 is relatively simple. Large numbers of newly derived deficiencies may readily be obtained. Many of these should be of approximately the same length. Since the mutants pale-yellow and white are due to specific deficiencies, these same mutants should appear repeatedly in the progeny of individuals that receive these newly derived deficient chromosomes. Special tests, conducted to determine this, confirmed this expectation.

LITERATURE CITED

BARBARA McCLINTOCK


1941b The association of mutants with homozygous deficiencies in *Zea mays*. Genetics 26: 547–571.


OLIVER, C. P., 1937 Evidence indicating that facet in Drosophila is due to a deficiency. Amer. Nat. 71: 560–566.


Perspectives on Nobel Laureate Barbara McClintock’s Publications (1926-1984): A Companion Volume
edited by Lee B. Kass

Perspective:¹

Citation: 55. *McClintock, Barbara. 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

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Part III: MOBILE GENETIC ELEMENTS: 

Corn & the Nobel Prize (1946-1987)

In Parts I and II we demonstrated that McClintock’s discovery of “mobile genetic elements” (also termed transposable elements, controlling elements, transposons) was built on cytogenetic investigations begun in 1926. In this section we present perspectives and reprints of McClintock’s studies of maize plants undergoing breakage-fusion-bridge cycles (BFB). By 1946 McClintock had identified a new type of “chromosome breakage variegation.” She named this “chromatin elimination locus” Dissociation (Ds) (1947), and she discovered that it was controlled by another locus she named Activator (Ac). Continued studies of the activity of the “Dissociation-Activator (Ds-Ac) system” [McClintock 1987a p. x; now termed Ac/Ds] led to her initial conclusion that mutable loci were caused by chromatin gains and losses (1948). Subsequently she hypothesized that the primary mechanism responsible for the origin and behavior of these mutable loci was a “transposition of the Ds locus from one location in the chromosome complement to another” (1949, p. 143), and that “Ac behavior is the same as that shown by Ds” (1949, p.147).

By 1950 McClintock presented her novel findings concerning transposition in the Proceedings of the National Academy of Sciences, “a journal with wide readership” (McClintock 1987a, p. x) and at a symposium (1951, published in 1952). Because she received few reprint requests for a second article on the origin of mutable loci, which she published in the peer reviewed journal Genetics (1953a), she decided to disseminate future results as brief reports in the Carnegie Year Books, the Maize Genetics Cooperation Newsletters, invited symposia proceedings, and as “unpublished written accounts” (McClintock 1987b, p. x). By 1954 she reported another mutable system, which she designated Spm for Suppressor-Mutator. Although the action of the Spm element was similar to that of the Ac/Ds two-element system, she did not observe chromosome breaks associated with Spm transpositions. Because of their distinctive modes of regulating the expression of genes (McClintock 1987b, p. xi), she soon called transposable elements “controlling elements” (1956a). When regulation of gene action (expression) was reported in prokaryotic organisms, McClintock published a comparison of her findings in maize with studies on bacterial gene control (1961a). She then resumed her custom of publishing in the Carnegie Year Books and in symposium proceedings (Table I, this volume).

Kass and Chomet (2009, p. 31) offer an explanation of why it took so long for the importance of McClintock’s novel discovery to be recognized by the extensive scientific community. McClintock’s conclusion that genes could move from place to place in the maize genome was accepted, but the concept did not seem broadly applicable until transposable elements were found years later in other organisms, first in bacteria and then in higher organisms. When the universal importance of McClintock’s discovery of “mobile genetic elements” was finally recognized, she was awarded the 1983 Nobel Prize in Physiology or Medicine. McClintock’s discoveries continue to inspire research into the phenomenon of transposition and its effect on genetic control and organismal evolution (Fedoroff 2013).

References:

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1 Perspective solicited.
2 For cross-reference purposes, this is the publication number in the annotated, chronological list of McClintock’s publications (Table I) in the Front Matter.
3 The symbol ② and the header 'Citation' indicate that this McClintock publication is not reprinted herein. However, in this case, an online URL is provided.
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1 Perspective solicited.
Perspective: Transposable controlling elements step out onto the broader scientific stage.

by Clifford Weil, Purdue University

[See also: McClintock’s challenge in the 21st century; Fedoroff, N. 2012 PNAS, 109(50): 20200-20203]

Online: http://www.pnas.org/content/109/50/20200.full


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Online: http://www.pnas.org/content/36/6/344.full.pdf+html?sid=ee5dc218-882a-4cf8-ac2b-2f8849f24e7f
Transposable controlling elements step out onto the broader scientific stage

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Introduction

McClintock’s (1950a) paper is one of her earliest syntheses of the first years of her work on a new interpretation of mutable loci (phenotypically unstable alleles) and their causes. What she had reported in great detail in the Carnegie Institution of Washington Yearbooks (McClintock, 1946, 1948, 1949), read and cited by few people outside the maize genetics community, and described in talks at a small number of conferences, was brought into the more general scientific press for the first time (McClintock 1950). McClintock states that her paper is intended to provide the general nature of her study of these “mutable genes” and some conclusions about the origins of mutable loci and interpretations of heterochromatin as a possible controlling factor (p. 347).

At the time, heritably variegated plants and animals and “mosaic” organisms had been reported for over 300 years and, at Cornell, Emerson (1914) had been characterizing them carefully since his initial description of variegated pericarp in maize, but an explanation for their phenotypes was still unknown. By 1950, the concept of the chromosome was well established and the idea that genes were factors carried on these chromosomes that could be rearranged by recombination and mapped to fixed positions was widely accepted. It remained unclear what a gene actually was or how genes and gene expression were controlled. The prevailing approach at the time was to understand what was happening when a gene at a given location was turned on or turned off, and what the differences in those two conditions might say about how genes were controlled.

McClintock (1950) reports on genes (loci) that showed altered expression (e.g., changes from dominant to recessive aleurone phenotypes). She also suggested factors that might be responsible for those changes: the loci could change their position on the chromosome, gain an ability to break chromosomes, or cause other genes to become unstable. The work is entirely by interpretation of progeny from genetic crosses, and cytogenetic observations of meiotic chromosomes. Given the complexity and sheer volume of the work she summarizes, and that it is a relatively concise report written for a more general scientific audience, this paper is a tour de force that has stood for decades at the center of transposable element biology. It would be another 30 years before the transposable elements, Ac and Ds would be isolated, to begin understanding their mechanisms at a molecular level. After that, a dozen years of work by a dozen labs around the world finally would find molecular validation for nearly every one of the mechanisms McClintock describes in this paper using just her keen powers of observation, deductive reasoning and talents as a geneticist.

Reflections on Mutable Loci

By 1950, McClintock had been puzzling over the nature of mutable loci arising in her maize stocks for several years. Similar sorts of mutable loci had been reported (Muller, 1930; Demerec, 1940); at the time, the most detailed work had been published by Lewis (1950), using Drosophila, on the phenomenon of “position effect variegation.” He and others had proposed that the apparent instability of gene expression they could observe, with patches of mutant and nonmutant tissue present in the same organism, occurred when genes were brought into close proximity with heterochromatin (defined in these early cases only as differentially staining regions of the chromosome visible in the microscope). Ephrussi and Sutton (1944) had then proposed that the variegation was the result of a physical change in the state of the chromosome that altered the expression of the gene. Belgovsky (1946) suggested that “…the differences in characters [variegating genes] display [depended] solely upon differences in biochemical reactions taking place in the immediate vicinity of genes.”
McClintock’s friend and colleague, Marcus Rhoades, had identified an unusual allele of the anthocyaninless (al) locus on chromosome 3 in maize that only showed this instability in the presence of another locus Dotted (Dt) on chromosome 9 (Rhoades, 1938). She herself had found a large number of genetically unstable (or “mutable”) loci arising in stocks she had been studying because of their tendency to undergo chromosome breakage-fusion-bridge cycles, and suspected the two phenomena might be related. A locus on chromosome 9 she had described [Dissociation (Ds)] was capable of breaking the chromosome and initiating breakage-fusion-bridge cycles, but required a second locus, Activator (Ac) to do that (McClintock, 1947). McClintock had the impression that “…the mechanism underlying the phenomenon of variegation is basically the same in all organisms” (McClintock 1950, p. 345), a reiteration of her original thoughts on this occurrence (McClintock 1946, p. 186).

A suggested mechanism for mutable loci in maize

McClintock (1950) grouped her mutable loci into two types, those that required a second factor to show the unstable phenotypes (just as she had observed with Ds), and those that could be unstable on their own. In addition, McClintock made what she called “…a fortunate discovery…” that allowed her to connect her observations on chromosome breakage to her observations on mutable loci. “The time and frequency of the breakage events occurring at this Ds (Dissociation) locus appeared to be the same as the time and frequency of the mutation-producing events occurring at the mutable loci” (citing Carnegie Yearbook nos. 41-48 [1942 -1948], p. 347). She then hypothesized that the same factor was controlling both the chromosome breakage events and the instability at her mutable loci. She had mapped Ac genetically and had shown, the previous year (McClintock 1949), that Ac was able to change its position in the genetic map, moving by “transposition.” Similar to the position effect variegation studies in Drosophila, she concluded that the mutable loci she was observing were not cases of genes being moved next to heterochromatin, as Lewis had described; rather, they might be cases of heterochromatin – Ac or Ds – moving next to genes.

She also classified her mutable loci into types based on the nature of the mutability. One type could go from the mutant phenotype to stable, wild type (or nearly so) expression. A second class also went from mutant to wild-type phenotype but remained unstable, able to go back to the mutant form again. A third class would go from the mutant phenotype to various, stable, quantitative levels of gene expression—a sort of partial reversion event. Finally, she described a fourth class that went from mutant to various quantitative levels of gene expression but that remained unstable. This fourth class could change to still other quantitative levels of gene expression ranging from a return to the mutant phenotype all the way to wild-type levels of expression. The molecular bases of these different types will be discussed below, but it is worth noting here that this represents nearly the full range of transposon-induced phenotypes that have been observed since.

Enzymes acting on DNA, the structure of DNA itself and the molecular details of chromatin had not yet entered the general discussions on variegation. McClintock sought to explain all her observations in a physical sense, attributing her results to a “stickiness” of the heterochromatin and listing several reasons she thought this was the case. Chromatid ends at the site of the Ds breakage event quickly stick to one another (fuse) to create dicentric chromosomes [having two centromeres] and acentric fragments [without a centromere]. In addition, when these elements move, they sometimes delete DNA that is adjacent to them, perhaps because it becomes “stuck” to what McClintock proposed was moving heterochromatin; she does note, however, that sometimes these deletions occur without loss of the element as well. She also observed a variety of rearrangements associated with and having one end at the Ds locus, ranging from translocations to inversions and ring chromosome formation. McClintock seems to suggest that the stickiness of heterochromatin arises as a consequence of breakage, either directly or indirectly, though she never says this outright; once Ds was broken, the sticky nature of the heterochromatin could attach itself (or the DNA adjacent to it) almost anywhere else in the genome, but these events did not seem to occur for heterochromatin all the time.

The last piece of evidence in her list related to a key observation she had made about Ds; its ability to change from something that had a strong tendency to break the chromosome, with all the possible results but rarely produced mutable loci to something that had a tendency to cause mutable loci but rarely broke the chromosome, something she termed a “change in state” (p. 348). With respect to her stickiness idea, she attributes these changes in state to changes in the amount of heterochromatin at the locus: more heterochromatin led to a higher frequency of rearrangements while less heterochromatin produced a higher frequency of mutable loci. One of these changes in state became an important clue.
McClintock describes a transposition event of \textit{Ds} from its position on the short arm of chromosome 9 between the \textit{Waxy} (\textit{Wx}) gene and the centromere to a new position farther out on the short arm of chromosome 9 near the \textit{Colored aleurone} (\textit{C}) gene [p. 349]. The transposition disrupts the function of the \textit{C} gene, turning a purple kernel yellow (or white, depending on the genetic background) and creates an unstable, mutant \textit{c} allele, \textit{c-m1} that can frequently revert back to \textit{C}, easily seen as a purple kernel if \textit{Ds} moves away from the \textit{C} locus and gene function is restored. \textit{Ds} characteristics were associated with the nonfunctioning \textit{c} allele and those \textit{Ds} characteristics were lost whenever the mutant reverted. A second transposition to the \textit{C} locus had a distinctly different phenotype, however. This second allele, which she names \textit{c-m2}, produces variegating kernels, disrupting \textit{C} function again, but this time with spots of purple color on the kernels having various intensities. The \textit{Ds} in \textit{c-m2} was a change in the state of \textit{Ds} as compared to both \textit{c-m1} and \textit{Ds} in its original position near \textit{Wx}. She describes a similar \textit{Ds} insertion creating a mutable locus at \textit{Wx}, \textit{wx-m1}, again with revertant sectors of kernel tissue having various levels of restored \textit{Wx} activity, which she could gauge easily by staining the kernel with iodine to look at starch. \textit{Wx} cells containing amylose starch stain blue while \textit{wx} cells with no amylose stain red; running the kernels under some hot water removes the red color from cells with no amylose starch but the blue color remains in cells that have amylose starch. Differences in the intensity of the blue color are correlated with different amounts of amylose starch and, accordingly, differences in \textit{Wx} activity (see Wessler et al., 1986).

All of the \textit{Ds} events McClintock (1950) described required the presence of a second factor, \textit{Activator} (\textit{Ac}), inherited as a single gene. Without it, \textit{Ds} was completely stable. McClintock’s \textit{Ac} also had the ability to transpose from place to place, it could create mutable loci on its own and, regardless of where \textit{Ac} was in the genome, it could cause \textit{Ds} to be active. She hypothesized that \textit{Ac} was also comprised of heterochromatin, and that it was responsible for producing the stickiness of the heterochromatin that might be at the basis of what she was observing. \textit{Ac} also had its own quirks. In particular, McClintock noted that the higher the genetic dosage of \textit{Ac}—one copy in a heterozygous plant, two in a homozygous plant and either one, two or three possible in triploid endosperm tissue depending on whether it had been introduced through the male, the female or both parents—the later in development transpositions seemed to occur. She assessed this timing by observing the size of revertant sectors on kernels. Larger sectors arose early in kernel development, producing, in the \textit{c-m2} example, a revertant, purple cell that then gave rise to a larger proportion of the eventual cells in the kernel; smaller sectors arose later, when there were fewer cell divisions left before the kernel finished development. As will be discussed below, this control of one locus by another and the capacity to regulate that control (in this case by \textit{Ac} copy number) became a central part of McClintock’s future studies.

Finally, with due acknowledgement of the \textit{Drosophila} work, of additional position effect studies in \textit{Oenothera}, and of Rhoades’ work on \textit{Dt}, McClintock turns to a discussion of what these elements are made of. \textit{Dt} is located in or very near the heterochromatic knob near the telomere of the short arm of chromosome 9, supporting her thought that heterochromatin was the source of genetic instability. But all stocks that had the heterochromatic knob on chromosome 9 did not have mutable loci or a tendency to develop them. Was there something that made the \textit{Dt} version different and, if so, what was it? McClintock hypothesized that chromosome breakage and the breakage-fusion-bridge cycles that followed it, might trigger the change in heterochromatin that led to mutable loci.

She then describes experiments using material she had developed nearly a decade earlier (McClintock, 1941, reprinted this volume) using a line with a rearrangement on the short arm of chromosome 9 that produced chromosome breaks, and that also carried the \textit{a1} allele on chromosome 3. She had gotten the \textit{a1} allele from Rhoades, but it had been completely stable in her crosses for years. She crossed this line to a line that had the same \textit{a1} allele, had a heterochromatic knob on the end of the short arm of chromosome 9 but had no \textit{Dt}; McClintock is quick to point out that she had never obtained the \textit{Dt} allele or used it in any of her crosses, so there was no chance of contamination. Her idea was to generate lines that had both the knob and a newly generated chromosome break to ask whether this caused the \textit{a1} locus to become unstable and generate purple, \textit{A1} revertants. Such a result would suggest that the chromosome break had altered the heterochromatin in the knob, in effect recreating \textit{Dt} or something like it. As she had predicted, McClintock found kernels that had purple sectors, ranging from those with one small spot to kernels that were every bit as spotted as with \textit{Dt}.

The paper closes with the question of why an alteration in heterochromatin might be expected to have these various effects. The role of heterochromatin in the cell was largely unknown at that point in time, and McClintock refers to studies suggesting it controlled exchange of material between the nucleus and the cytoplasm and that perhaps
these were changes in the rate of those exchanges and the types of material being exchanged (p. 354). She raises more questions than she answers, and even concedes that she may be taking her speculations on heterochromatin a little too far, but adds that the striking similarities amongst position effect variegation, $D_t$, heterochromatic knobs and her own observations of $Ac$ and $Ds$ are unlikely to be a coincidence.

**Discussion**

By the time her 1950 *PNAS* paper came out, McClintock had been puzzling over the quite unexpected observation that individual loci could transpose from place to place without the apparent need for large-scale chromosome rearrangement for nearly six years. There are several different facets of her work brought onto the broader stage with this paper, including transposition, transposable element changes in state, mutable loci, regulation of one genetic element by another, the capacity of these regulatory elements to move from place to place in the genome, potentially regulating a variety of genes and a proposed role for heterochromatin in all these things. The discussions by McClintock about a broader role for mobile “controlling elements” in normal development that grew out of results published in this paper, and that precipitated a great deal of controversy, did not take full flight until the following several years.

In 1950, the part of the story involving transposition (assessed by changes in genetic linkage of $Ac$ and $Ds$ to various well-known mutations) was quite solid. The control of $Ds$ by $Ac$ was equally solid. The link between these elements and mutable loci, and the capacity to change between a predominantly chromosome breaking state (her “state I”) and a predominantly mutable locus-causing state (her “state II”) are key contributions of this paper. Variegation had been known for centuries (Kaibara, 1709) and reports of genetic phenomena and mutants that might begin to explain variegation were also known (Emerson, 1914; Rhoades, 1938; Catcheside, 1947; Lewis, 1950). Not surprisingly then, citation of her examples of and ideas about mutable loci were quick to appear in the literature among those who had seen and were studying similar phenomena (for example, (Sager, 1951; Brink & Nilan, 1952) and her 1950 paper is cited in a number of papers presented the following year at the *Cold Spring Harbor Symposia on Quantitative Biology*. So-called “mutator” lines were known in *Drosophila* (Demerec, 1937), which had unusually high frequencies of mutation, though the means by which these mutations arose were not yet known. The idea that transposition and mobile heterochromatin might be the explanation for such lines began to gain at least some acceptance within two years of her report (e.g., Hinton et al., 1952; Plaut, 1953). Broader acceptance of and understanding of McClintock’s early work, by whom and to what extent will be discussed in detail elsewhere (Kass, in preparation). It is interesting to note here that transposons (transposable elements, mobile genetic elements), as major engines of mutagenesis, seemed to be easier for those thinking about evolution more than about molecular mechanisms.

McClintock’s background as a cytogeneticist had steeped her in the notion that chromosomes were dynamic, moving in the cell, breaking and rejoining. However, at a time when the gene itself was only just coming into focus, the prevailing idea was that, while chromosomes were dynamic, the loci governing traits that were on those chromosomes were not, like passive passengers on a moving train. Position effect variegation, the notion of rearrangements that could bring genes next to heterochromatin to alter their expression as a by-product of the rearrangement, was an extension of that idea. McClintock was able to keep an open mind about those dynamics, however, letting her data lead her to the conclusion that the heterochromatin itself might also be moving around while the genes remained still.

**Molecular Explanations for Transposable Elements**

Explaining these ideas and the data that led to them to students very focused on reducing the gene to its component parts can be difficult. A longstanding comment of students when assigned one of McClintock’s papers in a class is how dense they can be to read. Here, in the somewhat abridged version of the story, McClintock still describes no fewer than 11 different facets of her work. Some understanding of the eventual explanations of these facets is useful here.

**Mutable alleles arising in a stock showing a distinct site on chromosome 9 where chromosome breakage occurred.** Several features of $Ac/Ds$ elements had to be discovered before we understood this initial observation. We now know that the breakage site McClintock reported is a pair of transposons inserted one into the other, later termed a “double $Ds$” (Courage-Tebbe et al., 1983; Weck et al., 1984). The transposons have distinctive “left” and “right” ends with respect to how the transposase enzyme binds them, each end having a characteristic arrangement
of multiple transposase binding sites (Kunze & Starlinger, 1989). With McClintock’s original, double Ds, the two elements were in opposite orientations (Fig. 1). The binding sites have a DNA sequence that can be methylated; as a result, after DNA replication occurs, each site is in a hemimethylated form, with the newly synthesized DNA unmethylated and the older, template strand still methylated. The transposase enzyme binds preferentially to one of the two hemimethylated forms of the site (Kunze et al., 1988). This feature is important, as it means that only one of the two replicated copies is the preferred substrate for transposition. Furthermore it means that for two elements in opposite orientations with respect to one another, this preferred copy of the transposon will be on one chromatid for one of the elements and on the other chromatid for the other element (reviewed in Kunze & Weil, 2002). One of each transposon end, a left and a right, is required for transposition, and the transposase molecules bound to each end interact with one another to bring about transposition. Once the transposon has left, the cell then rejoins the chromosome at the excision site.

All of that information proves necessary to understand what is happening in McClintock’s corn. The mistaken recognition of a left end from one element and the right end of the other, attempting to excise that as a transposon and, more importantly, trying to repair the apparent excision site, creates a dicentric chromosome (Fig. 1; English et al., 1993; Weil & Wessler, 1993; English et al., 1995). That creates the chromosome break, and it always creates the break at that site.

The mutable loci arise from this same stock when one of the two transposons is recognized correctly as an individual element, excised from the chromosome and moved to a new position. The transposed element then acts like a single transposon. It is now clear that chromosome breaking arrangements of Ds (or of Ac) are not required to spawn mutable loci (indeed, they are the vast minority of cases), but they certainly can.

“Changes in state”: the capacity of a chromosome breaking (or State I) Ds to change into an agent of unstable mutations (or State II) Ds. McClintock proposed a change in the state of the heterochromatin, from a more sticky substance and back, as the explanation for differences in whether McClintock proposed that the mutability she observed in maize was caused by heterochromatin, which had recently been associated with genetic instability in Drosophila and in Oenothera. “Position effect variegation” had been described in fruit flies and in evening primrose flowers. Heterochromatin, loosely defined at that point as chromatin that stained differently under the microscope than the rest of the chromosome (the “euchromatin”), could cause varying levels of expression in a locus when that locus was positioned near the heterochromatin. These differences in expression could occur even within different cells of the same individual (Muller, 1930; Dobzhansky, 1936). It was not clear how this was happening, but McClintock tries to bring all these ideas together, thinking that the mutability she describes might have the same explanation. The relationships between transposons and heterochromatin would prove to be much more nuanced and complex, an entire field of study that is still very active 70 years later. We still do not completely understand the precise mechanism of position effect variegation, a dynamic expanding and receding of the heterochromatic state that can reach a gene and silence it or recede and allow it to reactivate. Large blocks of some transposons occur as heterochromatin, and this observation has contributed a great deal to our understanding of such things as centromeres (Presting et al., 1998; Hudakova et al., 2001; Sharma et al., 2013). At the other end of the spectrum small groups of transposon repeats can seed new regions of heterochromatin (Dorer and Henikoff, 1994). Given what was known at the time, McClintock’s hypothesis about heterochromatin was a good one; however, in this particular case, her interpretation turned out to be incorrect, as did her prediction that a physical “stickiness” of the heterochromatin would prove to be important. McClintock also hypothesizes that the Dotted (Dt) element
studied by Rhoades and her transposable elements, $Ac$ and $Ds$, were operating in a similar manner. Both proved to be transposable elements, though she concluded at this point that they were both simply manifestations of the same “heterochromatin.” $Dt$ also turned out to be two element systems, with a transposable element at the a1 locus on chromosome 3 (“rDt”) (Brown et al., 1989) driven by a second transposable element (“Dt”) located near the heterochromatin knob on chromosome 9.

**Autonomous and nonautonomous mutability.** McClintock introduces what we now know are the autonomous Activator ($Ac$) and nonautonomous Dissociation ($Ds$) transposons. Simply put, $Ac$ elements have both the ability to make a transposase enzyme and the binding sites that transposase needs to bind in order to cause transposition. $Ds$ elements have the appropriate binding sites, but not the capacity to make the transposase. In many senses, particularly before the molecular explanation was clear, this is the idea that is hardest to grasp at first. The notion that the genetic instability was a piece of DNA inserted into a gene, and that was capable of moving from place to place had not been thought of, let alone understood. Some of these DNAs can move themselves and can direct other pieces of DNA, which cannot move themselves, to move as well. Remember too that, at the time, the ideas we take for granted now about genes encoding proteins that are diffusible substances and can act at a distance from their point of origin had not been formulated yet.

**Germinal reversion events, in which a gene is restored to function throughout an individual of the next generation.** McClintock is generally associated with describing mutability in somatic cells (e.g., the purple, revertant spots that would arise on developing corn kernels or the green revertant stripes that would arise on yellow-green leaves). However, her earliest work on mutability are reports, some included in the 1950 PNAS paper, of completely revertant kernels. Mutation, or reversion of entire progeny kernels, termed “germinal” events, reflects changes in the gamete(s) that produce the kernel. The same kinds of reversion events that produce spots and stripes, when they arise in cell lineages that ultimately give rise to reproductive tissues and cells that produce gametes, could produce gametes that carry the reversion, and whole plants/seeds that show the revertant phenotype. These observations stood in stark contrast to observations in animals, and eventually helped in understanding that, unlike animals, plants do not set aside a germline early in development.

**Alleles that are unstable become stably restored to function, then once again become unstable.** In molecular terms, these alleles prove to be transpositions of an element from a site within a gene of interest (where it is causing an unstable phenotype) to some nearby site no longer in the gene, such that the gene is restored to function. Subsequently, the gene of interest can be remutated, if the transposon then moves again and reinserts itself into a new site in the gene. This phenomenon turns out to be a valuable way to create a series of alleles for a gene of interest (Brink & Williams, 1973; Moreno et al., 1992; Weil et al., 1992).

**Unstable loci that produce alleles with various levels of restored gene function.** Eventually, $cl$ and $al$ alleles that were only light purple, or $wx$ alleles that restored only light blue staining with iodine would prove to be the result of the DNA repair process used to rejoin transposon excision sites, resulting in nonhomologous end-joining (Saedler & Nevers, 1985). Transposable elements create short duplications of the sequence into which they insert. Once the transposon moves again, the cell repairs the broken site by a somewhat error-prone process that can add or remove bases. These changes can either eliminate gene function entirely, creating reading frame shifts, or result in the addition or removal of amino acids to a protein, altering its activity (Dennis et al., 1986; Wessler et al., 1986). DNA sequence analysis of thousands of transposon footprints since then has validated this idea. Ultimately, this same process of excising a DNA sequence, error prone repair of the site from which the excision took place, and the sequence variability it leads to, was found to account for a lot of the diversity in the vertebrate immune system (Lieber et al., 1988; Gellert, 1992; Schatz et al., 1992).

**The negative dosage effect of $Ac$, in which increasing copies of the $Ac$ element result in fewer and later transposition events.** McClintock observed that, whatever the Activator proved to be, increasing its genetic dosage seemed to decrease its activity. Making it homozygous in a line showed fewer revertant sectors than having it as a heterozygote, while having it absent altogether stopped any unstable behavior. Indeed, in maize, having a triploid endosperm, the range of genetic dosages was expanded to zero, one, two or three. Several theories about how this “negative dosage effect” occurs have been tested. Perhaps the best idea to date is that there is an optimum amount of transposase to have in the cell for maximum transposition (Scofield et al., 1993; Brutnell et al., 1997). Transposase protein molecules interact with one another (Heinlein et al., 1994). Eventually, having too many transposase molecules begins to interfere with the formation of productive transposase/DNA transposition complexes and
the ability of the elements to move is compromised. One aspect of this trait that remains unresolved is whether there is just a difference in the frequency of transposition or whether there is a difference in the timing during development of transposition, as McClintock initially suggests. With increasing doses of Ac, revertant sectors are smaller and less frequent. This may mean they are restricted to later times in development when there are fewer rounds of cell divisions left to produce a revertant sector, or it could mean that there needs to be many more cells dividing with chances to revert before the very low frequency reversion events are observed. For example, the Mutator transposon of maize produces many, very small revertant sectors, suggesting it is a clearer case of control by developmental timing (see Lisch, 2002 for a review).

The capacity of mutable loci (transposon excision) to generate large deletions from the site of insertion, sometimes with loss of the transposon and sometimes not. This observation is, to some extent, an extension of McClintock’s general finding that chromosome rearrangement can be associated with aberrant transposition events, as described earlier. However, the capacity to generate deletions from the point of transposon insertion has proven to be a very useful tool. Deletions of various lengths that start at a fixed position are valuable for understanding promoter function and for generating stable knockout alleles by deleting all or part of a gene. Interestingly, some genetic backgrounds are more proficient at generating deletions from transposon insertion sites than others, even for a specific transposon inserted into a specific gene, suggesting that an interaction between the “host” genome, particularly the DNA repair machinery, and the transposon will prove to be an important part of the mechanism (Witsell et al., 2010). Deletions can sometimes retain the element intact at its original position with the deletion extending in one direction, leaving the potential for repeated rounds of deletion. While less frequent for Ac/Ds elements than for some others, such as Mutator in maize, Tc1 in the nematode Caenorhabditis elegans (Zwaal et al., 1993) or Tol2 in zebrafish (Huang et al., 2013), transposon-induced deletions have been used to generate allelic series starting at insertion sites in both plants and animals.

Differences in phenotype can result when a transposon inserts into different positions within the same gene, e.g., the c1-m1 and c1-m2 alleles. Several reports of this phenomenon have appeared since McClintock’s (1950) initial observation (Brink & Williams, 1973; Coen et al., 1986; Moreno et al., 1992; Weil et al., 1992). The differences appear, in retrospect, to be differences in the importance to gene function of the transposon insertion site. For example, an insertion into a part of a gene that is relatively unimportant for function may disrupt that gene function but, if the transposon excises, virtually any repair product (transposon footprint) that restores the reading frame of the original gene will restore gene activity. In contrast, an insertion into a part of the gene that encodes a functionally important part of a protein (for example, the active site of an enzyme) may be unable to tolerate much alteration and still retain function. Many of the transposon footprints left at such a site will still be non-functional, making it appear as though there has been very little transposon activity (on the basis of the low frequency of revertant sectors).

Conclusion

With the benefit of hindsight, it is striking how much mechanism McClintock understands and predicts correctly long before the advent of the molecular tools to ask the questions directly, and long before the nature of genes, gene expression, the structure and function of DNA or the DNA repair mechanisms that figure into the results of transposition are understood. There are early hints here that she is considering that these events may be related to, even important in, controlling gene expression over the course of development. Interestingly, while the general use of transposons as gene controlling modules is no longer thought to be true, there are many cases where transposon sequences have either brought genes under the control of factors that impact transposon expression or brought genes under the control of promoters within the transposons themselves (Coen et al., 1986; Bai & Brutnell, 2011; Dooner & Weil, 2013; Wang et al., 2013, and see Schulman & Wicker 2013, p. 34).

References Cited


Kaibara, E. 1709-1715. *Yamato honzo (Japanese Materia medica)* 6 volumes. [republication of an earlier 1672 edition].


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**Fig. 1 Diagram of a state I chromosome breaking arrangement of two Ac/Ds elements in opposite orientation on the same chromosome.**

A) Centromere indicated by black circle. The end of the transposon nearest the 5’ end of the Ac transcription unit within the element is labeled as the left end (“L”) of each element.

B) Following replication, one of the two daughter elements for each copy of the transposon is competent to transpose (shown in black) while the other is not (shown in grey) (see text). Each competent element is on a different chromatid.

C) Transposase bound to the left end of one competent element interacts with the transposase bound at the right end of the other competent element, excises these two transposon ends in an attempt to transpose.

D) Repair of the two “excision site ends” fuses the two chromatids together (indicated by a star), creating a dicentric chromosome once replication is completed, leading to bridge-breakage-fusion cycles.
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Perspective: McClintock and epigenetics: changes in phase of transposition activity.

by Nina V. Fedoroff, King Abdullah University of Science and Technology & Penn State University

[Fedoroff’s perspective provides an overview of McClintock’s Suppressor-mutator (Spm) studies from 1953 through 1971. In 1953, McClintock first described these “units” from their control of genic expression at the a m-1 genetic locus (McClintock 1953b). The following year they were “tentatively designated as Spm” (McClintock 1954).]

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McClintock and Epigenetics: Insights from Changes in the Phase of Transposon Activity

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In the Carnegie Institution Year Book 70, McClintock (1971) wrote:

“It is now well recognized that the genomes of most organisms have many of the same basic operational tools: the same kinds of enzymes, the same cellular structures with the same functions, and the same overall gene products required to build and maintain these structures. The differences between organisms, therefore, must reflect to a large extent differences in regulation of their genomes.”

She went on to summarize and expand on what she called a “change in the phase of activity” of mobile controlling elements, now generally called transposons. In her initial characterization of the Activator (Ac) element, she noted that the Ac locus (as it was then called) could undergo what she called a “change in state” that heritably altered the timing and frequency of the chromosome breaks it induced at the Dissociation (Ds) locus on the distal arm of chromosome 9 (McClintock, 1948). It was not until her studies on the Suppressor-mutator (Spm) element that McClintock recognized and studied a different type of heritable, but reversible, epigenetic mechanism controlling transposon activity (McClintock, 1958). This important discovery has received little attention. The following discussion traces its genetic origins in McClintock’s work and its later molecular explication through the work in my laboratory.

Spm-suppressible alleles

Spm was named for the effect the transposon had in trans [denoting an effect independent of genomic location] on a group of mutant alleles of the A1 locus, designated the a1-m1 alleles (McClintock, 1953, 1954). Expression of the A1 gene is reduced, but not completely inhibited by what is now known to be an insertion of a transposition-defective Spm at an exon-intron boundary within the A1 gene, one of several genes required for anthocyanin pigment production in the kernel’s aleurone layer (Fig. 1A) (Schwarz-Sommer et al., 1985; Schwarz-Sommer et al., 1987). The low-level expression of the A1 gene in the a1-m1 alleles is completely suppressed in the presence of an Spm element elsewhere in the genome in response to what McClintock designated as the suppressor component of Spm (Fig. 1B). We later called this type of allele Spm-suppressible to distinguish it from insertions with a markedly different phenotype, as described below (Masson et al., 1987). The insertion is excised in some cells during development in response to the mutator component of Spm, restoring full A1 gene expression in tissue sectors derived from such cells (Fig. 1, right panel). Members of the series a1-m1 alleles were derived from an initial insertion and differed from each other in both the basal level of expression of the A1 gene in the absence of Spm, as well as in the timing and frequency of reversion to the wildtype level of gene expression. The various alleles, which McClintock designated “changes of state,” were derived from the original isolate and were heritable, like those she reported in the Ac-Ds element family (McClintock, 1956). These are now known to constitute a series of internal deletions in the transposition-defective Spm element (Schwarz-Sommer et al., 1985; Schwarz-Sommer et al., 1987). Subsequent studies on the Spm transposon revealed that the transposon-encoded TnpA protein binds strongly to the ends of the element (Masson et al., 1991; Raina et al., 1993; Raina and Fedoroff, 1995; Raina et al., 1998). Thus it appears a reasonable interpretation that the A1 gene is transcribed, but that the transcript is either inefficiently spliced or unstable, giving a low level of A1 gene expression in the absence of a trans-acting Spm, while transcription is blocked by TnpA binding to the insertion in the presence of an Spm element elsewhere in the genome (Schwarz-Sommer et al., 1985; Schwarz-Sommer et al., 1987)
McClintock subsequently identified similar mutant alleles of the A2 locus (McClintock, 1957). However, her analysis of these alleles was initially confused by the occurrence of an alteration in the trans-acting Spm element in these cultures that altered its “phase of activity,” as well as a “change in state” of the transposition-defective element at the locus that rendered it unable to excise (McClintock, 1958). Both of these alterations proved extremely important in understanding the changes in expression of the Spm element, as well as the communications between elements (McClintock, 1958). In the Carnegie Institution of Washington Year Book 57, McClintock (1958) wrote:

> “By means of various kinds of experiment with this state, it was first learned that the Spm element in the a2-m1 cultures may undergo frequent changes in activity during the development of a plant, each such change affecting its capacity to serve as a suppressor-mutator. Clearly, some regulatory mechanism controls the time of occurrence of such changes, although it is not yet understood…. The class II state of a2-m1 readily reveals these changes in action capacity of Spm, for, with this state, the Spm element in its active phase serves only to inhibit expression of gene action at a2-m1.”

Kernels having the genetic constitution a2-m1 (class II)/a2 (a2 is a stable recessive allele) are intensely pigmented in the absence of an Spm element (Fig. 2A) and colorless in the presence of a fully active Spm (Fig. 2B). Kernels of this genetic constitution vividly reveal changes in expression of the Spm transposon itself. McClintock (1958) wrote:

> “Changes in Spm action phase may alternate, and both the times and the types of change are revealed in the kernel phenotypes. In kernels having one Spm element, these alternating changes may be observed readily. For example, a large pigmented area may be seen in an otherwise colorless region of a kernel. Within this large pigmented area, smaller colorless areas may be observed and within these, in turn, specks of deep pigmentation. In this illustration, the sequence of changes of phase of Spm activity during development of the kernel was from active to inactive to active, and again to inactive.”

The types of pigmentation patterns typical of a2-m1 (class II) state in the absence and presence of a fully active Spm are shown in Figs. 2A and B, respectively, while the variegation pattern observed with a single Spm undergoing changes in its phase of activity during development, which I designated a cycling Spm (Spm-c), is shown in Fig. 2C (Fedoroff, 1983).

McClintock quickly discovered that the kernel pattern of variegation observed depends on the number of Spm-c elements present. By introducing the Spm-c transposon through the male, the female or both, she was able to observe the patterns produced with 1, 2, or 3 elements in the triploid endosperm. She found that the pigmented areas corresponding to the element in an inactive phase were progressively smaller as the number of Spm-c copies increased. This suggests that the deeply pigmented inactive-Spm phenotype requires all of the resident elements to be inactive, while the colorless active-Spm phenotype will result if any one of the elements is active. This observation, in turn, suggests that an active element produces a “suppressor” that acts in trans on the transposition-defective element inserted at the A2 locus to suppress expression of the A2 gene. As noted earlier, it is now known that it is the TnpA protein encoded by the Spm transposon that binds to the element ends (Raina et al., 1998).

McClintock had by this time observed that some plants that showed no evidence of an active Spm in the plant or in the ear produced on the main stalk could occasionally produce a few kernels on tiller ears that have an active Spm phenotype. Moreover, the inactive Spm (Spm-i) could be inherited in the inactive phase through many generations. She then discovered that when an Spm-c was introduced into a plant carrying such an inactive Spm, the kernels that received both the Spm-i and the Spm-c exhibited the variegation pattern expected for multiple copies of an Spm-c. She inferred that the active element could reactivate the inactive element and that the Spm-c and the reactivated Spm-i then underwent inactivation during development independently. This observation suggests that a transposon-encoded protein could act in trans to activate the inactive element. McClintock’s analysis of the
McClintock identified a second Spm insertion mutation in the A1 locus whose behavior was markedly different from that of the a1-m1 and a2-m1 Spm-suppressible alleles (McClintock, 1961). McClintock notes that Spm was inserted very close to the A1 gene in the original allele, but did not completely eliminate gene expression. Kernels were completely unpigmented only when the resident Spm was in the inactive phase (McClintock, 1961). McClintock then identified a number of derivatives that exhibited what she called a “two-element system of control of gene action,” in which a transposition-defective element capable of being mobilized in trans remained at the locus (McClintock, 1962). The phenotypes of one such allele, the a1-m2 7995 allele, are shown in Fig. 3. By contrast to the behavior of the Spm-suppressible alleles, pigmentation resulting from expression of the A gene is not observed in the absence (Fig. 3A), but is observed in its presence of an active Spm elsewhere in the genome (Fig. 3B).

In subsequent years, McClintock collected a number of similar derivatives differing in the level of A1 gene expression and the pattern of somatic reversion (McClintock, 1963). We designated these alleles Spm-dependent in view of the fact that A1 gene expression is observed only in the presence of an Spm elsewhere in the genome (Masson et al., 1987). All but one of the Spm-dependent alleles contained an internally deleted Spm element derived from the original one inserted in the promoter of the A1 gene (Masson et al., 1987; Schwarz-Sommer et al., 1987). The Spm-dependent expression of the A gene in these alleles suggested that the gene had come under the control of the Spm element’s auto-regulatory control system. Subsequent studies revealed that the Spm promoter is co-extensive with a subterminal, internally repetitive sequence immediately adjacent to the transposon’s terminal repeat (Raina et al., 1993). The 12-bp subterminal repetitive elements are the sites that bind the element-encoded transcriptional activator TnpA (Raina et al., 1993). They are present in both orientations and in multiple copies (9 and 15 in the 5’ and 3’ ends of the transposon), hence the ends of the Spm transposon are bi-directional TnpA-dependent promoters (Raina et al., 1993). The ability of the trans-acting element to activate expression of the A1 gene is likely to reflect the operation of the transposon’s positive autoregulatory mechanism.

Presetting

Certain of the a1-m2 alleles had an odd property which McClintock called “presetting” (McClintock, 1963, 1964). While such alleles showed no A1 gene expression when consistently maintained in the absence of an active Spm element (Fig 4A), in some kernels on ears in which the Spm is separated from the insertion allele by genetic segregation, some kernels exhibit continued, albeit irregular expression of the gene (Fig 4B). The Spm element in some way “presets” the gene containing the transposition defective element to continue expressing the gene in its absence. The likely explanation for this phenomenon leads back to the epigenetic mechanism that underlies the “change of activity phase” of the transposon itself.

Molecular analysis of the several a1-m2 alleles in McClintock’s collection identified one that appeared to have an intact element at the locus, designated a1-m2 8167B (Masson et al., 1987). The results of McClintock’s studies on the interaction between active and inactive elements indicated that the active element could trans-activate the inactive one, but she further reported that the activation was transient and that the inactive element reverted to the previous inactive form when segregated away from the active Spm (McClintock, 1959). However, our subsequent studies showed that an active element could heritably activate an inactive one (Fedoroff, 1989) and we were able to stably reactivate the resident Spm at the A locus in the a1-m2 8167B allele by maintaining it in the presence of an active Spm for several generations (Banks et al., 1988). By contrast, no spontaneous activation of this element was observed among several hundred thousand control kernels carrying the a1-m2 8167B allele produced on plants lacking an active Spm.
Molecular analysis of active, inactive and cycling Spm elements revealed that the transposon’s phase of activity was well-correlated with the extent of methylation of a short GC-rich sequence in the first exon just downstream from the transposon’s transcription initiation site (Fedoroff and Banks, 1988; Banks and Fedoroff, 1989). At one extreme, the deeply inactive element in the a1-m2 8167B allele is extensively methylated, while at the other extreme, a fully active element is unmethylated in this region, although the remainder of the element is methylated irrespective of its activity phase (Banks et al., 1988). Elements exhibiting an intermediate level of methylation in the GC-rich first exon sequence can also show regular and heritable patterns in the timing or pattern of activity, although these can also readily give rise to new, heritable patterns of element expression (Banks and Fedoroff, 1989). As noted above, an element in the stably inactive state we called cryptic, such as the one in the a1-m2 8167B allele, does not undergo spontaneous activation, but can be reactivated by an active Spm over several generations (Fedoroff and Banks, 1988; Banks and Fedoroff, 1989). The active state is also highly stable, although less so than the cryptic state and heritably inactive derivatives can be selected over several generations, exhibiting increasing methylation of the GC-rich sequence in each generation (Banks and Fedoroff, 1989). transposons with intermediate levels of methylation are inactive in the absence of a transacting element, but fully active in the presence of a trans-acting fully active Spm. Strikingly, such elements can remain transiently active immediately after segregation of the trans-activating Spm, suggesting an explanation for presetting (Fedoroff, 1989).

We have reported that the Spm transposon also becomes reversibly inactivated in transgenic tobacco plants (Schlappi et al., 1993). Of the two transposon-encoded proteins necessary for transposition, TnpA and TnpD (Masson et al., 1991), only TnpA is necessary to reactivate an inactive Spm in transgenic tobacco. A reporter gene expressed from the Spm promoter is inactivated and methylated if the promoter sequence includes the first exon GC-rich sequence, but not if it lacks the sequence (Schlappi et al., 1994). Moreover, the methylated and inactive promoter can be reactivated and demethylated in the presence of TnpA (Schlappi et al., 1994). Subsequent studies showed that TnpA promotes active demethylation of the promoter (Cui and Fedoroff, 2002). It should be noted that the sequence that undergoes methylation and demethylation is located downstream from the TnpA binding sites in the promoter, hence it is likely that the TnpA does not participate in demethylation directly, but rather recruits demethylating enzymes to the promoter.

In view of these findings, the likely explanation for presetting is that the transposon sequence inserted just upstream from the A1 gene in the a1-m2 alleles does not itself prevent expression of the gene, but does readily attract inactivating methylation to the promoter. The presence of a trans-acting Spm supplies TnpA, which recruits the demethylation machinery to the gene, allowing its expression. However, upon removal of the Spm by genetic segregation, the remaining transposition-defective element in the A1 gene’s promoter is not instantly remethylated, allowing its transient “preset” expression in some aleurone cells of kernels that do not receive an active Spm.

In summary, McClintock’s perceptive analysis of the “changes in the phase” of Spm activity constitutes one of the earliest genetic investigations of an epigenetic regulatory system, only now beginning to be understood at the molecular level (Lisch, 2009). The active negative feedback regulation, now known to be triggered by silencing RNAs derived from transposon transcripts and maintained by DNA methylation, is fundamental to keeping transposon activity in check and maintaining chromosome stability. At the same time, however, the Spm transposon encodes a positive regulatory system which is not only necessary for transposon expression, but is also capable of both overriding and actively reversing the DNA methylation that maintains the transposon in a silent state. All of this molecular complexity was presaged by McClintock’s genetic analysis of maize transposons and their interactions with genes.

References


Perspective:¹


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① Perspective solicited.
1. Spread of Mutational Change Along the Chromosome

When Ds is inserted just to the left of Sh1 in chromosome 9, it can subsequently affect mutational change in genetic materials located to either side of it. These occur only when Ac is also present in the nucleus. These effects appeared following 2 independent insertions of Ds at this location. A total of 56 mutational changes were examined. Seven affected the action of genetic materials located to the left of Ds and including 1, 37 affected the action of Sh1, located just to the right of Ds, and 12 affected the action of both Sh1 and B21, the latter located to the right of Sh1. The origin and general patterns of behavior of some of these mutants were reviewed in recent issues of the Year Book of the Carnegie Institution of Washington. In all 56 cases of mutational change, Ds was present and apparently unaltered in location by the event that produced the mutation. In many of these cases, however, it could be shown that the Ac present underwent a transposition at the time that the mutation-producing change occurred at Ds. Some of the changes affecting only Sh action are unstable in that the recessive mutant, sh, reverts to Sh but only when Ac is also present in the nucleus. These reversions are not accompanied by loss of Ds or by its transposition to a new location. The dominant, Sh, so produced may again mutate to sh, and again, only when Ac is present in the nucleus.
In one of the 12 examined cases of simultaneous change in action of both Sh and Bz (to sh and bz), the bz component of the double mutant proved to be mutable. Mutations to Bz occurred but only when Ac was present. The action of the sh component remained unchanged. It could be shown that Ds was located to the left of the mutable bronze locus and that reversions to Bz were not accompanied by loss or transposition of Ds. No evidence of crossing over within the sh to bz^m interval was obtained. Crossing over to either side of the double mutant was either normal or increased in frequency in comparison with the standard frequency.

In all of the 56 examined cases of Ds initiated mutations, Ds remained unaltered in location. This suggests that loss of Ds from this particular location, following its initial insertion just to the left of Sh, results in some lethal action. Some of these mutants have shown that the effects Ds induces on the action of genetic materials located close to it are not confined to local inhibitions of genic action. Some of the mutational changes that spread some distance along the chromosome not only produce an inhibition of the action of genetic materials within the affected segment, but also give rise to a dominant effect that produces a marked distortion in the morphology of the kernel and plant. Such altered growth patterns do not appear in kernels and plants that are hemizygous for the affected segment.

2. A Case of Ac-induced Instability at the Bronze Locus in Chromosome 9

A case of insertion of Ac at the bronze locus in chromosome 9 has been found that results in instability of action at this locus. It originally appeared in a C sh bz wx carrying gamete produced by a plant that was Ac I Ds Sh Bz Wx/ Ac C ds sh bz wx in constitution when this plant was crossed to one homozygous for Ac, C, ds, sh, bz, and wx. Mutations to Bz occur at this mutable bz locus. They are Ac controlled and the mutational response to doses of Ac is similar to that expressed by other Ac controlled mutable loci—the higher the dose of Ac, the later the time during development of a tissue that mutations occur. Tests to determine the location of Ac were conducted with 135 plants heterozygous for this mutable bz. In all of them, an Ac factor was present and situated close to or at the locus of bz^m. It could be determined that the mutational response to doses of Ac is dependent not only on the dose of bz^m that is present (Ac at the bronze locus) but also on that produced by additional Ac factors located elsewhere.

Several distinctly different phenotypes result from mutation at this bz^m locus. The most common of them gives rise to a Bz expression or to a stable bz expression, the latter occurring about five times more frequently than the former. Some of the mutations to Bz are stable in that no further mutations occur in the presence of Ac. Six such cases were examined and in all 6, Ac was no longer present at the Bz locus. Fourteen cases of mutation to stable bz examined and again, in these cases, it could be shown that the change was associated with removal of Ac from
the bz\textsuperscript{m} locus. In some of these cases, Ac was present in the chromosome complement but located elsewhere, either within chromosome 9 or at a position that gives no evidence of linkage with genetic markers carried in this chromosome. Three additional cases of mutation to Bz were examined. Each of them was characterized by instability of expression of Bz. Mutations to bz or to bz\textsuperscript{m} occurred. An Ac factor was found to be present in each case and located at or close to Bz\textsuperscript{m}. Analysis of the progeny produced by plants carrying one of these Bz\textsuperscript{m} mutants indicated that stability at the Bz locus could arise if Ac were removed from its immediate vicinity.

The Ac element originally present at this bz\textsuperscript{m} locus produced some chromosome breaks. They occurred with rather low frequencies in comparison with those mutations to bz or to Bz that are unaccompanied by gross chromosomal aberrations. However, a state of this Ac at Bz\textsuperscript{m} has appeared that gives rise to many dicentric-forming chromosome breaks and at rates that are comparable to those produced by known states of Ds.

In addition to the events described above that occur at this bz\textsuperscript{m} locus, other types of events also occur but with very much lower frequencies. Two of them have received some examination. Each appeared, initially, in a single gamete produced by a plant having bz\textsuperscript{m} and was detected because of a marked change in the appearance of the kernel produced by functioning of the gamete. In one case, the rate of mutation to Bz was strikingly increased in comparison with that usually produced by this bz\textsuperscript{m}. Tests of the plant arising from this kernel indicated that the mutations were no longer directly initiated by events occurring to Ac at the bz\textsuperscript{m} locus. The Ac factor present in this plant was located elsewhere. The evidence indicates that a two-factor system of mutational control is present and suggests that one of these factors is Ac.

The second type of altered pattern of mutation at the bronze locus was derived from a gamete of a plant that carried Bz\textsuperscript{m} (Ac at the Bz locus) in one chromosome 9 and a normal recessive, bz, in the homologue. The kernel showing the altered mutation pattern had a background coloration suggesting a weakened expression of Bz. Areas were present showing either a weaker or a stronger expression of Bz coloration. In the plant derived from this kernel, 2 Ac factors were present, one located close to this modified Bz\textsuperscript{m} locus, and the other located elsewhere. In the progeny of this plant, the pattern of mutation present in the kernel that gave rise to it was again repeated. However, present evidence is insufficient to indicate the mode of control of mutation.

3. Transposition sequences of Ac

In the study described above, it has been possible to follow transpositions of Ac through several sequential steps. Three of them occurred in the ancestry of the plant that gave rise to the mutable bronze condition. Ac was first present in a plant having the constitution 1 Sh Bz Wx Ds/C Sh Bz wx ds and it showed no linkage with these markers in chromosome 9. It
then appeared in an I Sh Bz Wx Ds carrying chromosome at a position that was approximately 20 crossover units to the right of Ds. I Sh Bz Wx Ds Ac. Ac was then inserted just to the left of I, and coincident with this was insertion of Ds to the left of Sh: Ac I Ds Sh Bz Wx. Ac then appeared at the bronze locus in a gamete of a plant having this last position of Ac, and it produced the mutable condition described above. From this position, in turn, its insertion at several other locations has been determined: to positions not showing linkage with markers in the short arm of chromosome 9, to a position close to sh, and to a position that is very close to wx. The removal of Ac from this last location coincident with its appearance at a new location, not showing linkage with markers in the short arm of chromosome 9, has also been followed.

4. A suppressor-mutator system of control of genic action and mutational change.

Several systems that control genic action and mutational change, other than that of Ds and Ac, are being examined. One of them has received a considerable amount of study and its pattern of behavior is now apparent. It is the system associated with control of genic action and mutation at the \( A_1^{m-1} \) locus. (Designation refers to a mutable condition that arose at the \( A_1 \) locus in the Cold Spring Harbor cultures.) It was originally thought that \( A_1^{m-1} \) was an "autonomous" mutable locus. This now appears not to be true and for reasons that will be apparent. An independently located factor, designated \( S_{pm} \) for Suppressor-mutator, is responsible for the observed behavior of \( A_1^{m-1} \). When this factor is present, anthocyanin development in kernel and plant is suppressed until a mutational change occurs at \( A_1^{m-1} \). These changes give rise to stable mutants distinguishable from one another by different levels of expression of anthocyanin pigmentation in kernel and plant. These range from no pigment formation to the apparent full \( A_1 \) expression. When \( S_{pm} \) is removed from the nucleus, either by a somatic loss or transposition, or by means of meiotic segregations, the \( A_1^{m-1} \) locus can express itself, producing uniformly distributed pigment in both kernel and plant. This expression is stable in subsequent generations as long as \( S_{pm} \) is absent from the nucleus. The degree of this expression varies with the particular state of \( A_1^{m-1} \) that may be present. Strikingly different states of \( A_1^{m-1} \) have appeared, one arising from the other through the influence of \( S_{pm} \) on the \( A_1^{m-1} \) locus. They are characterized by the types of mutation that occur, by the time during development when these occur, and by the type of pigmentation that is expressed in the absence of \( S_{pm} \). This latter ranges from almost none to very intense. When through appropriate crosses, \( S_{pm} \) is returned to the nucleus, the Suppressor-mutator action induces \( A_1^{m-1} \) is again apparent. The types of effects that it will produce are quite predictable if the state of \( A_1^{m-1} \) is known in advance.

The \( S_{pm} \) factor behaves much like Ac in that it occupies a definite position in the chromosome complement but may be transposed to a new position, remaining at the new location until a subsequent transposition occurs. Several different positions of \( S_{pm} \) within chromosome 6, within
chromosome 5 and within chromosome 9 have been found. As long as \textit{Spm} remains in a particular position, it gives clear-cut linkage relations with known factors. These are expressed directly in backcross tests or in progeny tests. It is in the progeny tests, however, that new positions of \textit{Spm} are discovered. Unlike \textit{A}_{1}, \textit{Spm} does not give a sharply defined dose action. Therefore, when 3 or more independently located \textit{Spm} factors are present in a plant carrying \textit{\textit{A}}_{1}^{m-1}, nearly all of the gametes carry one or more of them and, in test crosses, the \textit{A}_{1}^{m-1} locus appears to be "autonomous" in its mutation control. Progeny tests are required to separate the different \textit{Spm} factors and to determine the number present in the parent plant if more than 2 are present.

To summarize, \textit{Spm} is a chromosomal element, subject to somatically occurring losses from some nuclei or changes in location in others, that suppresses the potential action at the \textit{A}_{1}^{m-1} locus until a change occurs at this locus under the influence of \textit{Spm} that produces either an altered type of response to \textit{Spm} in subsequent cell and plant generations (a change in stage of \textit{A}_{1}^{m-1}) or a stable mutation that expresses a particular level of anthocyanin pigmentation in kernel and plant.

5. System responsible for mutations at \textit{A}_{1}^{m-2}

Although the system responsible for mutations at \textit{A}_{1}^{m-2}, another mutable condition that arose at \textit{A}_{1} in the Cold Spring Harbor cultures, is less well understood than that associated with \textit{D}_{t}, \textit{M}_{r}, \textit{D}_{a}, \textit{A}_{c}, or \textit{Spm}, its mode of action appears to differ from these other better known systems in one striking way. Present knowledge suggests the following interpretation: An independently located factor, subject to loss or to change in location in somatic cells, is responsible for maintaining one particular type of expression of anthocyanin pigment formation at \textit{A}_{1}^{m-2}. Following removal of this factor, either through a somatically occurring event or by means of meiotic segregations, a mutational change occurs at the locus of \textit{A}_{1}^{m-2} which results in a stabilized expression of this locus in subsequent cell and plant generations. The types of mutation-producing changes that occur fall into two distinct classes. One class contains mutants expressing different levels of anthocyanin pigment formation and these range from those giving nearly none to those that produce intense coloration in the aleurone layer of the kernel. All of the mutants in this class produce intense pigmentation in the plant but this is confined to certain of its tissues. The mutants in the second class give an apparent full \textit{A}_{1} type of expression in both the aleurone layer of the kernel and in the plant tissues.

Barbara McClintock
Perspective:¹

Citation: 72. *McClintock, Barbara. 1955b. Controlled mutation in maize: The \( a_{1}^{-1}-Spm \) system of control of gene action and mutation. Continued studies of the mode of operation of the controlling elements \( Ds \) and \( Ac \). *Reprinted in McClintock 1987

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1 Perspective solicited.
1. Further study of the $a_l^{m-1}$ - $Sp_m$ system.

A general outline of the system of control of gene action at $a_l^{m-1}$ was given last year in this News Letter, and transpositions of the controlling element, $Sp_m$, were mentioned. To determine $Sp_m$ constitutions in the cells of different parts of a plant, several ears of a single plant were utilized in test crosses. From 101 plants, tests of two ears per plant were obtained. In 95 plants, the number of $Sp_m$ elements was the same in the cells that produced each ear (63 with 1 $Sp_m$; 26 with 2 $Sp_m$; 6 with 3 $Sp_m$). In 6 plants, the $Sp_m$ constitution was not the same in the cells that gave rise to each ear (1 case of 1 $Sp_m$ in one ear...
and no Spm in the other; 3 cases of 1 Spm in one ear and 2 Spm in the second; 2 cases of 1 Spm in one ear, the second ear having a sector with no Spm. From 12 other plants, tests of three ears per plant were obtained and correspondence in number of Spm elements was evident in each of the 3 ears of 11 of them (6 with 1 Spm; 4 with 2 Spm; 1 with 3 Spm). In one plant, the cells that gave rise to two ears contained 1 Spm element but 2 Spm elements were present in the cells that gave rise to the third ear.

Tests were made of Spm constitutions in the progeny of plants in which 1, 2, or 3 Spm elements were known to be present. One test of 238 individuals derived from plants having 1 Spm element will illustrate the nature of the results obtained. The parent plants carrying Spm had been crossed by plants homozygous for a_{1}^{m-1} but having no Spm. Kernels on the resulting ears that showed the presence of Spm in the endosperm were selected and the plants grown from them were again crossed by plants homozygous for a_{1}^{m-1} but carrying no Spm. On the ear produced by 7 of these plants, no kernels having Spm appeared. One Spm element was present in 205 plants, 2 Spm elements were present in 20 plants and in 6 plants, 3 Spm elements were present. Also, tests were conducted to determine the position of Spm in the progeny of plants in which the location of Spm was known. In the majority of such tests, the Spm element occupied the same position in the chromosome complement as it had in the parent plant, with some exceptions, however, that were to be expected. In one such test, 103 individuals in the progeny of plants carrying Spm in chromosome 6 and showing approximately 35% recombination with Y, were examined. In 92 of these plants, 1 Spm was present and it showed the same linkage with Y as it had shown in the parent plants. Two plants had 2 Spm elements, one of which was linked with Y. Five plants had 1 Spm but it showed no linkage with Y, and 1 plant had 3 Spm elements whose linkage relationships could not be detected because of the high number of Spm elements that were present. In another test of 22 individuals in the progeny of a plant carrying Spm in chromosome 6 but showing, in this case, closer linkage with Y, 19 plants proved to have 1 Spm element and its location was similar to that in the parent plant. In two plants, 2 Spm elements were present and one of them was linked with Y. In the remaining plant of this culture, 1 Spm was present but it showed no linkage with Y. The table below will illustrate the nature of the tests conducted and the results obtained from them for this progeny of 22 plants.
\[
a_{1}^{m-1}/a_{1}^{m-1}; \\
\text{or} \\
Y/y \quad \varphi \ x \ a_{1}^{m-1}/a_{1}^{m-1}; \quad y/y; \quad \text{No Spm} \ \\
a_{1}^{m-1}/a_{1};
\]

<table>
<thead>
<tr>
<th>Plant No.</th>
<th>Pale aleyrune (No Spm)</th>
<th>Colorless aleyrune with spots of ( A_{1} )</th>
<th>Totals</th>
<th>Germinal Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( Y )</td>
<td>( Y )</td>
<td>( Y )</td>
<td>( Y )</td>
</tr>
<tr>
<td>A. One Spm, linked with ( Y )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>38</td>
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<td>114</td>
<td>44</td>
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<td>27</td>
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<td>19</td>
<td>52</td>
<td>172</td>
<td>171</td>
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</table>

**Totals for A**

<table>
<thead>
<tr>
<th>( Y )</th>
<th>( Y )</th>
</tr>
</thead>
<tbody>
<tr>
<td>683</td>
<td>3164</td>
</tr>
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</table>

**B. Two Spm elements, one linked with \( Y \)**

<table>
<thead>
<tr>
<th>Plant No.</th>
<th>Pale aleyrune (No Spm)</th>
<th>Colorless aleyrune with spots of ( A_{1} )</th>
<th>Totals</th>
<th>Germinal Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( Y )</td>
<td>( Y )</td>
<td>( Y )</td>
<td>( Y )</td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>17</td>
<td>63</td>
<td>29</td>
</tr>
<tr>
<td>21</td>
<td>30</td>
<td>101</td>
<td>252</td>
<td>152</td>
</tr>
</tbody>
</table>

**Totals for B**

<table>
<thead>
<tr>
<th>( Y )</th>
<th>( Y )</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>118</td>
</tr>
</tbody>
</table>

**C. One Spm, not linked with \( Y \)**

<table>
<thead>
<tr>
<th>Plant No.</th>
<th>Pale aleyrune (No Spm)</th>
<th>Colorless aleyrune with spots of ( A_{1} )</th>
<th>Totals</th>
<th>Germinal Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( Y )</td>
<td>( Y )</td>
<td>( Y )</td>
<td>( Y )</td>
</tr>
<tr>
<td>22</td>
<td>117</td>
<td>112</td>
<td>101</td>
<td>100</td>
</tr>
</tbody>
</table>
Similar results were obtained from tests of progeny of plants carrying \textit{Srm} in chromosome 5 and showing linkage with \textit{Fr}. However, in one other test of a small progeny of only 5 plants, quite aberrant results were obtained. In the parent plant, 1 \textit{Srm} element was present and from the ratio of kernel types on the ear it produced, there was no evidence of linkage of \textit{Srm} with alleles of \textit{Y}, \textit{Fr}, or \textit{Wx} which were also segregating. In one of the 5 plants in this progeny, 2 \textit{Srm} elements were present and one of them was loosely linked with \textit{Y}. In each of the remaining 4 plants, 1 \textit{Srm} element was present. It was very closely linked with \textit{Y} in one plant. In another, it showed 35\% recombination with \textit{Fr}. In the third plant, it gave 33\% recombination with \textit{Wx}, and in the fourth plant, no linkage of \textit{Srm} with any of these markers was noted. It is suspected that many transpositions of \textit{Srm} occurred in the parent plant and at a time that was late in the development of its sporogenous cells.

On test ears, such as those described above, an occasional kernel may appear showing a markedly altered pattern of mutation. Some of them arise from a change in state of the \textit{a1m-1} locus. Others, however, arise from modifications of another type. Several examples of the latter type of modification have received some study. One type appears relatively frequently and the evidence suggests that it may arise from a change in the \textit{Srm} element itself. In the presence of the modified element and in the absence of \textit{Srm}, plant tissues show pigmentation but the aleurone layer is almost totally colorless, only a few specks or dots of color appearing in it. When both the modified element and \textit{Srm} are present in the same plant, the action of \textit{Srm} is dominant to that of the modified element and clear-cut segregations of these two different controlling elements are observed. Like \textit{Srm}, the modified element may occupy different positions within the chromosome complement. Other types of modifiers have also arisen and in the same general manner. Their presence results in altered distributions of pigmentation in the plant tissues and in the aleurone layer of the kernels and also in an altered time and frequency of occurrence of mutations at the locus of \textit{a1m-1} in these tissues.

2. Further study of \textit{Ac} control of mutation at the bronze locus in chromosome 9.

In last year's News Letter, a case was described of control by \textit{Ac} of gene action at the bronze locus in chromosome 9. This case has been further examined and evidence was obtained suggesting a relationship between an apparent direct \textit{Ac} control of gene action and indirect control of this action, such as that exhibited by the \textit{Dg - Ac} two element system where the \textit{Dg} element directly controls types of modification in gene action but does so through the influence that \textit{Ac} exerts on it. As mentioned last year, the recessive \textit{bz} in this case was capable of mutating to higher alleles of \textit{Bz} as well as to stable recessives, and control of this process was found to be associated with the presence of \textit{Ac} at the locus. The relatively simple types of change in gene action are those that give rise to stable dominants or to stable recessives. Altogether, 14 independent mutations to a stable dominant were examined. In all 14 cases, mutation to \textit{Bz} was associated with removal of \textit{Ac} from
the bronze locus. In 6 cases, it was not present in the gamete that carried the \( B_z \) mutant. In the remaining 8 cases, \( A_c \) was present in the gamete but its location was altered. In 4 of these 8 cases, the \( A_c \) element showed no linkage with markers in the short arm of chromosome 9. In the 4 other cases, \( A_c \) was linked to these markers. In 3 of them, it was located several crossover units to the right of \( B_z \) and in one case it was located very close to \( W_x \).

Twenty-four cases of mutation to a stable recessive were examined. In 9 of these cases, \( A_c \) was absent in the gamete that carried the stable recessive. In 5 cases, one \( A_c \) was present but it showed no linkage with markers in the short arm of chromosome 9. In 9 cases, one \( A_c \) was present and it showed linkage with markers in the short arm of chromosome 9. In 2 of these 9 cases, \( A_c \) was located close to \( W_x \), and in one case, it was located very close to \( sh \). In the remaining 6 of these 9 cases, its exact location was not determined; it was linked with \( W_x \) and showed from 20 to 30% recombination with it. In the remaining case, two \( A_c \) elements were present, one located close to but to the right of \( b_z \), the other showing no linkage with markers in the short arm of chromosome 9.

Two cases were found in which control of mutation at \( b_z \) had changed from apparent direct \( A_c \) control to indirect control by this element. In all essential respects, the system of control of mutation in these two cases is the same as that exhibited by the \( D_s - A_c \) two element system. \( A_c \) is not present at the locus of \( b_z \) but its presence in the chromosome complement is necessary for mutations to occur there, and the time of their occurrence reflects the \( A_c \) dose in the cells.

In addition to the mutant types mentioned above, two cases of mutation to an unstable dominant, \( B_z \), were examined. In both of them, \( A_c \) was present and located at or close to \( B_z \). Examination of a number of derivatives of one of them was made and the types of modification found are listed below:

1. Change to a stable dominant associated with removal of \( A_c \) from the \( B_z \) locus—apparently a frequent occurrence but only 3 cases examined in detail.

2. Change to a mutable recessive, \( b_z \). 8 cases examined. All had \( A_c \) at the \( b_z \) locus. Mutations controlled by \( A_c \). Marked change in \( A_c \) dose action was exhibited by 4 of these cases.

3. A change in state of the \( B_z \) locus recognized by a very high rate of mutation from \( B_z \) to \( b_z \). \( A_c \) present at or close to the \( B_z \) locus and the mutation process controlled by it.

4. Appearance of a high rate of \( D_s \)-type chromosome breaks at a position a few crossover units to the right of \( B_z \). The \( B_z \) phenotype is stable. \( A_c \) occupies the locus where the \( D_s \)-type breaks are occurring. Twelve derivatives of this particular modification that showed no breaks
or a reduced frequency of them were examined. In 2 cases, Ac was not present in the plant. In 6 cases, the location of Ac was apparently unchanged but Ds-type breaks were very much reduced in frequency or did not occur. In the remaining 4 cases, 2 Ac elements were present, one at or close to the former location and one located elsewhere (the second Ac element was close to wx in two cases and not linked to markers in the short arm of chromosome 9 in one case).

5. Appearance of an intermediate allele giving a weak Bz expression. Ac no longer present at the locus. However, if Ac is present somewhere in the chromosome complement, mutations occur at this locus to give alleles expressing higher or lower levels of the Bz phenotype. This intermediate allele is stable in the absence of Ac.

6. Mutability of a component of the Bz locus detected in kernels that are C sh bz wx da/C sh bz wx da/I Sh BzAc Tx Ds in constitution. Breaks at Ds in the I Sh BzAc Wx Ds chromosome during development of the endosperm produce areas that are C sh bz wx in phenotype. When the normal Bz locus is present, such areas have rims showing the Bz phenotype due to diffusion of a substance produced in the surrounding I Sh Bz Wx cells in response to the presence in them of Bz. In the case here considered, only short, interrupted streaks of the Bz phenotype appear in the boundary rims of the C sh bz wx areas. In kernels that are I Sh BzAc Wx Ds/I Sh BzAc Wx Ds/C sh bz wx da in constitution, the majority of C sh bz wx areas show either no Bz streaks in the rim cells or only an occasional very small streak. However, in kernels that are C BzAc/C bz/C bz in constitution and in the plants derived from them, full Bz color appears. This suggests a possible dual activity of the genic materials at the Bz locus and, in this case, mutability is being expressed by only one of these components.

3. Degree of spread of mutation along the chromosome induced by Ds.

When Ds is located immediately to the left of Sh in chromosome 9 it induces, in the presence of Ac, changes in action of genic materials located to either side of it. Ds is retained following such an event and it is unaltered in its location. A number of mutations of Sh and simultaneous mutations of both Sh and Bz have been examined in the past, as well as those changes that affect the genic materials located to its left and extending into the I locus. In order to expand the study of spread of mutational change along the chromosome, plants having Ds-induced modifications of gene action in the segment which extends to its left and includes the I locus were used in crosses in order to isolate from these plants some cases in which Sh or both Sh and Bz, located to the right of Ds, were subsequently modified. Three cases of modification of Sh expression but not that of Bz were isolated and examined. In two other examined cases, both Sh and Bz were modified in their expression to give the recessives, sh and bz. In none of these 5 cases was the position of Ds detectibly altered as a consequence of the modifications.
in gene action it induced, nor was the previously modified action of genetic materials, located to its left, altered by these events. Cytological examination of plants carrying these 5 modifications gave no detectible evidence of alteration of chromosome components within the affected segment. These cases indicate that $Bz$-induced change in gene action can spread along the chromosome to include a segment of chromosome extending from the locus of $Bz$ to and including that of $I$.


A preliminary study was made of a modification affecting the organization of chromosome 9. This modified chromosome is composed of two independent segments. One segment includes the distal third of the short arm and will be called the fragment chromosome. The other segment is composed of the proximal two-thirds of the short arm and all of the long arm and will be called the deficient chromosome. The distal end of the fragment terminates in a knob and its proximal end is composed of a centromere from which a short piece of very deep-staining chromatin extends. These two independent segments carry the full genetic complement of chromosome 9. The locus of $C$ is carried in the fragment chromosome. That of $Sh$ is very close to the end of the short arm of the deficient chromosome. The deficient chromosome is transmitted through the female gametophyte but its transmission through the pollen occurs only when the fragment chromosome is also present in the tube nucleus. Study of this modification was undertaken because the fragment chromosome undergoes many changes in constitution in somatic cells: "misdivision" of its centromere leading to loss or non-disjunction of the fragment; loss of the deep-staining component adjacent to the centromere or duplications of this component; ring chromosome formation; deletion of segments of chromatin composing the fragment; attachment of the fragment at its centromere region to the end of another chromosome, its own centromere being lost in the process; attachment of its centromere to that of another chromosome resulting in loss of an arm of the other chromosome; etc. These events affecting the fragment chromosome appear to be regulated in a manner somewhat similar to that which controls mutation at a "mutable locus." This is made evident in some isolates by the patterns produced by patches of colorless aleurone in a colored background that appear in kernels having one or two normal chromosomes 9 carrying $C$ and a fragment chromosome carrying $C$. The colorless patches represent those areas in which $C$ has been lost from the cells. The broken end of the deficient chromosome also initiates modifications that affect its own organization and also that of other chromosomes of the complement but the frequency of occurrence of such events appears to be lower.

In structural heterozygotes, crossing over occurs between the locus of $C$ and the centromere of the fragment chromosome and isolates having $C$ in the fragment chromosome have been obtained as well as isolates in which the $C$ from the fragment has been introduced into a normal chromosome 9. Several sets of data suggest that a segment carrying $sh$ and $bz$
may be included in the fragment. If so, the constitution of this modification includes a duplication of the segment that carries the loci of sh and bz for both Sh and Bz are present in the deficient chromosome. Plants carrying Bz both in the normal chromosome 9 and in the deficient chromosome and also a fragment chromosome, when crossed to plants homozygous for bz have produced ears on which kernels showing the bz phenotype have appeared in constant proportions. This is made evident in B of the following table:

A. \( \Phi \) C. sh bz wx/C sh bz wx \( \times \) \( \sigma \) sh Bz wx; normal chromosome,
  Sh Bz Wx; deficient chromosome
  No Fragment.

B. \( \Phi \) \( \times \) \( \sigma \) Same as A but fragment present.

<table>
<thead>
<tr>
<th>Phenotype of kernel</th>
<th>A.</th>
<th>B. Plants 1 to 5</th>
<th>Totals for B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Sh Bz Wx</td>
<td>0</td>
<td>81</td>
<td>141</td>
</tr>
<tr>
<td>Sh Bz wx</td>
<td>0</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>sh Bz Wx</td>
<td>71</td>
<td>65</td>
<td>81</td>
</tr>
<tr>
<td>sh Bz wx</td>
<td>367</td>
<td>357</td>
<td>335</td>
</tr>
<tr>
<td>sh bz Wx</td>
<td>0</td>
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<td>1</td>
</tr>
<tr>
<td>sh bz wx</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Totals</td>
<td>438</td>
<td>514</td>
<td>576</td>
</tr>
</tbody>
</table>

\% bz among sh class 0.6 0.95 1.2 1.4 1.8 1.4

Substantiating evidence of inclusion of sh and bz in the fragment chromosome was obtained from crosses of several plants having a normal chromosome 9 carrying c sh Bz wx, a fragment chromosome 9 carrying C, and a deficient chromosome 9 carrying Sh Bz Wx. When crossed by plants homozygous for c, sh, bz, and wx, the following phenotypes appeared among the kernels on the ears of these plants: 84 C Sh Bz Wx, 4 C Sh Bz wx, 53 c Sh Wx, 12 c Sh wx, 1 C sh Bz Wx, 35 C sh Bz wx, 5 C sh bz wx, 18 c sh Wx and 294 c sh wx. Among the 41 sh kernels in which C, originally carried in the fragment chromosome, was present, 5 were bz in phenotype. The duplicated region must be very short for cytological evidence of it has been difficult to substantiate. Also, mutation at the locus of Bz in the normal chromosome 9 from some event at meiosis associated with synopsis of the fragment chromosome with its homologous segment in the normal chromosome cannot be excluded, for it is known.
from other studies of this modification that changes in expression of $\zeta$, of $Sh$, and of $B_2$ that cannot be accounted for by normal crossover processes, have occurred.

Barbara McClintock

The following publications by McClintock were not included in the list of recent maize publications given at the end of the News Letter:


Perspective:\(^1\)


*Reprinted in McClintock 1987

Online: [https://archive.org/stream/yearbookcarne55195556carn#page/322/mode/2up/search/McClintock](https://archive.org/stream/yearbookcarne55195556carn#page/322/mode/2up/search/McClintock)

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\(^1\) Perspective solicited.
Perspective: forthcoming


Online: http://symposium.cshlp.org/content/21
http://symposium.cshlp.org/site/misc/index_archive.xhtml
Perspectives on Nobel Laureate
Barbara McClintock’s Publications (1926-1984):
A Companion Volume
edited by Lee B. Kass

Perspective:¹


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¹ Perspective solicited.
1. Continued study of stability of location of Srm.

The mode of operation of the $a_1 m^{-1}$ - Srm system was outlined in the last two issues of this News Letter and evidence was presented indicating that the Srm element undergoes frequent changes in location. To obtain further evidence of the degree of stability of location of Srm, two additional tests were conducted this past summer. Each involved determination of Srm constitution and linkage relations in the progeny of a plant having one Srm whose location was known. In both cases, the location of Srm in the chromosome complement differed from that of other determined locations of it. In one parent plant, Srm was linked with wx in chromosome 9. In the other parent plant, it was located close to Y in chromosome 6. The history of the first mentioned parent plant is referable to a culture grown in the summer of 1954. The plants in this culture were Wx/wx and either $a_1 m^{-1}/a_1 m^{-1}$ or $a_2 m^{-1}/a_2 m^{-1}$ in constitution. In one plant of this culture, two independently located Srm elements were present, one of which was linked with Wx. When pollen of a plant homozygous for $a_1 m^{-1}$ and wx and having no Srm (standard Srm tester stock) was used on the silks of an ear of this plant, there appeared 130 pale colored kernels (no Srm) and 335 kernels that had $a_1$ spots in a colorless background (Srm present), indicating the presence in this plant of two independently located Srm elements. From the ratio of Wx to wx in each class ($100 \text{ Wx} : 30 \text{ wx}$ in the no Srm class and $123 \text{ Wx} : 212 \text{ wx}$ in the Srm class) it was evident that one of the two Srm elements was located in the wx carrying chromosome of this plant. In order to obtain plants with a single Srm element located in a chromosome 9 carrying Wx, and to test for its stability in this location, 29 plants derived from the variegated, Wx class of kernels on the above described ear were again tested by crossing them with plants that were homozygous for $a_1 m^{-1}$ and wx but in which no Srm was present. The first ear on the main stalk was always used for this test and when possible, other ears of the plant were so used. Among these 29 plants, 1 had no Srm; 20 plants had one Srm but it was not linked with Wx; 4 plants had two Srm elements.
that were not linked to each other but in 3 of these plants, one of the
two Srm elements was linked with Wx. One plant had three independently
located Srm elements. In the remaining 3 plants, a single Srm element
was present and it was linked with Wx. Among the 1918 kernels appearing
on five ears obtained from these three plants, the following types
appeared: 1 A1 Wx, 1006 uniformly pale colored (no Srm) of which 222
were Wx and 784 were wx, and 911 in which spots of A1 appeared in a
colorless background (Srm present) of which 747 were Wx and 164 were wx.
Linkage of Srm with Wx is obvious and the value of the "recombinant"
classes is 20.1%.

Thirteen plants derived from the Srm Wx class of kernels on one of
the five above mentioned ears were grown this past summer under culture
number 7285. Each was used as a female parent in crosses with plants
homozygous for a1 m-1 and wx and carrying no Srm, and all fertile ears
produced by each plant were so used. One of the 13 plants had no Srm
but in the remaining 12 plants, one or two Srm elements were present.
The number of ears obtained from each plant, the Srm constitution in
the cells that produced each ear, and the linkage relations of Srm and
Wx, are indicated in table 1. The tiller ear produced by one plant had
no Srm but in the remaining 25 ears obtained from these twelve plants,
one or two Srm elements were present. In 16 ears, one Srm element
linked with Wx, was present. In 5 ears, two Srm elements were present,
one of which was linked with Wx. In 4 ears, one Srm was present but
it was not linked with Wx. The ratio of kernel types appearing on these
ears is given in table 2 for each of these three categories of Srm
constitution and location. From table 1, it may be seen that corre-
spondence in Srm constitution and location is shown in the cells that
produced the 1st and 2nd ears on the main stalk. Differences with
respect to this were expressed only in tillers. This suggests that the
mechanism responsible for change in number and location of Srm elements
was operating relatively early in development of these plants.

The second test of stability of location of Srm was conducted with
the progeny of a plant having a single Srm element located close to Y
in chromosome 6. The parent plant was one of 5 in a culture and it was
the only plant in this culture that showed close linkage of Srm with Y.
This plant was homozygous for a1 m-1 and heterozygous for Y, Pr, and Wx.
It was used as a female parent in a cross with a plant that was homo-
yzous for a1 m-1, Y, Wx, and wx, and had no Srm. The ear this cross
produced had a small, well defined sector in which Srm was absent. All
the kernels within this sector were uniformly pale colored (no Srm); 21 were Y and 26 were x. Among the other 329 kernels on this ear, 167
were uniformly pale colored (no Srm) and 162 showed A1 spots on a
colorless background (Srm present). In the pale colored class, 10 were
Y and 157 were x. In the variegated class, 153 were Y and 9 were x.
It could be concluded, therefore, that a single Srm element was present
in the part of the plant that produced most of this ear and that this
element was closely linked with Y (5.6% "recombinants"). No linkage
with Pr or with Wx was expressed. This past summer, 17 plants derived
<table>
<thead>
<tr>
<th>Plant Number in culture 7285</th>
<th>Number of ears tested per plant</th>
<th>Position of ear on plant</th>
<th>Srm constitution and linkage with Wx</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-6, B-1, and B-6</td>
<td>1</td>
<td>1st ear, main stalk</td>
<td>1 Srm; linked with Wx (each ear)</td>
</tr>
<tr>
<td>B-4</td>
<td>1</td>
<td></td>
<td>2 Srm; one linked with Wx</td>
</tr>
<tr>
<td>A-5</td>
<td>2</td>
<td>1st and 2nd ear, main stalk</td>
<td>2 Srm; one linked with Wx (both ears)</td>
</tr>
<tr>
<td>B-2 and B-5</td>
<td>2</td>
<td>1st ear, main stalk; tiller ear.</td>
<td>1 Srm; linked with Wx (all four ears)</td>
</tr>
<tr>
<td>A-1</td>
<td>3</td>
<td>1st and 2nd ear, main stalk; tiller ear.</td>
<td>1 Srm; linked with Wx (1st and second ear, main stalk)</td>
</tr>
<tr>
<td>A-3</td>
<td>3</td>
<td></td>
<td>1 Srm; not linked with Wx (tiller ear)</td>
</tr>
<tr>
<td>A-4</td>
<td>3</td>
<td></td>
<td>2 Srm; one linked with Wx (1st and 2nd ear, main stalk)</td>
</tr>
<tr>
<td>A-2</td>
<td>3</td>
<td>1st ear, main stalk; ear on each of 2 tillers.</td>
<td>1 Srm; linked with Wx (1st ear, main stalk; 1 tiller ear)</td>
</tr>
<tr>
<td>A-7</td>
<td>4</td>
<td>1st and 2nd ear, main stalk; ear on each of 2 tillers.</td>
<td>1 Srm; linked with Wx (all four ears)</td>
</tr>
</tbody>
</table>
Table 2.

<table>
<thead>
<tr>
<th>Srm constitution of tested plants (Culture 7285)</th>
<th>Phenotype of kernel</th>
<th>Colorless with spots of A$_1$ (Srm present)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A$_1$ Pale color (No Srm)</td>
<td></td>
</tr>
<tr>
<td>1 Srm; linked with Wx</td>
<td>1</td>
<td>418</td>
</tr>
<tr>
<td>2 Srm; one linked with Wx</td>
<td>0</td>
<td>79</td>
</tr>
<tr>
<td>1 Srm; not linked with Wx</td>
<td>0</td>
<td>190</td>
</tr>
</tbody>
</table>

* 20.2% are "recombinants".

from the variegated, Y, Fr, Wx class of kernels on this ear were tested for Srm constitution and location. The silks of all fertile ears produced by each plant received pollen from plants that were homozygous for A$_{m-1}$, Y, Fr, and Wx and had no Srm. One ear was obtained from 3 plants, two ears were obtained from 4 plants, three ears were obtained from 7 plants, and four ears were obtained from 3 plants. That a single Srm element was present in all tested parts of each plant was indicated by the approximate 1 : 1 ratio of presence and absence of Srm among the kernels on each of the 44 ears. And, in 43 of these 44 ears, linkage of Srm with Y was expressed. Only on the ear produced by a tiller of one plant was evidence of this linkage absent. The proportion of kernel types with respect to presence and absence of Srm and to Y and y among the kernels appearing on the ears of 15 of the 17 plants is given in A of Table 3. One plant, number 17, was small and defective in appearance. The ear it produced was partially sterile and from the ratio of kernel types on this ear, it was evident that the Y chromosome carrying Srm was not being transmitted normally. Nevertheless, close linkage of Srm with Y is indicated (B, Table 3). The types of kernels appearing on each of two ears produced by plant number 2 is shown in C of Table 3. On the 1st ear of the main stalk, linkage of Srm with Y was clearly expressed. However, the ratio of kernel types that appeared on the ear produced by a tiller of this plant gives no evidence of such linkage. Also, there was no evidence of linkage of Srm with either Wx or Fr.
<table>
<thead>
<tr>
<th>Plant number in culture 7260</th>
<th>A&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Pale color (No B&lt;sub&gt;1&lt;/sub&gt;)</th>
<th>Colorless with spots of A&lt;sub&gt;1&lt;/sub&gt; (B&lt;sub&gt;1&lt;/sub&gt; present)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>25</td>
<td>345</td>
<td>370</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>16</td>
<td>308</td>
<td>324</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>14</td>
<td>389</td>
<td>403</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>2</td>
<td>55</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>1 Y</td>
<td>17</td>
<td>367</td>
<td>384</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>17</td>
<td>252</td>
<td>369</td>
</tr>
<tr>
<td>7</td>
<td>1 Y</td>
<td>16</td>
<td>530</td>
<td>546</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>19</td>
<td>318</td>
<td>337</td>
</tr>
<tr>
<td>9</td>
<td>1 Y</td>
<td>20</td>
<td>468</td>
<td>488</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>28</td>
<td>548</td>
<td>576</td>
</tr>
<tr>
<td>11</td>
<td>1 Y</td>
<td>24</td>
<td>271</td>
<td>295</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>19</td>
<td>302</td>
<td>321</td>
</tr>
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<td>13</td>
<td>0</td>
<td>5</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>7</td>
<td>122</td>
<td>129</td>
</tr>
<tr>
<td>15</td>
<td>1 Y</td>
<td>18</td>
<td>358</td>
<td>376</td>
</tr>
<tr>
<td>Totals</td>
<td>4</td>
<td>247</td>
<td>4708</td>
<td>4955</td>
</tr>
</tbody>
</table>

B. Plant No. 17

<table>
<thead>
<tr>
<th>Plant No. 17</th>
<th>Y</th>
<th>Y</th>
<th>Y</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>91</td>
<td>20</td>
<td>111</td>
</tr>
</tbody>
</table>

C. Plant No. 2

<table>
<thead>
<tr>
<th>Plant No. 2</th>
<th>main ear</th>
<th>Y</th>
<th>Y</th>
<th>Y</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>25</td>
<td>203</td>
<td>171</td>
<td>410</td>
</tr>
<tr>
<td></td>
<td>tiller ear</td>
<td>0</td>
<td>65</td>
<td>47</td>
<td>112</td>
</tr>
</tbody>
</table>

*5% are "recombinants"
With regard to stability of location of \( s_{rm} \), the results obtained from the two experiments, outlined above, differ markedly. The first gave evidence of relatively frequent changes in location of \( s_{rm} \). This is in contrast to the experiment just described where an unusual degree of stability of location of \( s_{rm} \) was made evident. Nothing is yet known about genetic or other factors that may be responsible for controlling the time during development of a tissue when change in location of \( s_{rm} \) will occur, or the frequency of this.


In last year's News Letter, a description was given of a modification affecting the organization of chromosome 9. Two chromosomes instead of one carry the substance of this chromosome. One of these is composed of the distal third of the short arm and it was referred to as the fragment chromosome. The centromere is situated at the proximal end of this component of chromosome 9. The longer segment is composed of the proximal two-thirds of the short arm of chromosome 9 and all of its long arm, and it was referred to as the deficient chromosome. Interest in this case was centered on the aberrant behavior of the fragment chromosome in somatic cells, and this was outlined briefly last year. Further examination of this case required more exact knowledge of the composition of the two components of this structural modification. Therefore, an extensive series of tests of this were continued during the past year. The fragment was known to carry the locus of \( C \) and preliminary evidence presented in the News Letter last year, suggested that it also carried the loci of \( sh \) and \( bz \). Since the deficient chromosome was known to have the loci of \( Sh \) and \( Bz \), with \( Sh \) situated very close to the end of its short arm, the genetic composition of the structurally modified chromosome 9 would then include a duplication of a segment composed of the region from the locus of \( sh \) to one that is proximal to \( bz \). Recent tests have confirmed the presence of \( sh \) and \( bz \) in the original fragment chromosome and they also have revealed the relative length of the segment that extends from \( bz \) to the centromere of the fragment. It is equivalent to a segment in the normal chromosome 9 that is 5 crossover units proximal to \( Bz \).

Genetic study of the constitution of the fragment and the deficient chromosome makes it clear that a segment in the fragment,—from the locus of \( sh \) to the centromere,—duplicates a segment in the deficient chromosome that is located at the very end of its short arm. Examination of the chromosomes at the pachytene stage in structural heterozygotes did not reveal the physical length of the duplicated segment with the desired degree of certainty. It can not include more than 1 or 2 small chromomeres, if matching chromomeres in synapsed regions may be used as a reliable criterion of homology.

In structural heterozygotes whose chromosome 9 components are appropriately marked for crossover studies (an example: normal chromosome 9 with \( 1 \ Sh \ Bz \ Wx/ \) deficient chromosome 9 with \( Sh \ Bz \ Wx/ \) fragment...
with C sh bz) an exchange occurring in the region between sh and bz of the fragment (region 1) or between bz and the centromere of the fragment (region 2) would give rise to a structurally normal chromosome 9 carrying sh and Bz or one carrying sh and bz. The presence of such a normal chromosome 9 was confirmed in 48 plants that were derived from kernels exhibiting a phenotype expected from a crossover in one or the other of these regions. In 9 of these 48 plants, an unmodified fragment chromosome carrying sh and bz was also present. (The fragment could not carry the reciprocal product of the crossover. If it had, the kernel would not have exhibited the crossover phenotype.) A normal chromosome 9 would be obtained from a crossover in region 1 or 2 either between the fragment and the normal chromosome or between the fragment and the deficient chromosome. Evidence obtained from the test crosses did not allow definite conclusions to be drawn regarding the relative frequency of the exchanges that occur in these regions between the fragment and the normal and deficient chromosomes. It did suggest, however, that most of the crossing over may take place between the fragment and the normal chromosome and that a crossover in either region 1 or 2 does not interfere with another occurring between the normal and the deficient chromosome. Evidence for the latter statement is conflicting, however, and some of the difficulties encountered in these analyses may derive from differences in behavior of the fragment among the tested plants, as illustrated below.

Four plants having two deficient chromosomes 9, each carrying Sh, Bz, and Wx, and a single fragment chromosome carrying C, sh, and bz, were used as pollen parents in crosses to plants that were homozygous either for C, sh, Bz, and Wx, or C, sh, bz, and Wx, or for C, sh, Bz, and Wx. The only functional pollen grains produced by such plants are those having either a deficient chromosome and the fragment, a deficient chromosome and the fragment that has become attached to the end of another chromosome (which sometimes occurs), or a structurally normal chromosome 9 produced by a crossover, however it may be initiated, between the homologous segments of the fragment and the deficient chromosome. Crossovers of this latter type would give rise to structurally normal chromosomes having either C sh Bz Wx or C sh bz Wx. The number of kernels having such phenotypes that appeared on the ears produced by test crosses with these four plants is given in A of table 4. In the cross entered in B of this table, only the sh kernels could be recorded for all of them received Bz from the female parent. Pollen used in the test crosses was collected from each plant over many days, and from tillers as well as from the main stalk. Regardless of the date or the part of the plant from which the pollen was collected, the frequency of appearance of the sh class of kernels on the test cross ears was the same for an individual plant. However, as table 4 shows, wide differences in this respect are exhibited among these plants. Such differences would not be anticipated unless it was known or suspected that some genetic system was controlling the type of behavior of the fragment chromosome. There is some evidence to suggest that this system may be related to the one that controls the behavior of the
fragment in somatic cells. In somatic cells, the fragment may undergo types of events that affect its non-disjunction or its removal during a mitotic cycle from one or both sister cells. Differences in type of genetic control exist and these may be recognized readily, for they give rise to different patterns of variegation in plant and endosperm cells when proper genetic markers are present to allow detection of those events that affect fragment distributions. The behavior of the fragment in the two plants in table 4 that produced the lowest percent of sh kernels, i.e., plants 6971A and 7174A-2, was similar in endosperm development. The pattern of variegation each produced indicated a low rate of loss of the fragment and these losses occurred late in endosperm development. On the other hand, the behavior of the fragment in plants 7169-10 and 7176B-3 resulted in a pattern of variegation in the endosperm that indicated frequent loss of the fragment and often this occurred early in development.

### Table 4.

<table>
<thead>
<tr>
<th>Plant No.</th>
<th>Total No.</th>
<th>Phenotype of kernels</th>
<th>% of sh kernels</th>
<th>Total No.</th>
<th>C sh Bz Wx</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C sh bz wx</td>
<td></td>
<td></td>
<td>C sh Bz Wx</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c sh bz wx</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fragment: C sh bz;</td>
<td>Fragments: C sh bz;</td>
<td></td>
<td></td>
<td>Fragment: C sh bz;</td>
<td></td>
</tr>
<tr>
<td>Deficient: Sh Bz Wx</td>
<td>Deficient: Sh Bz Wx</td>
<td></td>
<td></td>
<td>Deficient: Sh Bz Wx</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant No.</th>
<th>Total No.</th>
<th>Phenotype of sh kernels</th>
<th>% sh kernels</th>
<th>Total No.</th>
<th>C sh Bz Wx</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C sh Bz Wx</td>
<td></td>
<td></td>
<td>C sh Bz Wx</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|       | 371       | 1                       | 2             | 0.8       | 742    |
|       | 3294      | 9                       | 44            | 1.6       | 1589   |
| 7167-10 |           |                         |               |           | 23     |
| 7174A-2 | 3750      | 5                       | 14            | 0.5       | 1361   |
| 7176B-3 | 1638      | 7                       | 51            | 3.5       | 1763   |
| Totals  | 22        | 111                     |               |           |         |

That the kernels showing the crossover phenotypes received a structurally and functionally normal chromosome 9 from the male parent was demonstrated by cytological and genetical studies conducted with 2 plants derived from the C sh Bz Wx class of kernels and with 8 plants...
derived from those in the C sh bz wx class. Among the latter, two plants had received an unmodified fragment chromosome in addition to the structurally normal chromosome 9. It is of interest to note that the ratio of Bz to bz among the sh class of kernels in A of table 4 (22:111) is much the same as the ratio of these two phenotypes among the sh class that was obtained from heterozygotes (normal chromosome 9 with I Sh Bz wx/deficient chromosome 9 with Sh Bz wx/fragment with C sh bz) when these were used as pollen parents in crosses to plants that were homozygous either for C, sh, bz, and wx, or for C, sh, bz, and wx. This ratio was 57 C sh Bz (6 wx: 51 wx) to 206 C sh bz (27 wx: 179 wx).

Barbara McClintock
Perspective:¹

Citation: 79. *McClintock, Barbara. 1957b. Genetic and cytological studies of maize: Types of Spm elements. A modifier element within the Spm system. The relation between $a_{1}^{m-1}$ and $a_{1}^{m-2}$. Aberrant behavior of a fragment chromosome. *Carnegie Institution of Washington Year Book 56, 1956-1957: 393–401. ② *Reprinted in McClintock 1987

Online: https://archive.org/stream/yearbookcarne56195657carn#page/392/mode/2up

¹ Perspective solicited.
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Perspective: Comparative studies relevant to transposon function in plant development.

by Allan M. Campbell, Stanford University

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Comparative Studies Relevant to Transposon Function in Plant Development

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This paper (McClintock 1961) was an attempt by Barbara McClintock to convey the significance of her work on controlling elements to a broader audience than the geneticists who had previously followed it. Although the paper received 35 citations between 1962 and 1964 (ISI-SCI, 1984), so far as I know, the paper had no substantial impact; few if any investigators changed the course of their work because of it.

A word on the historical context: McClintock’s paper appeared in the wake of Jacob and Monod’s (1961) studies on bacterial gene control (the work that motivated McClintock to write it). A modern molecular biologist seeing it for the first time will note the assertion that bacterial repressors are not proteins (a mistake the Pasteur group made and later amended) and should bear in mind that the mechanism of Salmonella phase variation (for which McClintock cites some elegant genetics done by Lederberg & Iino) was not yet known to comprise a specific inversion of a DNA segment that included the promoter for the Phase 2 antigen (Zeig et. al., 1977).

The paper posed some special difficulties for those of us familiar with McClintock’s previous work. Her most relevant papers had seemed to invite geneticists to regard the maize transposable elements as active participants in plant development (McClintock, 1956a,b). She had shown clearly that the elements could transpose and that they could affect expression of nearby genes. She had also shown that a transposable element (i.e., an element able to move about in the genome; such elements are now generally called transposons) could affect the activity of another transposon of the same system from a distance (e.g., Ac affects Ds) (McClintock, 1956a,b). The question that remained (and to some extent still remains) is whether such effects arise solely from circuitry promoting molecular parasitism by the transposon (and selected for that purpose), which only incidentally spills over to influence expression of cellular genes.

In comparing her results to Jacob and Monod’s (1961), McClintock (1961) uses their terms “regulator” and “operator” (always in quotes). A “regulator” was a gene that makes a regulatory protein (in Jacob and Monod’s case, a repressor); an “operator” was a site adjacent to the target gene on which the “regulator” acts. Both “regulators” and “operators” were detected by mutations that altered or inactivated them. Experimentally, McClintock (1961) distinguished “regulators” from “operators” in the maize elements by the fact that a “regulator” could act from any location in the genome (as expected for a gene that produces a diffusible protein product), whereas an “operator” could only affect a target gene adjacent to it. In subsequent decades, geneticists have encountered a plethora of regulatory factors and proximity effects with various physical bases. For this reason, modern geneticists commonly say that regulatory mutations whose effect is independent of their position in the genome (and the elements in which such mutations occur) act in trans, whereas mutations that affect only a target gene closely linked to them are cis-specific.

The Pasteur group (see Jacob, 1960) had noted some similarities between transposable maize elements and bacterial episomes. Bacterial episomes are genetic elements (mostly phages or plasmids) that can replicate either separately from, or integrated into, the bacterial chromosome, sometimes using integration mechanisms analogous to those used by transposons (Jacob and Wollman, 1958; Campbell, 1962.) McClintock (1961), recognizing some of these similarities, compares the activation of expression of a maize gene when an element is removed from the gene’s locus by transposition to the activation of bacterial genes by derepression of a nearby phage (Buttin et al., 1960). The latter effect is now understood as unrelated to excision from the chromosome; it rather results from transcription from the major leftward promoter of phage λ into genes for galactose metabolism that lie in bacterial
DNA adjacent to the phage (Adhya et al., 1974). Understanding at this level was possible only after the mode of attachment to the chromosome (e.g., insertion or lateral attachment) was settled, which was not the case in 1961 for either bacterial episomes or maize transposons (Campbell, 1962).

While recognizing such parallels between the maize elements and bacterial episomes, McClintock (1961) emphasizes that regulatory effects on host gene expression do not require transposability: Transposability may be lost by mutation or rearrangement in plants where regulation remains. That’s why she stresses here the analogy of the regulatory effects in maize to the simpler operator/repressor interactions in bacteria reported by Jacob and Monod (1961).

McClintock illustrates the workings of the maize elements with examples from the Spm (suppressor/mutator) system [mostly taken from experiments previously reported by McClintock, 1956, 1957, 1958; see Fedoroff perspective this volume], pointing out that her other results from maize involved elements with a different specificity than Spm, both of the cis and trans components (see Fedoroff this volume). Apparently one reason she chose Spm for this essay is because its primary trans action on host genes is to repress rather than to activate gene expression. She concludes with the suggestion that some of the effects (especially the enhancement of mutation rates at specific loci) may play a role in normal plant development.

Scientists frequently cherish some dreams about the significance of their own work. The notion that transposons like Ds and Spm might act in normal maize development certainly appealed to McClintock. I can especially relate to it, because I myself have long been attracted to the idea that cooption of bacteriophage (or, more generally, of viral) functions could play an important part in host evolution. I recall once asking Barbara whether the maize elements might be mimicking, rather then causing, normal changes during plant development. I don’t remember her entire answer, but it ended with, “I have no evidence.”

That’s where it stood through the end of her career and beyond. A direct role of transposable elements in plant development remained attractive to her, but she was far too good a scientist to imagine she had proven it. In her Nobel lecture, McClintock (1984) did not mention that hypothesis (see Shapiro perspective this volume). Instead she emphasized the role of genomic stress in activating transposons and indications that transposons can play a role in the stress responses. The ramifications of her thinking along those lines form the basis for the most thoughtful current thinking on the stress response (Fedoroff, 2012; see Weil perspective this volume).

References Cited:


Shapiro, J.A. (this volume). The special character of Barbara McClintock’s Nobel prize address.

Weil, C. (this volume). Transposable controlling elements step out onto the broader scientific stage.

Perspective:¹

Citation: 85. *McClintock, Barbara. 1961b. Further studies of the suppressor-mutator system of control of gene action in maize: Control of $a_{m-2}^m$ by the $Spm$ system. A third inception of control of gene action at the $A1$ locus by the $Spm$ system. Control of gene action at the locus of $Wx$ by the $Spm$ system. Control of reversals in $Spm$ activity phase. Nonrandom selection of genes coming under the control of the $Spm$ system. Carnegie Institution of Washington Year Book 60, 1960-1961: 469–476. ²
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An Incremental Book

3.97
Perspective:¹


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¹ Perspective solicited.
1. Restoration of A1 gene action by crossing over.

Neuffer has undertaken an extensive study of $a_1^{m-3}$ and $a_1^{m-4}$, two independent inceptions of control of A1 gene action by the Ac system, to determine whether a controlling element, presumed to be associated with the A1 gene in each case, could be removed by crossing over, thereby restoring A1 gene action. His results were negative as are those that I have obtained during the course of studies of $a_1^{m-3}$ and $a_1^{m-4}$. My data, however, are limited. My studies of $a_1^{m-2}$, on the other hand, have given quite different results. Restoration of A1 gene action appears to arise from a crossover event which occurs relatively frequently with some states of $a_1^{m-2}$ but infrequently, if at all, with others.

Nelson (personal communication) has shown that by means of a crossover, Wx gene action may be restored in tests conducted with $wx^{m-1}$ and $wx^{m-5}$, two independent inceptions of control of action of the Wx gene by the Ac system, and also with $wx^{m-6}$, controlled by the Spm system. His method of analysis is precise in that it
allows placement of the component that is removed by the crossover.

Gene action at the $a_1m^{-2}$ locus is under the control of the $Spm$ system. Initially, $Spm$ was associated with this locus. Later, it was possible to isolate a number of instances in which no evidence was given of the presence of $Spm$ at the $a_1m^{-2}$ locus. Action of the $A_1$ gene, nevertheless, remained under the control of the $Spm$ system. States 79775 and 7995, Table 1, are instances of this. Many studies of $a_1m^{-2}$ are conducted with plants that are $a_1m^{-2} Sh_2/a_1 sho$ in constitution and many such plants have been crossed with plants that are homozygous for $a_1$ and $sh_2$. The $a_1$ mutant utilized in these studies is the standard recessive that responds to $Dt$, but not to $Ac$ or $Spm$. The majority of the tests that produced the data given in line 1 to 6 of Table 1 utilized the heterozygote as the ear parent. This table was constructed mainly to illustrate the frequency of appearance of the $A_1$ phenotype in the $sh_2$ class of kernels in some types of cross and their absence in this class in others. It should be stated that these data were obtained from crosses made in years in which no plants were present in the field that had $A_1$ and $sh_2$ in chromosome 3.

The data in line 1 of Table 1 were obtained from tests of $A_1$ mutants of $a_1m^{-2}$. These mutations occurred in a chromosome carrying $a_1m^{-2}$ and $Sh_2$ and in plants that had an $Spm$ whose transposition-inducing component acts early in plant development. All of these $A_1$ mutants were stable in the presence of $Spm$. Line 2 is constructed from data obtained from tests of plants carrying a stable mottled mutant of $a_1m^{-2}$. (This phenotype is described in Carnegie Institution of Washington Year Book No. 61, 1962.) These mutants do not produce a typical $A_1$ phenotype. However, in the test-crosses, 2 $sh_2$ kernels expressing a typical $A_1$ phenotype appeared. The data in line 3 came from testcrosses of plants that had $Spm$ associated with the $A_1$ locus but the transposition-inducing component of this $Spm$ acts late in plant and kernel development and, in this regard, it is very stable. (Kernels with this $Spm$ are illustrated in E, Plate I of my report appearing in the Carnegie Institution Year Book No. 65, 1964.) It does not allow any germinal mutations to occur at $a_1m^{-2}$ nor at $a_1m^{-1}$ or $wx^{-8}$ which have been tested for this. Nevertheless, 5 $sh_2$ kernels with very clearly expressed $A_1$ phenotypes appeared on the ears that contributed the data in line 3 in the table. None appeared in the $Sh_2$ class. In contrast to this, no kernels with this phenotype appeared in tests of plants having an inactive $Spm$ associated with the $a_1m^{-2}$ locus, either in the $Sh_2$ or $sh_2$ class.
<table>
<thead>
<tr>
<th>Phenotype of Kernels Produced by Crosses of Plants That Were Homozygous for $a_1$ and $sh_2$ with Plants That Had $al$ or $sh_2$ in One Homologue</th>
<th>$sh_2$ chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh2</td>
<td>Colorless</td>
</tr>
<tr>
<td>A1</td>
<td>12</td>
</tr>
<tr>
<td>Mottled</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>8,996</td>
</tr>
<tr>
<td>2</td>
<td>10,399</td>
</tr>
<tr>
<td>3</td>
<td>12,131</td>
</tr>
<tr>
<td>4</td>
<td>10,813*</td>
</tr>
<tr>
<td>5</td>
<td>16,033*</td>
</tr>
<tr>
<td>6</td>
<td>8,780*</td>
</tr>
<tr>
<td>7</td>
<td>3,626</td>
</tr>
<tr>
<td>8</td>
<td>3,990</td>
</tr>
</tbody>
</table>

### Constitution of Sh2 chromosome

1. A1; mutant of $al^m-2$
2. Mottled; mutant of $al^m-2$
3. Inactive $al^m-2$
4. State 79713 (in cross)
5. State 7995 (in cross)
6. $al^m-5$ (in cross)
7. $al^m-5$ (in cross)
8. $al^m-5$ (in cross)

* A few of these kernels received a crossover chromatid with $al^m-2$ but its presence in most such kernels cannot be detected visually.
The phenotypes of the \( S_h \) class in this cross, line 4, and in those in lines 5 and 6, have been omitted from the table because there are a number of different types and these would be difficult to arrange in this table. None of these, however, is \( A_1 \) in phenotype. States 7977B and 7995, lines 5 and 6, also produced some \( A_1 \) \( s_h \) kernels on the testcross ears. An active \( S_{pm} \) was not present in the heterozygous parents. In some crosses, it was introduced into many kernels by the \( a_1 \) \( s_h \) pollen parent that also was homozygous for \( w_x \). Some of the plants in lines 5 and 6 had \( w_x^{m-8} \) in one chromosome 9. Three of the 10 \( A_1 \) \( s_h \) kernels in lines 5 and 6 received \( w_x^{m-8} \) from the ear parent and \( S_{pm} \) from the pollen parent. The \( A_1 \) expression in these three kernels was completely stable but that of the \( w_x \) gene was not. \( w_x^{m-8} \) responded to the introduced active \( S_{pm} \) by producing a number of endosperm sectors exhibiting various levels of \( W_x \) gene action.

Lines 7 and 8 of Table 1 are included to illustrate that no \( A_1 \) \( s_h \) kernels appeared in testcrosses conducted with a state of \( a_1m^{-5} \) having an \( S_{pm}^{W} \) associated with it. This \( S_{pm}^{W} \) undergoes frequent mutation to a state that allows early occurring transposition and thus early occurring mutations to high alleles of \( A_1 \).

Whether or not a controlling element may be removed from a locus by crossing over may well depend on the "state of the locus", as suggested by the data in Table 1, and also upon the organization of components in the comparable region of the homologue.

Two other studies aimed at removing a controlling element from the vicinity of the genes it can affect are reported below.

B. McClintock
Perspectives on Nobel Laureate
Barbara McClintock’s Publications (1926-1984):
A Companion Volume
edited by Lee B. Kass

Perspective:\n

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① Perspective solicited.
2. Attempts to separate Ds from neighboring gene loci.

Early in the study of transposition of Ds to various locations within the short arm of chromosome 9, two instances of its insertion just distal to Sh1 were found, the first instance in 1948 and the second instance in 1949. In both instances, Ds remained in this location thereafter. Although it did not transpose away from this location, it responded to Ac by producing dicentric chromatids and also a series of changes affecting the genes located to either side of it. The types of change were described in the Carnegie Institution of
Washington Year Books Nos. 51 to 55 covering the years 1952 to 1956. In the presence of Ac, one of these changes induced a modification affecting the adjacent proximal chromosome segment carrying the genes Sh1 and Bz1. Gene expression of Sh and Bz was nullified. In the presence of Ac, however, return to high levels of Bz gene action occurred but no changes to Sh expression were ever noted. Tests indicated that the segment Ds sh bz was inherited as a unit, as illustrated by the data given in Table 2. It may be mentioned that the presence of this unit has a strong influence on crossing over in the chromosome segment proximal to it. Studies of this were made, initially, with 10 sister plants. Five of these were Ds sh bz Wx/Sh bz wx; no Ac in constitution, and five had normal chromosomes 9 with the markers sh bz Wx and Sh bz wx. The ears of these plants received pollen from plants that were homozygous for C, sh, bz, and wx. Crossing over between Sh and Wx amounted to 24.6% (3,332 kernels) in the former plants and 12.6% (3,423 kernels) in the latter plants. Crossing over between the Ds sh bz unit and C was near normal, amounting to 4.1% in a total of 5,470 kernels on testcross ears.

Altogether 16 plants were examined, each derived from a kernel that had received a germinal Bz mutant. These Bz kernels appeared on ears produced by crosses similar to those shown in Table 2. Tests were conducted with these 16 plants and extended tests were conducted with the progeny of four of them. These tests indicated that in each case, the mutation to Bz was not accompanied by removal of Ds nor did the responsible event alter the unit of inheritance which now was Ds sh Bz. In the presence of Ac, dicentric chromatid formations occurred just distal to the locus of Bz. Return to bz expression also occurred in all well examined cases although the frequency of this varied with the different mutants. In the absence of Ac, however, Bz gene expression was completely stable. The Bz mutants differed from one another and from the original Bz in strength of Bz gene expression. Crossing over between the Ds sh Bz unit and Wx again was very high, amounting to approximately 25% in backcross tests using the heterozygote as an ear parent, and approaching 30% when the heterozygote was used as a pollen parent.

In order to determine whether or not Ds could be removed from the vicinity of the mutant Bz locus by crossing over, tests were conducted with plants that were C Ds sh Bz Wx/C Sh bz wx and had no Ac. These were used as ear parents in crosses with plants that were homozygous for C, sh, bz, and wx and had no Ac. Among a total of 16,578 kernels this cross produced, the
Table 2
Phenotypes of Kernels on Ears of Plants That Were C Sh bz/C Da sh bz'm-4 in Constitution and Had 1 Ac, Not Linked With These Markers, Produced by the Cross with Plants That Were Homozygous for C, sh, bz, and wx, and Had No Ac

<table>
<thead>
<tr>
<th>Pigment in Aleurone Layer</th>
<th>Totally Bz</th>
<th>Spots of Bz in a Bz Background</th>
<th>Totally bz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh₁</td>
<td>0</td>
<td>0</td>
<td>9,177</td>
</tr>
<tr>
<td>sh₁</td>
<td>9</td>
<td>4,291</td>
<td>4,656</td>
</tr>
</tbody>
</table>

Table 3
Phenotypes of Kernels on Ears Produced by Reciprocal Crosses Between Plants That Were C Sh Bz Wx/C sh bz wx in Constitution and Had No Ac With Plants That Were Homozygous For C, sh, bz, and wx and Had No Ac or Were Homozygous for C, sh, bz, and wx and Had One or More Ac

<table>
<thead>
<tr>
<th>Parentage of heterozygote</th>
<th>Phenotypes of Kernels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sh Bz</td>
</tr>
<tr>
<td></td>
<td>Wx Wx</td>
</tr>
<tr>
<td>case I</td>
<td></td>
</tr>
<tr>
<td>Bar</td>
<td>1,201 318</td>
</tr>
<tr>
<td>Pollen</td>
<td>1,546 527</td>
</tr>
<tr>
<td>case II</td>
<td></td>
</tr>
<tr>
<td>Bar</td>
<td>91 24</td>
</tr>
<tr>
<td>Pollen</td>
<td>397 157</td>
</tr>
</tbody>
</table>


following phenotypes appeared: 8,337 sh Bz : l sh bz : 2 Sh Bz : 8,238 Sh bz. The one sh bz kernel was wx and the two Sh Bz kernels were Wx. Plants were grown from each of the latter two kernels. Both plants were C Sh Bz Wx/C sh bz wx in constitution and had no Ac. Thus, contamination was excluded as the reason for the phenotype of the kernel producing each of these plants. Both plants, and also the progeny of one of them (case I, Table 3), were crossed reciprocally with plants that were homozygous for C, sh, bz, and wx, and had no Ac and with other plants that were homozygous for c, sh, bz, and wx, and had one or more Ac. No evidence was given in the latter cross of the presence of Ds in the C Sh Bz Wx chromosomes. The phenotypes of the kernels that appeared on the ears produced by these crosses is given in Table 3. Transmission of the C Sh Bz Wx chromosomes is normal through pollen and egg and crossing over between the marked intervals conforms with that expected to occur between two normal chromosomes 9. It is evident from this series of tests that removal of Ds, or its effects, has restored normal crossover potentials between the loci of Sh and Bz.

That crossing over may have removed Ds in the above described cases is supported by a much more extensive series of studies that were conducted with selected progeny of plants carrying I Ds Sh Bz in this order in both chromosomes 9 and also Ac. Seven independent instances of Ds-induced nullification of gene action in the chromosome segment immediately distal to Ds, and including the I locus, were isolated and each examined extensively. (Descriptions of these cases are given in the previously mentioned Carnegie Institution Year Books.) The events responsible for these nullifications did not remove Ds. It remained just distal to Sh. The null segment in each case behaved as if it were a deficiency although no evidence of deficiency was given by the meiotic prophase chromosomes. It was decided to use these 7 cases in order to determine if crossing over could occur between the nullified region and Ds or between Ds and Sh. These tests were conducted in 1955 and 1956 but were not reported earlier because a part of the study was never completed.

Plants with no Ac that had a chromosome 9 with the null region and also the markers Ds Sh Bz Wx, and a normal homologue with the markers C sh bz wx, were used as ear parents in crosses with plants that were homozygous for c, sh, bz, and wx, and had no Ac. The resulting ears were examined for kernels in the Sh class that were Bz pigmented and for kernels in the sh class that were colorless. These were the kernels of importance to this study. The results of these tests are given in
summary form in Table 4. The percent crossing over between Sh and Bz (region 2) and Bz and Wx (region 3) observed in these tests is also given for reference. In the three cases where the same testcross was conducted both in 1955 and 1956 (cases 1, 4, and 7 in the table) a striking degree of consistency was noted with each case in the amount of crossing over that occurred in both years within each of the three tested regions.

Plants were grown in the summer of 1956 from the types of kernels indicated in the last three columns of Table 4. Tests conducted with the plants derived from the C Sh Bz Wx kernels, and continued with their progeny, were aimed at determining the following: presence or absence of Ds in the C carrying chromosome, percent crossing over between C and Sh, degree of transmission of the C Sh Bz Wx chromosome through the pollen, and the phenotype of the seedlings that are homozygous for this chromosome. Because the number of C Sh Bz kernels was significantly larger than the number of colorless, sh kernels in cases 3, 4, and 7, either contamination or some other cause was suspected to be the reason for this. Apparently, this is true. Three of the plants derived from the 20 selected C Sh Bz Wx kernels (1 from case 4 and 2 from case 7) had the same constitution as the ear parent plant. Another kernel produced a plant that had the null segment and Ds Sh Bz Wx in one chromosome 9 and c sh bz wx in the homologue. The Bz phenotype in the kernel producing this plant probably resulted from the action of blotched on the c gene as blotched segregated in one of the tested ears of this plant. A strong expression of blotched appears occasionally and unexpectedly in the cultures. The phenotype of the remaining 16 kernels did not result from contamination or misclassification. Each had received a chromosome 9 from the heterozygous parent with the markers C Sh Bz Wx. No evidence was given of the presence of Ds in any one of these 16 chromosomes. All appeared to be quite normal. Crossing over occurred with expected frequencies between the marked intervals, and the homozygotes were normal in appearance. It was concluded that each of these 16 chromosomes was produced by a crossover that had occurred between Ds and Sh.

All of the plants derived from the 14 colorless sh kernels were bz in phenotype. One plant was very small and produced no pollen or ear. Testcross ears were obtained from the remaining 13 plants, and from their progeny. All 13 plants had received a chromosome 9 with the null segment and also sh and bz. The presence of Ds in this chromosome was detected in the progeny of 4 of these initial 13 plants. Its exact location was not
Table 4
See Text for Explanation of Contents of Table

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Year of Cross</th>
<th>Total No. of Kernels</th>
<th>Phenotypes of &quot;crossovers&quot; in region 1</th>
<th>Percent crossing over+ Regions</th>
<th>Phenotypes of selected kernels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C Sh Bz c sh</td>
<td>1 2 3</td>
<td>C Sh Bz Wx c sh Wx</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1955, 1956</td>
<td>11,989</td>
<td>5 8</td>
<td>0.06 2.7 24.4</td>
<td>3 3</td>
</tr>
<tr>
<td>2</td>
<td>1956</td>
<td>7,261</td>
<td>6 10</td>
<td>0.16 2.9 27.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1956</td>
<td>6,066</td>
<td>17 8</td>
<td>0.54 2.4 31.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1955, 1956</td>
<td>12,910</td>
<td>15 8</td>
<td>0.23 2.0 24.2</td>
<td>8 2</td>
</tr>
<tr>
<td>5</td>
<td>1956</td>
<td>7,391</td>
<td>14 14</td>
<td>0.35 2.0 31.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1956</td>
<td>7,100</td>
<td>12 8</td>
<td>0.31 2.3 29.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1955, 1956</td>
<td>15,078</td>
<td>39 32</td>
<td>0.51 3.6 16.6</td>
<td>9 1 8</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>70,795</td>
<td>107* 88+</td>
<td>20</td>
<td>1 13</td>
</tr>
</tbody>
</table>

+ Calculated from the C carrying classes of kernels.

* Includes 5 double crossovers, regions 1 and 3

*+ Includes 5 double crossovers, regions 1 and 3
determined although it could be placed distal to the Wx locus. Tests of the presence of Ds in this chromosome in the progeny of the remaining 9 plants were not completed.

The project was discontinued at this juncture even though crosses had been made to obtain plants with proper constitution to determine the location of Ds in the chromosomes carrying the null segment. The main questions -- whether crossing over occurs and where this may occur -- appeared to be answered by the results already obtained. It was occurring, and between Ds and Sh and not between the null segment and Ds. At the time, it was considered that the rewards that could be expected by pursuing this project would be too meager to justify the considerable amount of effort involved in the pursuit. It should be emphasized, however, that this Ds, in the presence of Ac, causes modification in expression of Sh, located proximal to it, and this has occurred to Sh in those chromosomes that have the null segment located just distal to Ds.

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Online: https://archive.org/stream/yearbookcarne64196465carn#page/526/mode/2up/search/mcclintock

1 Perspective solicited.
Perspective:¹

Citation: 92. *McClintock, Barbara. 1965d. The control of gene action in maize. pp. 162–184. In: *Genetic Control of Differentiation, Brookhaven Symposia in Biology: No. 18*. Biology Department, Brookhaven National Laboratory, Upton, N.Y. [No editor listed; H. H. Smith Chairman of the Symposium Committee.] ²

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Perspective: forthcoming

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Perspective: The special character of McClintock’s Nobel Prize address.

by James A. Shapiro, University of Chicago

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See also:


The Special Character of Barbara McClintock’s Nobel Prize Address

James A Shapiro

Introduction

Her choice of content makes Barbara McClintock’s 1983 Nobel Prize address an extraordinary scientific document. Despite the fact that she received the most prestigious of scientific honors “for her discovery of mobile genetic elements,” her speech reviews her career since 1931 (McClintock 1931; Birchler, this volume) without describing any of the evidence for transposition, the genome restructuring process cited as her major achievement. She simply tells us that many of her observations involving transposable controlling elements have been repeated in numerous organisms, but she does not explain how she demonstrated their genetic mobility in maize. Her text treats the underlying mechanisms as well-established science not requiring specific review.

Why did McClintock relegate what the Nobel Prize committee considered her crowning discovery to so few words? The answer, I believe, is that she had something she considered far more important to discuss. In conversation, McClintock frequently said that regulation, not transposition, was the major focus of her research from the 1940s onward. She did not understand regulation simply as a molecular mechanism. Rather, she thought of regulation as a deeply biological phenomenon, exemplifying the vital processes of sensing, evaluating, responding, repairing and adapting.

The Nobel Prize address contains words professional scientists rarely apply to living cells and organisms, like “thoughtful” and “wise.” As the neurobiologist Dennis Bray points out in the introduction to his recent book, Wetware: A computer in every living cell, McClintock was the first modern biologist to ask that future research “determine the extent of knowledge the cell has of itself, and how it utilizes this knowledge in a “thoughtful” manner when challenged.”

Since we recognize McClintock as among the 20th Century’s greatest biologists, we need to ask how she validated and clarified her use of such unabashedly anthropomorphic language in a speech about the genome. This question leads us immediately to the heart of her true subject matter and also to the special problems a 21st Century reader faces in understanding this paper.

McClintock viewed her 60+ year career studying the maize genome as a never-ending series of lessons about the amazing sophistication of cellular cognition and control. At the same time, she felt that others could only appreciate what she had learned if she described each lesson in detail. Because of McClintock’s need to be straightforward about the import of her conclusions and also precise in her descriptions of the underlying observations, her papers in general (and this one in particular) make special demands on the reader, who frequently has to shift back and forth between detailed accounts of maize cytogenetics and the broadly stated implications of her findings.

One further feature of McClintock’s thinking and writing has proven challenging for contemporary readers of her work. Today, we are accustomed to papers that conclude with summarizing models and explanatory schemes. McClintock, on the other hand, was deeply skeptical of what she called “the NOW explanation.” Her long career exposed her to many concepts and ideas that were inevitably superseded by later discoveries and theoretical formulations. She expressed how her own experience in the founding years of cytogenetics had provided her “the
pleasure of witnessing and experiencing the excitement created by revolutionary changes in genetic concepts that have occurred over the past sixty-odd years. I believe we are again experiencing such a revolution.” Given this background, she preferred to state the meaning of her observations without attempting to provide explanations that would ultimately prove inadequate.

McClintock was comfortable in saying, as she did in this address, that certain well-documented phenomena “are beyond our present ability to fathom.” This was not a statement of futility but rather a well-founded recognition that science had to progress both technologically and conceptually before certain problems could be fruitfully investigated. Remember that McClintock received the Nobel Prize over a decade before the scientific community started to become aware of the importance of RNA-directed regulation in general (and of epigenetic states in particular; see Fedoroff 2013 & this volume). The revelation of this unexpected layer of complexity in cell control systems would not have seemed unusual to her because she had already lived through six decades of comparable revelations. To her, new and surprising shifts in thinking were recurring, inevitable and enjoyable.

The Cognitive Lessons McClintock Learned From Maize

McClintock organized her presentation in the chronological order in which key observations and realizations occurred. She left out studies on the mechanics of transposition because she was interested in explaining how her maize plants responded to “shocks” experienced in the course of genetic experimentation. Her main discoveries concerned the ability of plant cells to sense and repair genome damage. The major surprise in her work was the totally unanticipated discovery that maize genomes contain latent elements that can be activated to alter patterns of genome expression and restructure chromosomes. Recognizing that her focus was on how maize cells sense damage and respond appropriately makes the paper easier to follow and helps illuminate the logic of her narrative.

McClintock’s introduction gives the reader an overview of her argument. She uses the contemporary examples of heat shock and “SOS” responses to remind us that we take certain programmed genome adaptations to damage stimuli for granted, but she immediately places them in the cognitive context she will employ throughout (“Some sensing mechanism must be present in these instances to alert the cell to imminent danger…”). Then she moves on in a very condensed fashion to mention less programmatic responses to damage, her own surprising experience “in the mid-1940s” and the study of X-ray mutagenesis, before she reminds us that we are in the midst of an upheaval in our “views of components of cells and how they operate…” This section brings in many relevant topics but only makes sense once the reader has become familiar with the whole story McClintock is telling (see Kass & Chomet 2009).

Following the introduction, McClintock moves to a brief and technical description of the 1944 experiment that led to her discovery of mobile genetic elements (Kass & Chomet 2009, p. 27). These findings led to her Nobel Prize, which may be the reason she brings them in outside of the historical sequence that would have made them easier to follow. She tersely explains the crosses that she designed to use her previously acquired knowledge of chromosome breakage and repair to isolate deficiencies (deletions) removing segments from the short arm of chromosome IX. Instead of the expected deficiencies, she recounts how she was surprised to obtain genetically unstable plants that produced variegating sectors in the leaves and other parts of the plant. Noting that these sectors often appeared in pairs (“twin sectors”) gave her the idea that allowed her to track down the source of variegation as transposable controlling elements “that could regulate gene expressions in precise ways.”

But McClintock does not really explain how she solved this intricate puzzle of genetic mobility. That story is recounted at length in the 1987 collection of her papers. She had a different point to make about cell sensitivity, which required a historical narrative of her studies on responses to chromosome breakage. Accordingly, the next three sections of the address tell the story of how McClintock learned that maize cells sense the presence of broken chromosome ends and activate latent transposable elements when they cannot easily repair them.

Lesson One

Historically, the first lesson about genome repair came from her experience beginning in 1931 with Stadler’s mutant X-irradiated maize stocks (McClintock 1931b, Birchler this volume). This was a time, she noted, when “our
knowledge of chromosomes and genes was limited. In retrospect we might call it primitive.” Nonetheless, she was eager to study them, being “delighted to do so, as this would be a very new experience.” She realized that the altered phenotypes obtained with X-rays were not due to the expected “gene mutations” but resulted from deficiencies and other rearrangements. These changes were explicable as the results of fusions of two chromosome ends following X-ray induced breakage events. This was McClintock’s first demonstration of genome repair capabilities, and she confirmed her ideas by studying the behavior of ring chromosomes; these were subject to forming double dicentric rings by recombination that ruptured to produce two broken ends in each daughter cell that then resealed after cell division.

Summarizing this first lesson about break repair from her cognitive perspective, McClintock writes: “The conclusion seems inescapable that cells are able to sense the presence in their nuclei of ruptured ends of chromosomes, and then to activate a mechanism that will bring together and then unite these ends, one with another. And this will occur regardless of the initial distance in a telophase nucleus that separated the ruptured ends. The ability of a cell to sense these broken ends, to direct them toward each other, and then to unite them so that the union of the two DNA strands is correctly oriented, is a particularly revealing example of the sensitivity of cells to all that is going on within them. They make wise decisions and act upon them.”

Lesson Two

A second major lesson came when McClintock decided to find out what happened when there was only a single broken chromosome end in a cell. Again, in this section of the address, she explains the experimental procedures and chromosome events in terse, technical language and provides three figures to illustrate her experiments.

She discovered that chromosomes with one broken end replicate, fuse their ends to form a dicentric chromosome and then undergo breakage again during mitosis (the “breakage-fusion-bridge” or BFB cycle) in haploid microspore and triploid endosperm cells. However, in diploid zygotes and embryo cells, the broken end is quickly “healed” so that no further fusions or breaks occur. McClintock mentions that she found a recessive mutation (now lost) that prevented the healing process. These experiments showed that a broken end could be sensed and capped with a telomere in embryo cells but that the repair process is not expressed in other cell types.

Lesson Three

The most important lesson about sensitivity to broken ends was the surprising outcome of McClintock’s “failed” experiment looking for chromosome IX deficiencies that unexpectedly led to the discovery of transposable controlling elements.

Where did these previously unknown elements come from? McClintock reasoned that they must have been latent in the genome and become active in response to the sensing of an uncapped broken end in the microsporocyte divisions before fertilization. To confirm this hypothesis, she looked for activation of the Dotted (Dt) element that Rhoades had shown to generate variegated expression of the standard recessive a allele of the A locus.

Because homozygous a/a plants produced colorless kernels that reverted to display dark spots in the presence of Dt, new activations should be easy to detect. McClintock found such activations in endosperm nuclei fertilized by plants that had undergone the BFB cycle in the previous haploid generation. Doerschug later confirmed this result by using the same method to obtain active, transposable Dt elements in embryos.6

McClintock’s conclusion was: “Activation of potentially transposable elements, as well as other structural modifications of the chromosomes not considered here, are recognizable consequences of the cell’s response to the continuing trauma.” In other words, genome monitoring does not only involve turning on repair functions; it also activates systems that create new genome configurations. In McClintock’s perspective, hereditary change is a cognitive response to damage.

How Widely Applicable Are The Observations And Conclusions Drawn From Maize?

After laying out the experiments that convinced her of maize cell sensitivity to “genome shocks,” McClintock

addresses the extent to which her findings have parallels in other organisms. In keeping with her treatment of transposition as a well-established phenomenon not needing fuller explanation, she only makes a few terse references to the detection and application of mobile genetic elements in a wide variety of other organisms.

Instead of discussing the mechanisms of genome restructuring, McClintock places the emphasis on “the numerous homeostatic adjustments required of cells.” She takes a very broad view of this subject and links it to developmental genome control during the morphogenesis of multicellular organisms. Here she brings in a whole new series of stimuli that induce complex but programmatic responses: the formation of insect-induced leaf galls and bacterially-induced root nodules in plants. She links these “reprogrammings” to the ability of “a single genome” to encode “two brilliantly designed organisms, the caterpillar and the moth.”

Her point is that we are still at the beginning of understanding how cells extract information from their genomes: “…we know little of the potentials of a genome. Nevertheless, much evidence tells us that it must be vast.” Since McClintock often suggested insect galls as potential material for molecular study, her goal seems to be to set out key elements of a future research agenda on whole-genome regulation.

After pointing out how much remains to be learned about realizing “the potentials of a genome,” McClintock shifts the focus to genome modification and restructuring “when confronted with unfamiliar conditions.” She cites examples of genome-wide changes that scientists in the early 1980s rarely thought about (but which we currently view from an epigenetic perspective): nuclear reprogramming of tissue culture cells and the shift from somatic to flower (germinal) development in plants.

McClintock links these genome-wide changes to the need for whole genome regulatory modifications during normal sexual reproduction (gamete formation, fertilization, and zygote development) as well as during vegetative plant reproduction from cuttings. Then she moves on to the abnormal generation of whole plants from tissue culture cells. It is in this abnormal situation that phenotypic and genomic changes arise (a process often called “somaclonal variation”). McClintock emphasizes that such changes “could be potent sources for selection by the plant breeder, and incidentally, for theoretical ponderings by the biologist.”

From tissue culture and plant regeneration, McClintock moves on to other unpredictable but nonetheless common events that lead to genome restructuring, such as the activation of transposable elements by RNA virus infection and interspecies crosses. She places particular emphasis on the role of interspecific hybridization in formation of a new plant species, Triticale (x Triticosecale Wittm.), and how important genome restructuring has been in animal evolution, citing the chromosome fusions in Muntjak deer and movements of heterochromatic blocks in copepods of the Cyclops group.

Considering unusual events triggering genome change leads McClintock to propose a connection between “shocks,” chromosome restructuring, and species change. Clearly, she ranks evolution among the areas subject to “theoretical ponderings by the biologist.” In her concluding summary she returns to this point when she says that “illustrations from nature are included because they support the conclusion that stress, and the genome’s reactions to it, may underlie many species formations.”

**How Has McClintock’s Cognitive Perspective Held Up Since 1983?**

It is now over 27 years since McClintock received the Nobel Prize. In that interval, there has been tremendous progress in our knowledge of molecular mechanisms of sensing, regulation, DNA repair, mobile genetic element activities and genome rearrangements. In addition, whole genome sequencing has provided unimpeachable documentation of all these classes of genome restructuring events involved in evolutionary transitions (Shapiro 2011). Altogether, these developments have reinforced McClintock’s views on genomic change in response to stress and begun to answer the challenge she posed at the very end of her address, when she said, “We know nothing, however, about how the cell senses danger and instigates responses to it that often are truly remarkable.”

Among the many developments in the molecular cell biology of the genome that have emerged since 1983, the following are particularly relevant to McClintock’s observations and the conclusions she drew from them:

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(i) The importance of genome monitoring and checkpoint execution in maintaining genome stability. The articulation of the checkpoint concept by Weinert & Hartwell in 1988 introduced a cognitive element (viz. damage sensing, signaling and adaptive regulatory response) to the molecular biology of cell cycle control. Our knowledge of the molecular components of checkpoint monitoring and signaling systems has increased tremendously since then.9

(ii) The discovery of DS break repair centers in eukaryotic nuclei. Key among genome monitoring modalities in eukaryotes are detection of broken DNA molecules by ATM-related proteins, their marking with a complex of proteins unique to broken ends, transmission of information about the presence of the break, and active transport of the broken ends to subnuclear repair centers for correction by homologous recombination (HR) or non-homologous end joining (NHEJ).10 NHEJ of broken ends from different sites and chromosomes is fundamental to the kinds of genome restructuring McClintock described in her maize plants.11

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(iii) The documentation of whole genome duplications (WGDs) at key points in evolution. McClintock’s view that shocks such as interspecific hybridization play a key role in formation of new species fits well with sequence data documenting the occurrence of WGDs in evolution of new species, genera and families ranging from yeasts and protozoa to vertebrates and many groups of flowering plants (whose rapid diversification seemed to be “an abominable mystery” to Darwin). WGDs are commonly observed in the allotetraploid progeny of interspecific hybrids, and their occurrence is widely seen as a source of genome instability and rapid phenotypic diversification.

(iv) The elucidation of RNA-directed epigenetic control regimes for silencing mobile genetic elements. McClintock’s address places great emphasis on the activation of silent mobile elements in the genome as a totally unpredictable cognitive response to the detection of a single broken end that cannot readily be repaired. Although quite a few distinct control mechanisms have been documented for individual mobile elements, the most general regulatory mode for transposons and retrotransposons in eukaryotes involves their incorporation into silent heterochromatin. This epigenetic control is directed by small siRNAs transcribed from specialized DNA segments that preserve a genomic memory of earlier element invasions.

It is therefore of the highest significance that we are learning of how sensitive genome stability and epigenetic chromatin configurations are to many kinds of stimuli, or “shocks” (to use McClintock’s term). A tabulation


of these stimuli can be found online at http://shapirobsd.uchicago.edu/TableII.7.shtml and http://shapirobsd.uchicago.edu/TableII.10.shtml. In other words, the molecular study of mobile genetic elements has brought us to see them as reflecting a cell’s ability to sense disturbances and modify the epigenetic regulatory status of its genome.

Considering our growing knowledge of sensory processes in cell cycle regulation, DNA repair, and the control of genome stability, there can be no question that molecular biology is bringing us ever closer to McClintock’s cognitive view. In addition, genome sequencing is confirming her insight that cellular responses to shocks of all kinds have played key roles at major steps in evolution (Shapiro 2011).

Since 1983, we have made major advances towards deciphering “how the cell senses danger and instigates responses to it that often are truly remarkable.” Nonetheless, our knowledge remains fragmentary; it is composed more of a long and often confusing parts list than of functional insight into how cellular cognition operates. If we are to succeed in formulating a comprehensive picture that integrates our molecular discoveries with the adaptive survival capabilities of living organisms on all time scales, we will certainly need to ponder deeply why McClintock calls cells “thoughtful” and “wise” when it comes to using their genomes. In practical terms, this means developing novel information-processing concepts and experimental models to explore the extraordinarily sophisticated capabilities that living cells apply to extracting and writing genomic data and inherited biological programs.

References

Birchler J. this volume. Summary and Analysis of “Cytological observations of deficiencies involving known genes, translocations and inversions in Zea mays.”


Fedoroff, N. this volume. McClintock and Epigenetics: Insights from Changes in the Phase of Transposon Activity.


204. Trauma as a Means of Initiating Change in Genome Organization and Expression. B. McClintock, Carnegie Institution of Washington, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

Responses of genomes to shock may be programmed or they may be improvised and result in various types of genome modification. Cells of maize in which one arm of one chromosome is continuously restructured over a limited number of successive mitotic cycles respond by activating potentially transposable elements that previously could not be identified as such. This sensing occurs almost immediately as evidenced by activations detected as early as the second mitotic division following initiation of the cycle. Insertion of such an element at a gene locus may start a sequence of altered types and patterns of gene expression. Besides such activations the trauma may initiate major chromosomal reorganizations. Evidence from various sources supports the notion that trauma ("genome shock") may be effective in providing a wide range of new and unanticipated organizations and expressions of a genome.
Perspective:¹


¹ Perspective solicited.
INTRODUCTION

Transposable elements were discovered in maize long before their presence was recognized in other organisms. This discovery was the logical outcome of a series of observations of maize chromosomes, each of which revealed an unanticipated and significant aspect of their behavior. These studies began with my observations of the events occurring to ring-shaped chromosomes during chromosome replication. As initially reported in 1932 (12), the replication of ring-shaped chromosomes usually produces two sister chromatids that separate freely in the following mitotic anaphase. However, as the amount of chromatin in the ring increases, replication is increasingly accompanied by a sister-chromatid exchange that produces a double-size ring with two centromeres. In this case, when the centromeres of the two sister chromatids are pulled to opposite spindle poles in the following anaphase, two chromatid bridges are formed between them. These bridges come under increasing tension as the centromeres approach the poles. The tension eventually becomes sufficient to rupture the two bridges, with the break occurring at an unspecified place that is generally different in each chromatin strand. Each telophase nucleus therefore receives a linear chromosome whose two ends have just been created by rupture events. What happens next is extraordinary and proved to be highly significant for later studies: the two ruptured ends find each other and "fuse" (are permanently ligated together). This event reestablishes a ring-shaped chromosome with a single centromere, but one whose genetic composition usually is modified because of the non-equivalent locations of the breaks in the two anaphase bridges. In the period from 1932 to 1941, some of the consequences of these genetic modifications were examined (17, 18, 19, 23). Two general aspects of chromosome behavior discovered in the 1932 study would turn out to be crucial for later transposition studies: the approach of broken ends of chromosomes towards one another within the nucleus and the precise ligation of the ruptured ends.

An additional aspect of chromosome behavior that was important for the discovery of transposable elements relates to the behavior of a single ruptured end of a chromosome after it enters a telophase nucleus. This end has no partner to fuse with. In the spring of 1937 the question was asked: What would occur to such a single broken end during chromosome replication, as deduced by viewing this chromosome in the following mitosis? A means of generating such chromosomes was available. It utilized plants that were heterozygous for an inversion in the long arm of chromosome 4 of maize. A crossover within the limits of the inverted segment during meiotic prophase produces a dicentric chromosome plus an acentric fragment, each missing a large segment of chromosome 4. The acentric fragment is lost from the nucleus, either during a meiotic mitosis or in a subsequent mitosis, because it lacks a centromere to direct it to a spindle pole. However, the dicentric chromosome attaches to the spindle, and the movement of its two centromeres towards opposite spindle poles at the first meiotic anaphase produces a single chromatid bridge that is ruptured as the tension on it increases. A chromosome with a single, newly ruptured end then enters each telophase nucleus.

The fate of such a chromosome could be observed by examination of the chromosomes of the generative cells that are formed after male meiosis. In the maize tassel, meiosis occurs in cells of anthers in the florets. The meiotic process initially gives rise to four haploid spores. Each spore then undergoes two mitoses. The first of these results in a tube nucleus and a generative cell;
the generative cell then divides to form the two sperms of the pollen grain that will function in fertilization. During the first of these two mitoses, the chromosomes are readily observed from prophase through telophase. Among the spores undergoing mitosis, those that had received a chromosome with a newly broken end could be detected. In them the event that had occurred to the ruptured end was revealed. Replication had produced two sister chromatids. However, instead of being free to separate from each other at anaphase, these chromatids were joined together at the end that had been ruptured in the previous meiotic mitosis. This gave rise to a newly formed dicentric chromosome made visible by a chromatin bridge at the spore anaphase. Rupture of this bridge could be observed. Again, a newly ruptured end entered each telophase nucleus. Descriptions of this sequence were reported in 1938 (20) and reviewed in 1984 (73).

It seemed imperative to determine whether such chromatid fusions at positions of previous rupture would continue in subsequent mitoses. For this purpose, it was necessary to produce functional pollen grains whose sperm nuclei had received a chromosome with a newly ruptured end. These were not provided by the pollen grains produced in the experiment just described, since they lacked a segment of the long arm of chromosome 4 that prevented their functioning. Fortunately, just when needed, a special type of chromosome modification was isolated that could provide such pollen grains. It was found during a study of the crossover products of a chromosome 9 that had undergone a complex rearrangement of segments. Although this study was not extensively reported until 1941 (22), experiments with the fortunate isolate obtained from it were started without delay and the results reported in 1939 (21). A breakage-fusion-bridge cycle was described that continued from initiation of the break at a meiotic anaphase through the development of the gametophytes and gametes, and then could be detected during development of the endosperm of kernels that had received one such gamete. However, in the zygote nucleus in these kernels, or more likely in the division that followed, this cycle ceased. The broken end had "healed" and was replaced by a new and stable telomere that behaved thereafter the same as any normal end.

The ability to obtain functional gametes that carry a chromosome with a single newly ruptured end also made it possible to learn whether fusions of the broken ends of two chromosomes would occur if each gamete contributed one such chromosome to the zygote. The fusion (ligation) of the ruptured ends of a ring-shaped chromosome in plant tissues, as described earlier, suggested that this might occur. However, this would contrast with the behavior of a single broken end introduced into the zygote, which was shown to "heal" shortly thereafter. Detection of kernels on an ear that had received one chromosome with a newly broken end from each parent requires some means of recognizing such kernels. A chromosome 9 with a similar type of organization as that used in the above described tests would be ideal providing that it carried detectable dominant alleles. While at the University of Missouri I had discussed this problem with Dr. L. J. Stadler. Shortly thereafter, he showed me some kernels on an ear in his stocks whose phenotypic expressions suggested to him the presence of a chromosome with just the right gene markers and chromosome organization. Cytological examination of plants derived from such kernels confirmed the presence of a chromosome with just the right structural organization. Crosses were then made between plants with similarly organized versions of chromosome 9 that carried contrasting genetic markers. On ears so produced there were kernels whose phenotypes revealed that a chromosome 9 with a newly ruptured end had been contributed by each parent. Fusions (ligations) occurred between these broken ends, forming a dicentric chromosome composed of the two chromosomes 9 joined together at their positions of previous rupture. Initial reports of this fusion and its consequences appeared in 1942 (25, 26). It
was an expansion of this test, conducted in the summer of 1944, that produced the plants whose progeny revealed the unexpected presence of transposable elements. Why this expanded test was conducted needs to be explained.

The behavior of ruptured ends of chromosomes during mitotic cycles gives rise, in a number of instances, to recessive "mutations." Each "mutation" reflects the presence of a viable homozygous deficiency, and each can be recognized by a specifically altered phenotype it produces. Such "mutations" were noted initially in studies of ring chromosomes where each modified phenotype reflected a change in the chromatin content of the ring. These phenotypes were described in publications dating from 1937 through 1944 (17, 18, 19, 23). The newer type of breakage-fusion-bridge cycle likewise produced deficiencies that were viable when homozygous, and they likewise could be recognized by the modified phenotype associated with each. A large percentage of these were minute deficiencies located at the end of the short arm of chromosome 9. Their nature was described in the Carnegie Institution of Washington Year Books from 1942 through 1947 (25, 26, 27, 29) and in Genetics in 1944 (28).

The many examples of mutant phenotypes, already determined to be reflections of minute deficiencies, suggested another possible means of obtaining such deficiencies distributed at many different sites along the short arm of chromosome 9. The start of the test was to grow plants from kernels that had received from each parent a newly broken end in the chromosome 9 short arm. Because of fusion of the two ends, a dicentric chromosome would form in the zygote, resulting in initiation of the chromosome type of breakage-fusion-bridge cycle. A viable plant would come from those cells in the seedling in which the broken ends had "healed," causing this cycle to cease. These plants, in turn, would be self-pollinated and their progeny examined for segregation of new mutants. A total of 677 kernels that received a newly broken end of chromosome 9 short arm from each parent were sown in the summer of 1944. The appearance of the seedlings that these kernels produced is described in volume 10 of the Stadler Symposium (70). The plants that survived were self-pollinated, and the ears produced were examined for kernels with mutant expressions. Then, forty such kernels from each ear were sown in a seedling bench in the greenhouse early in 1945, and the seedlings examined for segregation of new mutants. These appeared. However, the phenotypic expressions of some of them were totally unexpected. The segregants were variegated for distinctive grades and types of chlorophyll distribution in the young leaves. Between cultures the patterns of this varied widely, but within a culture the pattern among the segregants was the same.

Seedlings with variegated chlorophyll patterns from some of these cultures were transplanted to pots in order to observe the pattern of variegation in the new leaves as the plant developed. I soon recognized that the changes in patterns of variegation that appeared in sectors on these new leaves held the key to an understanding of the events that were responsible for initiating variegation in the first place. Most significant in this regard were twin sectors, obviously derived from sister cells, in which the pattern changes in the twins were reciprocals of each other. For example, a reduced frequency of mutations to give full chlorophyll expressions on the pale or white background in the surrounding leaf tissue was matched in the twin with a much increased frequency of such mutations. My conclusion from these twin sectors was that during a mitotic cycle one cell had gained some component that the sister cell had lost, and that this component was responsible for regulating (i.e., controlling) the mutation process: that is, its time and its frequency of occurrence in the plant tissues.

On examining kernels on ears of plants grown in the summer of 1944, one ear was found in which loss of genetic markers carried in the short arm of chromosome 9 was occurring in a patterned fashion. Here, also, twin sectors appeared in some of the ker-
nels; these expressed reciprocal relationships with regard to the loss patterns, as if initiated by one cell gaining what the other cell had lost. This suggested that the chromatin loss patterns were controlled by the same general regulatory mechanism that was controlling the patterns of variegation among chlorophyll mutants in the leaves. Because of the clear expression of chromatin losses in these kernels, study of plants derived from them was initiated in 1945.

The discovery of an ear segregating kernels with distinctive patterns of coupled loss of gene markers proved to be most fortunate. These patterns reflected the presence in the parent plant of the Dissociation–Activator (Ds–Ac) system of transposable elements. The initial observations were reported in the Carnegie Institution of Washington Year Book No. 45, issued in December 1946 (33). The symbols Ds and Ac were applied to these elements, and a discussion of their modes of operation appeared in the Carnegie Institution of Washington Year Book No. 46, issued the following year (34). During 1947 transpositions of both Ds and Ac were recognized, including two instances of transposition of Ds to the C locus in chromosome 9 short arm. These transpositions were reported in the Carnegie Institution of Washington Year Book No. 47 (35), issued in December 1948, along with an insertion of a Ds into the Wx locus in chromosome 9.

The study of insertions of a Ds into known gene loci had progressed sufficiently by 1950 to warrant publication in a journal with a wide readership (37). The report was titled "The Origin and Behavior of Mutatable Loci in Maize." It was clear from responses to this report that the presented thesis, and evidence for it, could not be accepted by the majority of geneticists or by other biologists. Genetics was still in an unformed state compared to the rapid changes in concepts that occurred subsequently in the 1950s and 1960s, and there was no clear notion of the nature of the gene. It remained a hypothetical unit until proven otherwise. A long report in the Cold Spring Harbor Symposium for 1951 represented a second attempt to present the "mutable loci" story as it was progressing at Cold Spring Harbor (40). The response to it was puzzlement and, in some instances, hostility. A third attempt to support the thesis of the origin of mutable loci in maize appeared in 1953 in the widely read journal Genetics (42). It was titled "The Induction of Instability at Selected Loci in Maize." This article appeared before copying machines practically eliminated requests for reprints. At the time, reprints were distributed to a selected few, and to others on request. In this instance I received a total of only three requests for this reprint! By then I had already concluded that no amount of published evidence would be effective. As a consequence, beginning in January 1949, those projects that had reached a state allowing conclusions to be drawn from them, or were in need of data assembly and comment, were treated to an unpublished written account, with tables of data, diagrams when needed, and a discussion of the significance of the findings. Only the highlights of these studies were reported in the annual Year Books of the Carnegie Institution of Washington.

In 1952 R. A. Brink at the University of Wisconsin and in 1953 P. A. Peterson, now at Iowa State University, each published their experiences with transposable elements in maize. Even so, I was not convinced that transposable elements were viewed, generally, with confidence of their reality. There were many vocal skeptics. Therefore, the method I had chosen to record data and conclusions from them was continued into the early 1960s. Some topics, however, were dealt with and illustrated in symposia articles (46, 50, 63, 66, 69, 70, 71). Also, beginning in 1958, special topics were discussed in the annual reports of the Carnegie Institution of Washington, as the titles indicate.

In retrospect, it appears that the difficulties in presenting the evidence and arguments for transposable elements in eukaryotic organisms were attributable to conflicts with accepted genetic concepts. That genetic elements could move to new locations in the genome had no precedent and no place in these concepts. The genome was
considered to be stable, or at least not subject to this type of instability. A further difficulty in communication stemmed from my emphasis on the regulatory aspects of these elements. In the mid-1940s there was little if any awareness of the need for genes to be regulated during development. Yet it was just this aspect that caught my attention initially. Unquestionably, some genetic mechanism was controlling the patterns of gene expression, as clearly illustrated by the twin sectors in leaves and kernels. In these instances, the responsible mechanism appeared to be associated with some event occurring during a mitotic cycle. It was my intention in the summer of 1945 to attempt to find out what this regulatory event might be. It was not until fifteen years later that the regulation of gene action began to gain credibility due to the elegant experiments of Jacob and Monod that were carried out in bacteria. Their studies began a new era in genetics. The integrated systems of transposable elements in maize, which I called "controlling elements" because of their distinctive modes of regulating the expression of genes, turned out to represent a quite different mode of gene regulation from that described by Jacob and Monod. Only now, more than forty years after the discovery of transposable elements, are we beginning to understand enough about the ways that they can affect genes to decipher some intriguing new aspects of gene control from their study.

Barbara McClintock

Publisher's Note: The above introduction was written by Barbara McClintock to show how the concept of transposable elements evolved, and to comment on subsequent investigations of these elements. The papers in this volume were selected because of their relevance to this topic. For the discovery of "Mobile genetic elements" she received the Nobel Prize for Physiology or Medicine in 1983.
Part IV: ORIGIN AND DIVERSITY OF MAIZE IN THE AMERICAS (1957-1987)

In 1957, Barbara McClintock was invited by the Committee on Preservation of Indigenous Strains of Maize (National Academy of Sciences -- National Research Council, USA) to organize a cytological survey of Peruvian races of maize. During two visits to Peru (1957, 1958), she provided “technical guidance to the cytologists engaged in this work” (Grobman et al. 1961, pp. vi, 3) at the Universidad Agraria, La Molina, Peru (Grobman & Moreno 1958, Moreno et al. 1959). The primary purpose of this study was to describe the “variability, racial configuration, relationships, origin, and evolution of the races of maize in Peru” (Grobman et al. 1961, p.1).

During the winter of 1958-59 McClintock was invited again to South America to continue cytological studies of maize races from Ecuador, Bolivia, Chile and Venezuela (McClintock 1959). There, laboratory facilities were provided by the Universidad Nacional, Facultad de Agronomía e Instituto Forestal, at Medellín, Colombia, South America. Her examination of the chromosome knobs of plants from various geographical locations led to her preliminary conclusion that “present-day maize may have derived from several different centers.” Similar studies in Mexico funded by the Rockefeller Foundation (McClintock 1960) proved important for the preservation of indigenous strains of maize there. Soon these cytological investigations of chromosome knob patterns were reported in studies of races of maize in Bolivia (Ramirez et al. 1960), Chile (Timothy et al, 1961), Ecuador (Timothy et al. 1963), and Peru (Grobman et al. 1961).

As consultant to the Agricultural Science Program of the Rockefeller Foundation (1962 to 1969), McClintock supervised the research activities of Foundation Scholars at North Carolina State College (now University). They continued cytogenetic studies related to the origins of maize and its diversity (McClintock 1978a, McClintock et al. 1981). In this section readers are fortunate to have the perspective by one of McClintock’s former students and collaborators about their work on the origin and diversity of maize in the Americas (Kato Y this volume). Years of studying maize chromosome knobs, correlated with their geographic distribution patterns, laid the foundation for their conclusions. Timothy’s book review of their pioneering contributions (Timothy 1983, reprinted here) offers another perspective on this ground-breaking work.

References


Perspective: ¹


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¹ Perspective solicited.
² For cross-reference purposes, this is the publication number in the annotated, chronological list of McClintock’s publications (Table I) in the Front Matter.
³ The symbol ➀ indicates that the McClintock publication is reprinted herein.
5. **Study of chromosome morphology of races of maize in Peru.**

Advances have been made in the study of the number, position, size and shape of chromosome knobs of the races of maize in Peru, trying to determine patterns for differentiating races (see first report in Maize News Letter 32:25).

Differentiating features have been obtained, and are being studied further for ample confirmation, in the frequency of appearance of knobs in certain chromosome arms, the shape and size of such knobs, the frequency of presence of the abnormal -10 chromosome, and the frequency of high number of B-chromosomes. The highest number of B's per plant, found so far, was 4. Generally speaking, B-chromosomes are found in highland races, with low number of knobs.

Ulises Moreno
Alexander Grobman
Barbara McClintock
Perspective:


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1 Perspective solicited.

2 The symbol ② and the header 'Citation' indicate that this McClintock publication is not reprinted herein. However, in this case, an online URL is provided.
Perspective:  Barbara McClintock’s Cytogenetic Studies of Chromosome Constitutions in Races of Maize in the Americas.

by David B. Walden, The University of Western Ontario

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Barbara McClintock’s cytogenetic studies of Chromosome Constitutions of Races of Maize in the Americas.

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Barbara McClintock would have been proud to be part of the community of maize geneticists who lived to witness the presentation of the sequence analysis of various maize genomes (Schnable et al. 2009, Gore et al. 2009). Particularly significant is the evidence supporting her early contributions and the identification of transposable elements (TEs, McClintock 1951). It is now known that nearly 85% of the maize information genome may contain up to 855 families of TEs (Schnable et al. 2009, Fedoroff 2012). The work of today's maize molecular new-order geneticists provides evidence for McClintock’s insights, developed when investigators were restricted to interpretations of breeding experiments and microscopic-based evaluations of chromosomal complexes. Future studies of genome sequence analysis of many of the races of maize that McClintock and others studied should complement the extensive data reported in her 1975 symposium presentation (McClintock 1978; also in several contributed poster papers and abstracts) and add the dimension of TE composition of races and their migratory pathways.

First a personal note: I was drawn to the Cornell University Department of Plant Breeding (Murphy & Kass 2007, 2011), in part because of the legacy of the maize genetics group, and during my career did meet personally with 4 of the ‘Big 5’ (CR Burnham, MM Rhoades, GW Beadle, B McClintock, less RA Emerson; see cover photo) – including a course with LF Randolph, at Cornell and a Post-Doc with Marcus Rhoades, who did his doctoral research with Emerson. My Post-Doctoral work and association with the Indiana University and University of Illinois groups in the 1960s and 1970s led to the collaborative efforts to recognize the 75th anniversary of the rediscovery of Mendel’s work via a gathering of the maize geneticists in Urbana, Illinois, in 1975. Thus the resulting volume (Walden 1978) was the culmination of my efforts to record the history of maize breeding and genetic research to that date, and Dr. McClintock’s (1978) contribution appears along with those of many active maize geneticists.

As the contributions in Walden (1978) reveal, less is known about McClintock’s use of descriptive research of maize to identify anthropological history through the use of maize race reconstructions than is known about McClintock’s experimental maize research leading to the identification of TEs (McClintock 1951 and earlier; this volume) and her role in eukaryotic genome evolution (Fedoroff 2012). She and her colleagues worked for more than 30 years using cytogenetic technologies to assess the hypotheses of the day regarding the origin of maize (Zea mays ssp. mays L.) and the galaxy of races that have appeared, mostly in the Americas, in the past 10,000 years. However, a brief search of recent publications on the domestication of maize found only one paper that cited McClintock’s work in this area (Tian et al. 2009). Edward Buckler seems to be one of only a few young maize geneticists who recognize McClintock’s contributions to the studies on maize domestication. If one goes to the website for the Buckler laboratory at Cornell University (MaizeGenetics.net), one will find a section titled “Brief History of Maize Domestication Studies.” Barbara McClintock’s contribution is included therein.

Studies purporting to identify the origin of maize, and its myriad of races, have been underway for over 100 years and can be classified into four, generation-dependent phases, each phase characterized by new technology: phase 1 - morphological and metrical data; phase 2 - cytogenetic data; phase 3 – electrophoretic data; and phase 4 - DNA sequence data. Goodman (1978) reported that the Rockefeller Foundation and the National Research Council contained nearly 12,000 accessions of races of maize by the early 1960s. These collections formed the resources for the work of McClintock and colleagues that would eventually culminate in their monumental contribution many years later (McClintock et al. 1981; see also Timothy 1983 reprinted this volume; and Kato Y perspective this volume). Large numbers of races were in collections from Argentina, Bolivia, Brazil, the Caribbean, Columbia, Chile, Ecuador, Guatemala, Mexico, Peru, and Venezuela, and a lesser number from the U.S. and Canada. It is instructive to remember that organized breeding (development of inbred lines and hybrids), chiefly but not exclusively in several (USA) state experimental stations, was underway during phase 1 and throughout phase
2, and continues today in corporations employing new genetic technology and information. Directed breeding programs harnessed, but also reduced diversity - making the collection of races and their maintenance all the more valuable.

Virtually all of the cytogenetic contributions by McClintock and her colleagues to the elucidation of the diversity and differences in the Central and South American races of maize are based on the analyses of late pachytene preparations, a technique first observed and published for maize by McClintock in 1930 (see Phillips perspective this volume; Kass & Bonneuil 2004; Coe & Kass 2005 this volume), in particular the heterochromatic knobs and chromomere patterns. This cytogenetic analysis exploited the stability of the euchromatin and heterochromatin features at this stage of meiosis, characterizing each race as to the presence or absence of a specific knob, the size of the knob, and most importantly the assignment to the specific chromosome arm on which the knob was located. Abnormal 10 (K10) and B chromosomes, if known to be present in a particular race or complex, were also added to the analyses.

McClintock (1978) incorporates data derived from Blumenschein (1973), Kato Y (1976), Longley and Kato Y (1965), McClintock (1959, 1960), Ting (1958) and Wellhausen et al. (1952), plus previously unpublished data from her laboratory. McClintock also acknowledges these colleagues for their support and collaborations, and refers her readers to an extensive bibliography and summary of the races of maize published by Brown and Goodman (1977).

In all, McClintock (1978) reports and integrates the data from thousands of individual accessions: approximately 115 from North America, 700 from Central America and the remainder from South America. She presents the data (specific heterochromatin constitution per sample) in a series of 17 maps, each documenting the presence of a specific cytogenetic constitution (complex) in a specified geographic region. Using these data, she is able to propose convincingly the trait(s) and path(s) of migration through which many races (highlands vs. lowlands, etc.) became established in their niches. She has also been able to identify many instances of introgression along some of these paths.

From the data and studies reported in this chapter, McClintock’s (1978) primary conclusion was, “… it is the Mexican teosintes … that have played a major role in the history of maize, suggesting that maize started its expansive development in those areas of Mexico where teosinte prevailed, that is, central and southwestern Mexico” (p. 184). It has only recently been accepted that maize was domesticated from Zea mays ssp. parviglumis teosinte of southwestern Mexico (Matsuoka et al. 2002). However, the contributions of Zea mays ssp. mexicana teosinte are still being elucidated (van Heerwaarden et al. 2011; Sawers & Leon 2011). In addition, much discussion still surrounds the origins of maize from teosinte (Kato Y this volume).

Now it remains for future DNA sequence studies of maize information genomes and epigenomes to confirm this intriguing, intertwined history of plant evolution and distribution in the Americas.

Literature Cited:


Chapter 11
SIGNIFICANCE OF CHROMOSOME CONSTITUTIONS IN TRACING THE ORIGIN AND MIGRATION OF RACES OF MAIZE IN THE AMERICAS

Barbara McClintock

Examination of chromosome constitution in plants of many races of maize has revealed extensive diversity in origin of their germplasms. Their chromosomes have distinctive structural components that derive from more than one initial source. The sources differed from each other in the presence or absence of a particular component and also in the morphological organization of the component, if present. The components of these initial sources are now distributed among the races, and in distinctive combinations. Recognition of them provides a means of determining degrees of relationship among the races, and estimates based on chromosome constitutions both confirm and supplement those based on morphological physiological, and genetic considerations.

The examinations have also revealed clearly defined geographic centers in which maize with a particular combination of initial germplasms was propa-
gated. This maize “migrated” into new territories, often along well-defined paths, or was introduced into a distant location where it could then initiate a new center of distribution. As a consequence of migrations and introductions, introgressions took place between maize originating in different centers, and these initiated new combinations of basic germplasms. Not only did new races arise through modification of genetic expression of maize with a particular germplasm combination, they also arose as a consequence of introgressions between maize strains having different combinations. In some territories an initial introduction was followed by later introductions, and in some instances it is possible to discern the sequence of arrivals. It is the purpose of this chapter to present briefly the nature of the evidence that is responsible for the above statements.

The studies reviewed here commenced with some initial observations of mine conducted with plants of races of maize derived from Ecuador, Bolivia, Chile, and Mexico, and with plants of a few additional races indigenous to the Central American countries, Venezuela, and the Caribbean Islands (McClintock, 1959, 1960). These studies revealed the highly conservative nature of the distinctive chromosomal components. This suggested that knowledge of the distribution of these components to the races of maize in the Americas could reveal much about the history of maize—the centers of origin of particular germplasm combinations, migration paths from these centers, introductions and their sources, and introgressions that have occurred. In these respects the fuller knowledge proved to be rewarding.

Extensive recordings of chromosomal constitutions of maize races began with the studies of Longley and Kato Y. (1965), who examined races collected in the Central American countries and from the Caribbean Islands. In addition, a few races derived from Mexico, Colombia, and Venezuela were examined. Subsequently, Blumenschein (1973) examined the chromosome constitutions of plants of various races collected in Venezuela and in central and eastern South America. His studies reveal much about the origin of races in these areas. Dr. Kato’s extraordinary talents for unraveling complicated combinations of basic germplasms, as expressed in the pachytene chromosomes, has been of inestimable importance for this study. His examinations include many races of Mexico, some strains of Indian maize from the southwest and central United States, and maize of formerly important open-pollinated, commercial varieties from the southeastern parts of the United States. All of the maize from the United States was provided by Dr. William L. Brown, who has generously cooperated in these studies since their very beginning. Recently, Dr. Kato determined chromosomal similarities and differences among many teosintes collected in Mexico and Guatemala. The information he obtained from these teosintes makes it possible to appreciate just how close the relationship must be between germplasms of Mexican teosintes and those of maize. It suggests that
Figure 1. Number of collections, within each bounded area, of maize races of strains whose chromosome constitutions were determined. To indicate the number of examined collections from each Department of Guatemala, an enlarged outline of this country is inserted to the right. This same placement of Guatemala appears on each map of North America. It represents the format devised by Dr. Kato.

the Mexican teosintes are the sources of all the basic germplasms of maize. The Guatemalan teosintes appear to have contributed little or not at all initially.

The number of examined plants of a single collection from a given locality varied considerably. In the extended studies of Kato and Blumenschein, this averaged between four and six. The numbers were increased for collections of those races considered to be of particular importance in the history of maize, or among collections of different races coming from a restricted territorial region and considered to be of special significance in this regard. The number of examined plants from such collections ranged within 7–24. Examination of plants from different collections of the same race, either from the same general area or distantly located, was much increased for those races considered to have contributed to the origin of other races, or to have been extensively distributed. The total number of collections and the areas from which they were taken are shown in Figures 1 and 2.
Figure 2. Geographic locations in South America of those collections of maize races from which chromosome constitutions were determined. An enlarged outline of Ecuador is inserted just to the left of Chile to indicate clearly the locations of examined collections. This format was devised by Dr. Blumenschein and appears on all maps of South America.

This report is based largely on the observations of Drs. Longley, Kato and Blumenschein. Although we may share a common mode of interpreting them, I must take full responsibility for the conclusions drawn from them that are expressed here.

**CHROMOSOMAL COMPONENTS CONTRIBUTING TO THE STUDY OF RACE RELATIONSHIPS**

The basic chromosome constitution of maize consists of 10 chromosomes, numbered according to their relative lengths from the longest, chromosome 1, to the shortest, chromosome 10. Among the examined races, these length rela-
tionships were found to be constant, except that plants of some races carried an aberrant chromosome 10 (called Abnormal-10) having a heterochromatic extension of its long arm. Each chromosome is divided into two arms whose relative lengths are determined by the location of the centromere. These locations also appear to have remained relatively constant, as no strains were observed that had a readily detectable modification in the location of the centromere. Slight shifts, however, could have been overlooked. Each arm exhibits a specific pattern of chromatin compaction at the stage of the meiotic cycle, late pachytene, that best served the purpose of this study. The chromatome patterns so formed also are basically conservative; they are quite similar in most examined plants. There are, however, several locations in the chromosome complement that may have an enlarged chromatome. One or another of these enlarged chromomeres may be present in a strain and be either homozygous or heterozygous with a smaller homologous chromatome. Although such chromatome variations were noted, no effort was made in this study to systematically record them.

The nucleolus organizer is another chromosome component that exhibits variation among maize strains. Not only does the size vary, but the location within the organizer where the nucleolus is initiated at telophase also varies. Although notes were taken when exceptional modifications of the organizer were observed, no attempt was made to record the state of the organizer within each plant.

Another variable in chromosome organization relates to rearrangements of segments of chromosomes, such as inversions, duplications, and translocations. The examined races were free of gross rearrangements, with the exception of the inversion in the short arm of chromosome 8, whose presence in maize and teosinte has been known for many years (McClintock, 1933; Ting, 1958). Occasionally a modification was noted in an individual plant or in plants of a single collection, but none has spread throughout populations as has the inversion in chromosome 8.

The most detailed observations and recordings of variation of chromosome organization involve the knobs in terms of their presence or absence at any one knob-forming region and, if present, the size class to which a knob belongs. The locations of these knob-forming regions in each of the maize chromosomes are shown in Figure 3. At any one of these locations in any one plant of a race, a knob may be present or absent in one or both homologs, and this reveals an aspect of the ancestral history of the plant. If present, a knob might be small or large or some size in between, and in one or both homologs. The exceptions are the knobs designated 8L-2, 10L-1, and 10L-2 in Figure 3, which were always observed to be relatively small. Again, the size and shape as well as presence or absence of any one knob depends on the ancestral history of the plant. It is the distinctiveness of each of these many knobs that has provided the most detailed and useful data for judging degrees of relationship between races and for
reconstructing the probable past history of some of them. Examples illustrating this are given after two other sharply exhibited variables in chromosome constitution are discussed. These are the B-type chromosome and the Abnormal-10 chromosome.

**DISTRIBUTION OF ABNORMAL-10 AND THE B-TYPE CHROMOSOME INITIAL CONSIDERATIONS**

The heterochromatic component that lengthens the long arm of chromosome 10 allows ready detection of the presence of Abnormal-10 in a plant (see...
chromosome 10a, Figure 3). Although some variation in organization of the heterochromatic component was noted, only in one small area in the Argentine Andes was any conspicuous modification observed in the organization of the B-type chromosome (see chromosome B, Figure 3). It had an elongated euchromatic segment.

One or the other or both of these distinctive chromosomes appeared in plants derived from collections made in various parts of the Americas, as shown in Figures 4 and 5. Each circle refers to plants derived from a collection made at the marked geographic location. It is obvious at a glance that each of these chromosomes has a distinctive distribution pattern and that overlapping is confined to restricted areas. There can be no question that plants having a B-type chromosome share at least one ancestor in common, as do plants that have Abnormal-10. The question then turns to the degree of relatedness among plants having either one or the other of these chromosomes. This may be answered by comparing other components of their chromosome constitutions.

MIGRATION PATHS IN NORTH AMERICA OF COMPONENTS OF ORIGINAL GERMPLASMS

The distribution patterns of the B-type chromosome in Mexico and Guatemala (Figure 4a) illustrate both old and relatively recent migration paths of maize germplasms. These paths diverge from definable areas, and several paths may diverge from a single area. Some of the paths are sharply revealed by those knobs that have restricted distributions. The geographic distribution of several of these knobs will serve to illustrate one mode of detecting migration paths.

Paths to and along the Pacific Coast of Mexico

One of the older paths is along the Pacific coast of Mexico, from its border with Guatemala into Sonora. Figures 6 and 13a define this path. Another path starts in the area that includes northern-central Guerrero, which is adjacent to Morelos, and the state of Mexico and runs into southern Guanajuato and northern Michoacan, from which it enters Jalisco and Nayarit, where it may join the Pacific coastal path to the north. No one knob alone illustrates this path but its existence is recognizable on viewing a number of knob distribution maps. It can be discerned in Figures 4a, 7, 8, and 9.

The path from Central Mexico to the U. S. Border

A relatively recent path begins in the Bajío of Mexico, directly north of Mexico City, and extends into Chihuahua. The territory covered by this migration broadens toward the north, resulting in a cone-shaped distribution pattern with
Figure 5. Locations of collections having plants that carry the Abnormal-10 chromosome (solid circles represent those collections in which the B-type chromosome was also present): (a) North America and Caribbean area; (b) South America. [In states located along the Pacific coast of Mexico the maize races are as follows: in Sonora: Onaveño-Cristalino and Cristalino de Chihuahua; in Sinaloa: Harinoso-de-Ocho; in Nayarit: Harinoso-de-Ocho, Jala, and one unclassified race; in Jalisco: Reventador, Celaya, Tabloncillo, Tuxpeño, and Pepitilla (two collections); in Guerrero: Nal-Tel (two collections) and Vandeño-Olotón; in Oaxaca: Bolita (two collections), Zapalote Grande (one collection), Zapalote Chico (four collections), and Nal-Tel (two collections); in Chiapas: Nal-Tel, Zapalote Grande, and Tepecintle.]
its apex in central Mexico (see Figures 7–9 and their legends). The race contributing the major fraction of germplasm to the maize in this cone-shaped area is Cónico Norteño, itself a product of earlier racial crosses that combined and integrated distinctly different basic germplasms.

The origin of Cónico Norteño, as projected by Wellhausen, Roberts, et al. (1952), starts with a hybrid between two strikingly different races (Palomero Toluqueño and Cacahuacintle), both of which are grown within the state of
Mexico. Stabilization of this hybrid produced the race known as Cónico, also grown in central Mexico. Not only are the two parental races strikingly different morphologically; their chromosome constitutions also are strikingly different, emphatically demonstrating basic differences in their germplasms. The chromosome constitution of Cónico contributes direct support for Wellhausen’s projection of its origin. Wellhausen, Roberts, et al. (1952) also projected that Cónico was taken north into the Bajío, where introgression occurred with a race from the west (Celaya). This series of mixing gave rise to the successful combination of germplasms that appears in the race designated Cónico Norteño. All of the projected racial combinations thought to be responsible for the origin of Cónico Norteño are fully supported by the chromosome constitutions of putative ancestral races and by Cónico Norteño itself. Clearly, it has germplasm components that are shared with each parental race but totally carried by no other single race.

Figure 7. Distribution of the small knob at position 1 in the long arm of chromosome 6. This knob was found elsewhere only in plants of one collection from the Territory of Roraima, Brazil, adjacent to Venezuela.
Figure 8. Locations in the Americas of collections having plants carrying the large- and/or the medium-sized knob in the long arm of chromosome 1. Solid triangles indicate the large knob; open circles indicate the medium-sized knob. Underlined triangle with circle indicates that both types of knobs were found among plants of the same collection. The two collections in Oklahoma are from the Kiowa and the Mescalero Indian tribes. Both of these tribes are related to the Apache tribe in Arizona. Chromosome constitutions of plants from collections of these two tribes indicate that their maize came mainly from the Apache tribe in the southwestern part of the United States, although the Kiowa tribe has some maize from the northcentral states. Note the relation of maize in the southwest United States to that in central Mexico. The two races in the highlands of Guatemala having the large knob are Nal-Tel Blanco and Imbricado.

A Path along the East Coast of Mexico

The distribution of the race Tuxpeño defines a relatively recent path along the eastern coast of Mexico. The path runs north, parallel to the coast, from the lower part of the state of Veracruz into the state of Tamaulipas. Toward the north the Tuxpeño germplasm has spread westward through the northern Mexican states. The spread was accomplished by introgressions with maize having other basic germplasm combinations, which had arrived in these states by other migration routes or by direct introductions. This path is illustrated by
the distribution of the 9L large knob shown in Figure 10a. The triangles refer to collections of the race Tuxpeño proper. The distribution of this knob within the described area of Mexico should be compared with that of the race Tuxpeño and its introgressions shown on the map of Figure 90 in Wellhausen, Roberts, et al. (1952). They are the same except for the states of Campeche and Yucatan. Tuxpeño strains arrived in these two states by an entirely different route, and they contained a few distinctly different basic germplasm components.

Tuxpeño's initial germplasm undoubtedly was formulated from a succession of introgressions between maize strains having different combinations of basic germplasms. The successful combination that produced the race now known as Tuxpeño appears to have occurred in southwestern Guatemala or in the adjacent state of Chiapas, Mexico. From this general area of origin, its migration took several distinctly different paths. One of these carried it through

Figure 9. Distribution of the small knob at position 2 in the long arm of chromosome 10. The open triangles represent collections of the race Tuxpeño. The collection from Virginia (U. S. A.) is of the strain White Tuxpan. The three collections from the highlands of Guatemala are of the races Serrano, Nal-Tel, and Imbricado. This knob was not found elsewhere in the Americas.
Figure 10. Distribution in the Americas of the large knob in the long arm of chromosome 9 (open triangles represent collections in Mexico of the race Tuxpeño): (a) North America and Caribbean area and (b) South America and Caribbean area.
Chiapas into and across Oaxaca, where it entered the state of Veracruz at Oaxaca's northeastern border. It then went north along the eastern part of Mexico, as just described and illustrated. In the course of its migration, it picked up bits of other basic germplasm components from maize growing along its route, without however suffering serious loss of character identity. Such an instance is illustrated in Figure 9.

**Distribution Paths of B-Type Chromosome**

The distribution of the B-type chromosome in the upper two-thirds of Mexico (Figure 4a) follows along the described western and central migration paths and to a lesser extent along the eastern path. This could be anticipated if the B-type chromosome originated in central Mexico and was integrated into the early maize races that were being propagated in this area. [The B-type chromosome is present in some races considered to be ancient (Palomero Toluqueño, Chapalote, Reventador, Pepitilla, Zapalote Chico, Nal-Tel). It is also present in teosintes located in western-central Mexico (Kato Y. 1975). Its origin in a teosinte is possible, if not probable].

From the southern border of the Mexican state of Nayarit on the western coast to the southern coast of Oaxaca, plants from only two collections along the coastal region had B-type chromosomes. One of the collections is of the race Nal-Tel, and the other is of the race Vandeño, and this is significant. In Oaxaca plants from nine collections had this chromosome. The collection from the far eastern part of this state is of the race Tuxpeño. Of the eight along the coast, seven are of the race Zapalote Chico, and the eighth is a hybrid of the related race, Zapalote Grande, with the race Bolita. In the adjacent state to the south, Chiapas, there are seven collections having plants with B-type chromosomes. Three are of Zapalote Chico, two are of Zapalote Grande, and there is one each of Vandeño and Comiteco. In these states the B-type chromosome is concentrated in the race Zapalote Chico and its close relative, Zapalote Grande. (In Oaxaca, the chromosome constitutions of seven races were examined: Bolita, Bolita-Zapalote Grande, Cónico, Nal-Tel, Tuxpeño, Zapalote Chico, and Zapalote Grande. In Chiapas, 13 were examined: Comiteco, Nal-Tel, Olotillo, Olotón, Tehua, Tepecintle, Tepecintle-Olotón, Tuxpeño, Tuxpeño-Tepecintle, Tuxpeño-Vandeño, Vandeño, Zapalote Chico, and Zapalote Grande.) In these studies Zapalote Chico proved to be an extraordinary race. It is a repository for components of germplasms of several races thought to be relics of early races (Nal-Tel, Chapalote, and Reventador). In addition, it has many of the components of a presumed early race that cannot yet be identified. It also may be viewed as a "point of departure" for the north coastal and southern distributions of some of the rare chromosomal components (e.g., see Figures 6, 11, and 13a, and their legends).
For the anthropologist, the geographic path described by the B-type chromosome is of some significance. This chromosome, as well as other specific chromosomal components, depicts the likely mode of entrance of maize into the Mayan territories of Campeche and Yucatan. Its path in Guatemala is in the form of an arc, passing through the southern Departments. It then curves toward the northwest into Jalapa, El Progresso, and Baja Verapaz, proceeding north into El Peten. From El Peten it enters Campeche in Mexico and follows northeast into Yucatan (see Figures 12a and 14a).

From Guatemala south, the paths described by the B-type chromosome again are instructive. These paths are extensions from the southern Guatemalan arc. They enter El Salvador (B-type chromosome in six of nine collections) and Honduras (in eight of 24 collections). The path stops with the two
Figure 12. Distribution in the Americas of the small knob in the short arm of chromosome 2: (a) North America and Caribbean area; (b) South America and Caribbean area.
Figure 13. Distribution in the Americas of the large knob terminating the short arm of chromosome 7. [The races in states of Mexico are as follows. In Sonora: Cristalino de Chihuahua; in Sinaloa: Chapalote; in Nayarit: unclassified; in Jalisco: Celaya; in Michoacan: Reventador (both collections); in Guerrero: Nal-Tel and Pepitilla; in Oaxaca: all are from collections of Zapalote Chico; in Chiapas: Zapalote Chico (one collection), Zapalote Grande (two collections), Tuxpeño–Tepecintle (one collection); in Veracruz: Tuxpeño; in Campeche: Nal-Tel–Tuxpeño. The races in Guatemala are mostly Nal-Tel Blanco or hybrids with it.]
collections located in the northwestern corner of Nicaragua, adjacent to Honduras. The appearance of the B-type chromosome south of Nicaragua reflects an introduction from the north of maize that had this chromosome in it. The significance of the scattered distributions of the B-type chromosome in the Caribbean area, the clustered distributions in the Andean regions of Ecuador, Bolivia, Argentina, and Chile, and its appearance in the race Cateto in Guyana, Uruguay, and eastern Argentina will become evident later during the discussion of other chromosomal components that suggest the geographic locations from which maize with the B-type chromosome could have been introduced into each of these areas.

**Origin and Distribution of Abnormal-10**

The distribution of Abnormal-10 (Figure 5) differs from that of the B-type chromosome. In states directly north and northeast of the central Mexican area the appearance of Abnormal-10 relates to the race Celaya, either directly in representatives of that race or through its contribution to the formation of the race Cónico Norteño, whose germplasm distributions were discussed. Celaya is grown in Guanajuato and Jalisco and has been introduced into northeastern Mexico. In fact, all three examined collections from Nuevo Leon are of this race, and all have Abnormal-10 (the legend to Figure 5a names the races on the western coast that have this chromosome).

I suspect that Abnormal-10 may have originated from some event occurring to an ancestor of the race Tepecintle, located at the time of the event in southwestern Mexico or adjacent Guatemala. (That Abnormal-10 may have entered the race via teosinte cannot be excluded, as it is present in some of the Mexican teosintes.) Its presence in Zapalote Chico and Zapalote Grande would not, then, be surprising as Tepecintle and Zapalote share many of the same basic germplasm components. Through subsequent and successive introgressions, Abnormal-10 was delivered to other races within the range of distribution of Tepecintle in Guatemala, and it now appears in some of them (Nal-Tel Ocho, Nal-Tel Blanco, Nal-Tel Amarillo, Serrano, Olotillo, and Comiteco). It also appears in some of the successful races or strains produced by hybridization with Tepecintle (Tepecintle-Salvadoreño, Tepecintle-Comiteco, and Nal-Tel–Tepecintle). These are the main races in Guatemala in which Abnormal-10 was found. The germplasms of these races are distributed along the previously described paths into El Salvador, Honduras, and Nicaragua, and into Yucatan by the described northern route.

**Migration Path from the Guatemalan Highlands and Lowlands into Central America and Its Relation to Maize in the Caribbean Area**

Another migration path, repeatedly recognized in these studies, takes the germplasm of maize of the highlands of Guatemala and also of two of the lowland
Departments (Jutiapa and Chiquimula) into the Central American countries. In Costa Rica and Panama introgressions then established new germplasm combinations. Some of these produced the races and strains that were taken to the Caribbean Islands. Also, some populated a large area in northwestern Venezuela, either directly as a racial type or indirectly after introgressions with maize previously introduced from the north (e.g., see Figure 12a). The general absence of the 2S small knob in the Caribbean Islands would postulate its general absence in eastern South America, because most of the maize in that part of South America relates to maize in the Islands. Its distribution in South America (Figure 12b) supports this postulate.

The map in Figure 12b conspicuously illustrates a correlation that was observed repeatedly. It shows a relationship between the maize of Venezuela and that of the Territory of Roraima, Brazil. The distribution of other knobs narrows this connection to maize located in northwestern Venezuela. These other knobs are mainly the small ones. Although they occupy the same locations in the chromosomes as the large knobs of the “Venezuelan complex” (see next section), the size differences are in marked contrast. The main center of these small knobs is in the highlands of Guatemala, and a group of them constitute the “small knob complex” (knobs in 2S, 2L, 3L, 4L, 5L, 7L, 8L-1, 9S, and 10L-1).

The distribution of the 2S small knob in Mexico (Figure 12a) also illustrates a pattern that is repeatedly expressed. This pattern relates to maize containing components of the small knob complex. Knobs of this complex appear in maize of the Central Mesa of Mexico. There can be no doubt that exchanges of maize occurred between inhabitants of the highlands of Guatemala and those of the Central Mesa of Mexico. Both chromosomal constitutions and racial characters give evidence of this. The distribution of these knobs from and about the Central Mesa follows the previously described paths diverging from this center, although the concentration of the knobs is much reduced within the migrant territories.

**ORIGIN OF THE “VENEZUELAN COMPLEX” AND THE SIGNIFICANCE OF ITS DISTRIBUTION PATTERN**

The knobs appearing in much of the maize of Venezuela suggest that there had been two early introductions preceding the one just described. One entered the western highlands (a spur of the Andes) and the other entered more easterly (Caribbean territory). The sources were related to each other although they differed in some of their basic germplasm components. Their commonly shared chromosomal components resemble some of those now commonly shared between the race Nal-Tel Blanco in Guatemala and Zapalote Chico in Oaxaca and Chiapas, Mexico. Both of these races have a wide assortment of large
knobs, and it is these knobs that characterize the Venezuelan complex. They are the large knobs in 2L, 3L, 4L, 5L, 7S, 7L, 8L-1, 9S, and 9L.

Several of the enumerated large knobs have restricted distributions in the Americas, and this simplifies the process of detecting the probable source of the Venezuelan complex. One is the large knob in the long arm of chromosome 9. Its distribution in Mexico (Figure 10a) was discussed with reference to the race Tuxpeño. In South America (Figure 10b), its concentration within the boundaries of Venezuela is conspicuous. It is well represented in maize of the adjacent islands of Trinidad and Tobago, as is each member of this complex. It should be noted that it is present in Costa Rica and Panama, in the two examined races in Colombia (Pollo and Pira), and in several collections from Ecuador. It is also present in the race Perola of Bolivia, but only in this race. In the east it has been introduced into the race Cateto.

Another large knob, with a distribution in northern South America resembling that of 9L, terminates the short arm of chromosome 7 (Figure 13b). Clearly, it is concentrated in Venezuela and adjacent eastern islands. In eastern South America it appears only in four collections of the race Cateto, three from the Guianas, and one from Uruguay. Its near absence in South America below the Venezuela–Roraima territory probably relates to its near absence in Central America. Central America provided much of the maize of the Caribbean islands, which, in turn, provided much of the maize of eastern Brazil. It also provided the examined maize that entered Ecuador along the Pacific coast. In western South America this 7S large knob is totally absent except in the two marked collections in Bolivia, both of which are of the race Perola. This race must have been introduced into Bolivia quite recently. It has the full complement of large knobs of the Venezuelan complex, and these knobs are not yet integrated into maize of other races grown in its vicinity. I suspect that this race may have been introduced into Bolivia from a location in the highlands of Colombia, possibly close to the border with Venezuela.

The two collections in Panama having plants with the 7S large knob are of related races: (a) Coastal Tropical Flint-Pira and (b) Coastal Tropical Flint-Tusón. The races in the Caribbean Islands that have this knob are related to the Panamanian races, sufficiently so to be given similar racial designations: Coastal Tropical Flint, Coastal Tropical Flint–Tusón, and Tusón. In Mexico it is concentrated in the race Zapalote Chico, but it also is present in the western Mexican Nat-Tel and in its close relatives. Thus, both the 9L and the 7S large knobs associate the germplasm components of the Venezuelan complex with those in these two races from the north.

It is evident that most of the knobs of this Venezuelan complex appear in maize that now is spread over much of Venezuela and the adjacent eastern islands of Trinidad and Tobago. In South America the distinctive germplasm of this complex is not distributed much beyond the territory of Venezuela proper.
This probably reflects the early introduction of maize into the Colombian and Venezuelan territories and the late arrival of maize in territories to the southeast. When the latter territories were populated with maize, it came mostly from the Caribbean Islands, and it entered along the eastern coast at least as far south as the examined collections extend. These introductions occurred at different times and places, bringing with them maize having various different combinations of the basic germplasms. Those having distinctive components of the Venezuelan complex are now distributed to the Catetos of the Guianas, Uruguay, and eastern Argentina, as the maps in Figures 10b and 13b show.

THE DISTRIBUTION AND INFLUENCE OF THE "ANDEAN COMPLEX"

In the early study of chromosome constitutions of races of maize from Ecuador, Bolivia, Chile, and Venezuela a result was obtained that seemed extraordinary at the time. With few exceptions, all of the examined races from collections made at high elevations in Ecuador, Bolivia, and Chile, but not Venezuela, had the same or nearly the same chromosome constitution. It was a simple one; there was a small knob in the long arm of chromosome 7 that was homozygous, and a small knob at position 3 in the long arm of chromosome 6 that could be homozygous, heterozygous with a knobless chromosome 6, or totally absent. From north to south, the highland territory in which this "Andean complex" prevailed—either exclusively as in Chile, or with few exceptions, as in Ecuador and Bolivia—extended approximately 3000 km. The outstanding exception appeared in the race Perola of Bolivia. The germplasms of most of the other exceptional races were highly diluted by the Andean complex. One of these is the race Pisinkalla of Bolivia. Its plant characters suggest a connection with maize of central Mexico. Support for this relatedness is given not only by the types of its few non-Andean knobs, but also by the presence of the same inversion in the short arm of chromosome 8 that is known to be distributed among maize races of central and western Mexico. A second exception, the race Canguil of Ecuador, also must have a connection with maize from the north. It is one of the two races in Ecuador that were found to have the Abnormal-10 chromosome (Figure 5b). The other is Uchima, grown in southern Ecuador at lower elevations.

Other constitutions began to appear in the lower elevations in Ecuador and Bolivia. The races had more knobs distributed among their chromosomes, and these ranged in size from small to very large. In the lowest elevations along the Pacific coast of Ecuador, the races had many large knobs in their chromosomes. Some of them were very large indeed, resembling in this regard some of the
knobs of the Venezuelan complex. In the lower elevations in Chile, chromosome constitutions other than those in Ecuador were noted, suggesting distinctly different sources for their germplasms. In each of these countries, some of the races collected from regions of intermediate elevation had chromosome constitutions that suggested origins initiated by introgressions between lowland and highland maize.

The very extended distribution of the Andean complex throughout the highlands of Ecuador, Bolivia, Chile, and Argentina points to an early introduction of one type of maize into the highlands. This maize was then distributed over a vast territory. Unlike the maize of Mexico, whose history is marked by many introgressions with teosintes having distinctly different knob constitutions, this maize suffered no extensive modification of its chromosome constitution until other maize was brought in very much later. Successive introgressions with the initially introduced maize diluted the constitutions of the secondarily introduced maize. Such dilutions are evident in the race Pisinkalla. Thus knowledge of chromosome constitutions of the Andean races makes it possible to project the probable sequence in time of arrivals of introduced maize—a very early introduction that gave rise to the Andean type maize, a later introduction eventuating in the race Pisinkalla, and a recent introduction, the race Perola, not yet modified by introgressions with maize carrying the Andean complex.

**INFLUENCE OF THE ANDEAN COMPLEX ON MAIZE OF CENTRAL AND EASTERN SOUTH AMERICA**

Andean maize has had an extraordinary influence on maize now growing in central and eastern South America. Its chromosome constitution appears unmodified, or nearly so, in some maize of Paraguay and of the Mato Grosso of Brazil. In eastern Bolivia, parts of Paraguay, and regions south and west of this central area, Andean maize has come in contact with maize migrating inland from the Atlantic coastal areas, or directly introduced into previously isolated areas populated only by maize with the Andean complex. It is evident that Andean maize also has influenced maize of northeastern Brazil and as far as the Atlantic coast, as if the Andean maize had been brought from the highlands to the lower lands along river routes. Introgressions that must have occurred of this Andean maize with maize from the east would be responsible for new character expressions, and thus for the appearance of incipient new races.

That much of the maize in Brazil, Paraguay, and Argentina has been influenced by Andean maize is revealed by the wide prevalence of the knobless state in all chromosomes except chromosomes 6 and 7, and by the exceptionally
wide distribution of the Andean type small knob in 7L. The distribution of this knob is shown in Figure 14b.

Figure 14a reveals an uneven distribution of the 7L small knob in North America. It is concentrated in the highlands of Guatemala, in Costa Rica, in northwestern Venezuela, and in the Caribbean Islands. Since it is a component of the small knob complex, its distributions are typical of members of this complex. They include the introductions into central Mexico from the highlands of Guatemala, as previously described. On the map the triangles represent collections of the race Tuxpeño that exhibited this 7L small knob. It may be noted that triangles appear not only in the east, but also in west Mexico in the states of Michoacan and Colima. Many of the knob distribution maps suggest the source of these westerly located Tuxpeños. They relate to the area in northern Veracruz where the triangles are located (see also Figure 9.)

The Andean-complex is exceptional in having so few knobs—only two—each of which is small. Present information would suggest that the initial introduction into the Andes came from the highlands of Guatemala. Not only is this region the center of the small knob complex, as defined earlier, it also is the center of the knobless complex. Maize of the Guatemalan highlands strikingly exhibits both these complexes. Morphologically, Andean maize resembles some maize types peculiar to the Guatemalan highlands. Anthropological considerations, along with those of chromosome constitutions and morphological and genetic characters, could point directly to the source.

CONCLUDING STATEMENT

The purpose of this chapter is to indicate by specific examples the usefulness of knowledge of chromosome constitutions in helping to resolve some of the problems associated with the origin of maize and its present-day races. This has involved reconstructions of its biological history, which often must have been associated with man's migration patterns and his commerce. As the discussions indicate, anthropological projections are central to the reconstructions. This association applies equally well to the maize of the United States that has not been specifically mentioned here. Relationships are fascinatingly revealed by the chromosome constitutions—the southwest maize with races of central and western Mexico (see Figure 8 and its legend), the northern maize with that of the highlands of Guatemala, the southeastern maize that was constructed from combinations of northern United States maize with some of the developed races of central and eastern Mexico, and so on.

In this chapter emphasis is placed on the importance of Mexican teosintes in supplying the basic germplasm components that were followed in these studies.
It should be emphasized again that it is the Mexican teosintes, not the Guatemalan ones, that have played the major role in the history of maize, suggesting that maize started its expansive development in those areas of Mexico where teosintes prevailed, that is, central and southwestern Mexico.

ACKNOWLEDGMENTS

Those of us who participated in these studies are indebted to the Rockefeller Foundation and to the International Maize and Wheat Improvement Center (CIMMYT) for the support these organizations provided. We also are particularly indebted to Dr. E. J. Wellhausen, formerly Director of CIMMYT, for his continuing support and enthusiastic responses as the work progressed. Personally, I am indebted to Drs. David H. Timothy, Lewis M. Roberts, William H. Hatheway, and Ing. Ricardo Ramirez E. for their generous collaboration during the initial phase of the study.

REFERENCES


Perspective: Significance of McClintock’s pattern concept in the analysis of chromosome knob distribution in the races of maize.

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Significance of McClintock’s Pattern Concept In The Analysis of Chromosome Knob Distribution In The Races of Maize

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Maize is one of the most important foods of humankind. However, its origin and diversification are still very controversial, even today. Several theories have been in existence since Ascherson proposed teosinte as its ancestor in 1895 (Mangelsdorf and Reeves, 1939). Among these theories, the most reasonable one is that called the Multicentric Theory, proposed by McClintock (1978), McClintock et al. (1981) and Kato (1984). It gives a rational sequence of events for racial diversification in the Americas after its domestication from teosinte in Mesoamerica (Kato et al., 2009). McClintock’s initial chromosome studies in corn permitted future understanding of its origin.

Transposable Elements, Chromosome Knobs, and Maize Patterns

Early in her career, McClintock worked on ring chromosomes (McClintock, 1932, 1938a) and broken chromosomes at meiosis and mitosis (1938b, 1939, 1941, 1942) in maize. She discovered that these phenomena also induced loss of gene containing segments and these frequently caused phenotype variegation observable on the surface of leaves and kernels. She later correlated these findings with her observations of newly arising mutable loci (unstable genes, variegations, mosaics) in maize. McClintock (1951, p. 15) wrote:

“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.”

These studies led to the discovery of the first transposons (originally called unstable loci — later designated “controlling elements,” McClintock 1956) $Ds$ (dissociation) and $Ac$ (activator) (McClintock, 1947, 1950). In all these years of intense investigations McClintock learned that every change on the chromosomes resulted in a specific type of variegation. In this way she theorized the underlying mechanism for mutable loci by simply analyzing the pattern of phenotype variegation, even within a single kernel: she reported that a “study of $Ac$ and the $Ac$-controlled mutable loci has made it possible to interpret the many patterns of variegation exhibited” by such unstable loci; “The variegated pattern is an expression of the time and frequency of occurrence of visible changes in the phenotype” (McClintock, 1950, p. 352).

The investigations made and summarized by McClintock (1951) explain that several loci governing various endosperm traits have displayed several mutant expressions, particularly loci affecting development of anthocyanin pigments. The mechanism causing these mutations is activated at different times and frequencies during mitosis. This becomes evident by the formation of tissue sectors with differences in appearance and size. She also discovered in many cases that these mutations are reversible to the original phenotype. Then she concluded that this kind of mutability need not change the structure of genes, but only modify their way of association and interaction between various chromosome components that are associated with the factor or factors at a given locus. This behavior pattern is what McClintock exploited while studying the action in time and space of different “controlling elements.”

Now, if changes of pattern-controlling mechanism can modify temporarily or permanently gene expression in individual plants and their adaptability to changing environmental conditions, it is conceivable that they also can modulate behavior of populations and races, according to the variations of environmental factors, without causing major changes in the structure of genes. McClintock (1951) also discovered that transposable elements are found in heterochromatin. Since knobs are known to be composed of this kind of chromatin in the maize genome, she could conclude that knobs were the supplier of transposable elements (later called “controlling elements”). If the knobs are just the “containers” of the chromosome elements that induce changes in gene expression, it is possible...
that the knob constitution of a race may be what modulates its reaction to environmental conditions. Now that transposons have been universally accepted, many researchers have made studies confirming such gene expression variation at the molecular level; among these are Ananiev et al. (1998a, b), who discovered and confirmed that retrotransposons are contained within the chromosome knobs in maize, in addition to the main repetitive DNA short sequences of 180 bp and 350 bp (TR-1). So, the idea of genetic modulation is quite credible. Furthermore, changing the chromosome knob constitution (for example, modifying knob size) in a maize race could induce the modification capacity of the race behavior. Then, the knob pattern would serve for identifying relationships among races located in different geographical areas. Changes may arise when hybridizations occur with other maize germplasm in the new location.

**Chromosome Knobs and Maize Origins**

By the end of the 1950s, McClintock became involved in a project on chromosome knob constitutions of the races of maize. Based on her experience gained on the pattern concept in the studies of transposons in maize (McClintock, 1951) and preliminary studies made on chromosome constitutions of races of maize in South America and Mexico (McClintock 1959, 1960), she decided to accept the big challenge of the maize cytological project that was offered to her by the Rockefeller Foundation (McClintock 1960). McClintock’s preliminary work in South America was crucial to the later cytological project on the races of maize in the American continent, because there she was able to apply the pattern concept she gained while analyzing the color variegation on the surface of maize kernels; but now she employed patterns formed on a continental level and was concerned with geographic and racial distributions of chromosome knobs of maize populations.

McClintock discovered that knob constitutions in races of maize in the highlands of the Andean region varied between areas belonging to Ecuador, Bolivia and Chile, compared to areas in Venezuela: the former had knobless chromosomes with the exception of the presence of small knobs in two positions, the long arms of chromosomes 6 and 7; she called this the “Andean complex.” The Venezuelan highlands’ populations, on the contrary, had many large knobs on the 10 maize chromosomes. A third discovery was that in the coastal regions of Ecuador, Bolivia and Chile large knobs were present in maize genomes, exhibiting a sharp difference from the highland Andes. With these discoveries McClintock made the following conclusion: “The impression gained from these preliminary studies is that present-day maize may have derived from several different centers. Migration from such centers in the past was followed by hybridization. Examination of the chromosome knobs of plants from various geographical locations may help to identify some of these centers” (McClintock, 1959, p. 456). In the same context, when she analyzed samples of races of maize from Mexico and Central America, she was able to discover the existence of several knob complexes: the “no-knob” complex in the central highlands of Mexico, the “large knob” complex in west central and northwest Mexico, the “small-knob” complex in the highlands of Guatemala, etc. (McClintock, 1960). These knob complexes were confirmed later in an extensive work described below. Also, she concluded:

“…it is both useful and necessary to classify present-day maize into distinctive races, on the basis of defined sets of morphological and complex characters kept together through propagative methods. By means of such classification, it has been possible to apprehend degrees of genetic relationship among plants of different types growing either in one area or in widely separated areas. For a clearer understanding of these relationships, a precise genetic and cytological study of certain races should be undertaken, to determine and compare the types of genetic components that have contributed to the establishment and success of each race” (McClintock, 1960, p. 472).

These statements were prophetic, because two decades after 1960 they became a reality, as briefly summarized below.

McClintock (1978), McClintock et al. (1981) and Kato (1984), applying her general methodology of distribution patterns to the chromosome knob constitutions of the races of maize plotted on maps of the Americas, were able to discern many aspects of maize origin, diversification, migration, introduction, and hybridization. This pattern analysis of chromosome knob data ultimately became the only effective methodology for understanding, in a wide and clear way, their geographical and racial distribution. This perspective describes how Barbara McClintock and her collaborators were able to disclose the history of maize evolution from its origin and domestication up to the present racial distribution throughout the Americas.
Mesoamerica

In order to understand the whole picture about the origin and distribution of cultivated maize (*Zea mays* L.) in the American continent, the analysis of the knob constitutions of both Mesoamerican maize and teosinte, its wild ancestor, became central and most important. This is so because teosinte populations are found only in this territory and, at present, there is almost general consensus among maize researchers that Mexican diploid annual teosinte is the ancestor of maize (Kato et al., 2009).

Teosinte is present in Mesoamerica in various forms: a) the most common is the Mexican diploid annual form [*Zea mexicana* (Schrader) Kuntze (Family Poaceae)], races Nobogame, Central Plateau and Chalco for the highlands of Mexico; race Balsas for lowland Mexico; races Huehuetenango and Guatemala for northwestern and southern Guatemala, respectively (Wilkes, 1967: 100-120); b) the perennial forms, one diploid (*Z. diploperennis* Iltis, Doebley & Guzmán), and one tetraploid (*Z. perennis* (Hitchcock) Reeves & Mangelsdorf), are both found in western Mexico.

In relation to the origin of maize, Kato (1976) gave cytological evidence, not previously considered, which clearly indicates that maize originated from Mexican diploid annual teosinte and not from any of the other teosintes: a) teosinte pachytene chromosomes have exactly the same metrical characteristics (such as chromosome length and arm ratios), plus some conspicuous chromomeres useful in identifying pachytene chromosomes, both in maize and in teosinte; b) the knob positions on the maize chromosomes are the same as those of teosinte, but the latter have positions not found in the former; c) the two races of teosinte from Guatemala have only terminal knobs on all the chromosomes, which, with a few exceptions, have been found neither in maize nor in Mexican diploid annual teosinte; in the latter cases pachytene chromosomes have mostly intercalary knobs.

It has been proposed by Kato (1984), McClintock (1978), and McClintock et al. (1981) that maize was domesticated in several regions between Mexico and Guatemala (Mesoamerica), the so-called Multicentric Hypothesis. This extraordinary discovery was based on the fact that racial and geographical distribution of certain chromosome knobs showed very particular distribution patterns, which led to the determination of five centers of origin. In each of these centers a primitive maize germplasm or knob complex was domesticated. At present, extant populations of the annual Mexican teosinte are known to exist in the lowlands of Mexico from the state of Nayarit all along the Pacific coastal regions to the state of Oaxaca. In the highlands they currently grow from Morelos, Puebla, Tlaxcala, the state of Mexico, Guanajuato, Durango to southwestern Chihuahua. This teosinte distribution makes multicentric domestication and differentiation possible. Because each of the knobs with restricted distribution belonged to a specific territory, they gave the clues that indicated how the primitive germplasm moved, forming distinctive migration paths. These five centers are as follows:

1. Mesa Central Complex.

This knob complex is exemplified by the distribution of the medium knob at the 6L1 position [L & S represent long & short chromosome arms; chromosome numbers precede arm lengths], concentrated in the central highlands of Mexico (Mesa Central) showing a migration path toward the north and the west of Mexico. Races included are Cónico, Palomero Toluqueño, Arrocillo Amarillo, Chalqueño, and Cónico Norteño. The distribution of this knob follows that of the maize races Chalqueño, Cónico and Cónico Norteño, the predominant present day races in this territory. Additionally, small, medium and large knobs at positions 1S2, 1L1, 2S1, 2L1, 3S1, 3L1, 4L1, 5L1, 6L1, 7L1, 8L1, also follow this migration path. On the other hand, the race Cacahuacintle, which shows very few knobs in its chromosomes, needs further study, because it provides evidence that a knobless germplasm might have also originated in this territory. In general, knobless chromosome knob positions are concentrated in the Mesa Central region of Mexico.

2. Pepitilla Complex.

The Pepitilla knob complex is represented by the distribution pattern of the large knob at the 6L3 position, which is centered in the intermediate altitude regions of the states of Morelos, Mexico and Guerrero and specifically found in the races Pepitilla and Maíz Ancho. It migrated toward the north to Guanajuato, Zacatecas, and Durango and then west into Jalisco from the Bajio. In addition, this original germplasm also had medium and large knobs in

4.42
different chromosome positions: 1S2, 2L1, 3L1, 4L1, 5L, 7L1, 8L1, and 9S.

3. Tuxpeño Complex.

This knob complex is typically represented by the large knob located at the 9L2 position. In addition, this germplasm had medium knobs at chromosome positions 1S2, 2L1, 3L1, 4L1, 5L1, 7L1, 8L1, 9S, and 9L2. Some large knobs also are present at chromosome positions 2L1, 3L1, 4L1, 5L1, 7L1, and 9L2. The 9L2 knob position is concentrated in the western region of the state of Oaxaca, migrated especially to the states of Veracruz and Tamaulipas on the east coast of the Gulf of Mexico, and then to the north-central states of Nuevo León, Coahuila and Chihuahua; it also appears in part of the Tuxpeño race germplasm in these territories. This primordial knob complex also migrated west from its center of origin into Guerrero, Michoacán, Jalisco, and then into the highland states of Guanajuato, Hidalgo and Querétaro, where it formed part of the Vanendeño race in the west and the Celaya race in the center of Mexico.


Knobs at positions 4S2, 5S1 and the large and medium size knobs at the 7S position are typical of the Zapalote complex. However, knobs of different sizes at positions 1S2, 2S1, 2L1, 3S1, 3L1, 4L1, 5L1, 6L2, 7L1, 8L1 and 9S are also present. They are centered on the race Zapalote Chico and races related to it, Zapalote Grande and Bolita, in Oaxaca and Chiapas. From this territory they were distributed to Guerrero, Michoacán, Jalisco, Nayarit, Sinaloa, and Sonora, marking their migration path toward the northwest of Mexico all along the Pacific coast; related races are Harinoso de Ocho, Tabloncillo, Reventador, Chapalote, and others. To the south these knobs migrated in races of Nal-Tel and Salvadoreño following the lowlands of Guatemala and into the other Central American and northern South American countries.

5. Highlands of Guatemala Complex.

The typical races of the highlands of Guatemala, San Marceño, Serrano, Quicheño, Olotón, Nal-Tel de Ocho, Negro and Salpor, are characterized by the predominance of a combination of small-knobbed and knobless chromosomes. The specific knob of this germplasm is the small one at the 10L1 chromosome position. This knob seems to be more frequent in races as Quicheño and San Marceño and infrequent in Salpor and Imbricado. Other chromosome knob positions having a predominance of knobless and small knobs are: 1S2, 2S1, 2L1, 3L1, 4L1, 5L1, 6L2, 6L3, 7L1, 8L1, 8L2, and 9S, distributed differently among diverse races. It is centered in the highlands of northwestern Guatemala and spread into the states of Chiapas and Oaxaca in Mexico and from southeastern Guatemala into the highlands of other Central American and western South American countries.

The determination of the five complexes, their centers of origin, and their migration paths also made it possible to localize the centers of further racial diversification in Mexico and Guatemala. The reasoning is as follows: where two or more migration paths met, the primordial germplasms had the opportunity to hybridize, so germplasms with new genetic combinations were formed from which Native Americans could have selected new racial types. If this event occurred this way, then those territories that received more primordial germplasms also gave origin to a greater amount of racial variation. Under this argument, four major centers of racial diversification were uncovered: a) Oaxaca-Chiapas-Guatemala, where Tuxpeño, Zapalote and Guatemala Highlands complexes met; b) western Mexico around the state of Jalisco, received four primordial germplasms, Zapalote, Tuxpeño, Pepitilla and Mesa Central complexes; c) the germplasm of the Mesa Central complex was influenced by the Pepitilla and Tuxpeño complexes in the Central highlands of Mexico; and d) in the state of Chihuahua three primordial germplasms met, namely the Mesa Central, Pepitilla, and Tuxpeño complexes.

The patterns of differentiation in Mexican annual teosintes seem not to be the same with respect to maize of the same geographical region or indeed like any extant maize races at all. One example of this is that in the Chalco-Amecameca region in the southeastern state of Mexico, sympatric maize-teosinte populations show predominance of large knobs at 4S2, 5S1, and 7S positions in teosinte, while maize populations do not have any of these knobs. On the other hand, in the Oaxaca-Chiapas region, the reverse situation is found: maize has high frequencies of these knobs and teosinte has none of them. These findings have been interpreted by Kato (1976, 1997) to mean that maize and teosinte, in spite of the fact that they can form spontaneous fertile hybrids when they grow in...
simpatry, generally do not interchange genes, implying that they are largely genetically isolated from each other. There are many more examples, like the one just given, on the genetic relationship between maize and teosinte, which suggest the need for more studies in this direction (Kato & Lopez, 1990; Kato & Sánchez, 2002). This partial genetic isolation may cause differences in patterns of differentiation between maize-teosinte sympatry.

The above general description of the multicentric differentiation of maize and how it occurred in Mesoamerica by means of migration paths followed by primordial germplasm is a rational and understandable explanation of how cultivated maize developed there to the present time. This theory on maize evolution in Mesoamerica, however, is completely different from that proposed by Wellhausen et al. (1952): a) based on a theory that teosinte was a hybrid product of a hypothetical wild corn with Tripsacum (Mangelsdorf and Reeves, 1939). Wellhausen et al. (1952) proposed a group of four extant races (Chapalote, Palomero Toluqueño, Arrocillo Amarillo, and Nal-Tel) as primitive ones originating from a hypothetical wild corn. Since no evidence has been found for the existence of such wild corn, this set of hypotheses appears unlikely; b) Wellhausen et al. (1952) also proposed that most present day races originated first, by the hybridization of the primitive races with other races whose origins were unknown, with teosinte and with further new races obtained by different combinations of these hybrids. This type of racial phylogeny is very incomplete because frequently there is no congruence between geographical distribution of the parental races and no real possibility for crosses between them. A good example is their proposed origin of Bolita as the product of Zapalote Chico x Tabloncillo, two races very distantly separated geographically. Since Zapalote Chico is distributed in Oaxaca and Chiapas and the Tabloncillo distribution goes north from southern Jalisco, their theory is unlikely. Also, according to their hypothesized racial origin, Tepecintle is a cross between Harinoso de Guatemala x teosinte; however, Harinoso de Guatemala is hypothetical and Tepecintle does not possess terminal knobs, which are typical of the Guatemalan teosintes. The racial hybridization phylogeny of Mexican races of maize as given by Wellhausen et al. (1952) does not work well, is based on invalid assumptions, and shows conflicting aspects within itself.

South America

After the origin and initial diversification of the races of maize in Mesoamerica were determined by analysis of chromosome knob distribution patterns, the knowledge obtained helped the interpretation of many of the knob patterns of the South American races of maize. Two large South American territories were crucial for understanding the origin and diversification of these races: 1) the northern territory, including Venezuela and adjacent areas of southern Central America, and the eastern Caribbean islands, and 2) the western territory, which comprised the Andean highlands, extending from southern Colombia through Ecuador, Peru, Bolivia, northern Chile, the highlands of Argentina, and the western coastal regions from Ecuador to southern Chile.

Northern Territory

It was found that three primordial Mesoamerican germplasms were introduced into Venezuela in ancient times, the Zapalote complex, the Guatemalan (or possibly Mexican) Highland complex and the Tuxpeño complex, which represented the large knob germplasm, the small and knobless germplasm and the medium size knob germplasm, respectively. The three Mesoamerican complexes were not introduced to Venezuela independently, but diverse mixtures arrived at different times and probably by means of various routes. However, each of the initial knob combinations is still recognizable: The large knob germplasm is distributed all over northern Venezuela and is observed in races such as Tusón and Coastal Tropical Flint/Costeño. It is composed of the large knobs at positions 2L, 3L, 4L, 5L, 7S, 8L1, 9S, and 9L2. Some knobs of the Zapalote complex of Mexico, such as those at the 7S position, are present especially within the western Venezuelan races, Aragüito and Guaribero. Most of the large knobs on the chromosome long arms are present in the Catetos (a group of strains similar to the Caribbean Flints [Cuban Flint, Tusón, and Coastal Tropical Flint]), grown along the Atlantic coastal region of South America as far south as Uruguay and Argentina. Cateto populations differ in knob constitutions due to introgressions introduced from races of the Central region of South America such as Caingang, Cristal, Pichingá and Pontinha.

In contrast, the knobless and small knobbed chromosomes at the 2S, 2L, 4L, 5L, 7L, 8L1, 9S positions, which also occur in the highlands of Guatemala, are concentrated in the western region of Venezuela. The small knob at the 10L1 position, characteristic of the Highlands of the Guatemala Complex, was not found in Venezuela nor in the limited data obtained from maize of Colombia. Not all races growing in the Guatemala highlands have this knob; it is frequent in races of San Marceño and Quicheño and infrequent in Salpor and Imbricado. If this was the
condition when maize was introduced to Venezuela, then, one possibility is that the maize brought to this region did not have this specific knob.

The large knob at the 9L2 position, as mentioned before, is typical of the Tuxpeño complex. However, this complex has medium size knobs at many other positions. In Venezuela, the 9L2 large knob has been found in high frequency in the northern part of the country together with other large knobs, indicating that Tuxpeño and Zapalote complexes were introduced together.

Western Highlands

The western highlands refer to the Andean region extending from southern Colombia to northern Chile and the highlands of Argentina. The races of this territory proved to be very uniform; their chromosomes were almost knobless, with the exception of two small knobs at the 6L3 and the 7L1 positions. This complex was originally found by McClintock (1959) in her studies for the chromosome project of the races of maize in the Americas. She called it the Andean Complex. The Highlands of Guatemala is a region in which maize races have a concentration of knobless and small knobbed chromosomes. It is reasonable, therefore, to consider this region to have contributed the original germplasm to the Andean territory. The small knobs at chromosome positions 6L3 and 7L1 are present in high frequencies in all Guatemalan highland races (Quicheño, Imbricado, Serrano, San Marceño, Negro de Chimaltenango, Salpor, Olotón, and Nal-Tel de Ocho); alternatively, the small knob at the 10L1 position, although characteristic of the germplasm of this territory, has a relatively low frequency.

In this same study, McClintock also found that the chromosome constitution of this Andean Complex was completely different from the races of maize of the Venezuelan segment of the Andes, which possess different knobs in high frequencies. The races having the Andean Complex were those characteristically classified (by morphological traits of the plant and ears) as Altiplano, Kulli, Huilcaparú, Checchi, Cuzco Boliviano, and Cuzco-Huilcaparú races of Bolivia (Ramírez et al. 1960), Marcame, Polulo, Negrito Chileno, Chulpi, Capio Chico and Grande, Capio Negro, Chutucuno Chico, Chutucuno Grande, and Harinoso Tarapaqueño of Chile (Timothy et al., 1961) and Sabanero, Sabanero Ecuadoriano, Mishca, Kcello, Kcello Ecuadoriano, Chillo, Chulpi Ecuadoriano, and Huandango of Ecuador (Timothy et al. 1963). Another characteristic of these races is that their kernels usually have floury endosperms. The original germplasm introduced to the Andes most likely came from the highlands of Guatemala, where the knobless and small knobbed chromosomes predominate, and also where races having floury endosperm, such as Salpor and Salpor Tardío, are known (Wellhausen et al., 1958).

This Andean complex influenced maize races of the non-Andean regions of Ecuador on the west and lowlands of Chile on the south; it also greatly influenced races such as Entrelaçado of central and eastern Mato Grosso, and other races in the lowlands of eastern Bolivia, Paraguay, Argentina, and the Brazilian states of Paraná, Sao Paulo, and Minas Gerais.

The maize races outside this Andean region have chromosomes with several knobs of various sizes which introgressed into the typical Andean races where they met (Ramírez et al., 1960; Timothy et al., 1961, 1963). In addition to the many knobbed chromosomes present, they frequently show two endosperm traits: 1) sugary, in races like Chulpi in Ecuador and Chile, Dulce in Chile, and Chuspillu in Bolivia, and 2) beaked kernels in the races Canguil in Ecuador, Choclero in Chile, and Pisankalla and Paru in Bolivia. These races have different germplasm from the typical Andean ones; some were likely introductions to the lowlands of the west coast of South America from the western territory of Mexico. This is so, because these traits are currently present in parts of western Mexico in races such as Maíz Dulce, Pepitilla, and Semi Pepitilla. Recently, Tracy et al. (2006) have found that the sul (Maíz Dulce) phenotype has at least four alleles in Mexican and U.S. maize populations. However, the sul allele found in the race Chulpi from Peru did not have any of those four alleles. This was taken as evidence that this sul allele mutated independently in South America and was not brought there with maize introduced into South America from Mesoamerica. Unfortunately, only one accession of Maíz Dulce from Mexico was included in their study (Guanajuato 181) and this race is widely distributed in the western states (Jalisco, Nayarit, Michoacán, Guanajuato, Zacatecas, Durango) of Mexico; so the allelic variation of sul in Mexico is not yet known.

Many of the beaked maize kernels of South America resemble Pepitilla race kernels more than the pointed kernels of the Palomero Toluqueño race of the highlands of Mexico, or of the Imbricado race of Guatemala and Colombia (Wellhausen et al., 1952, 1958).
This condensed description of the general pattern distributions of chromosome knobs and races of maize in South America and their relationships, clearly shows the powerful effectiveness of McClintock’s pattern concept applied as a visual method for analyzing complex chromosome knob data: a) for separating different knob complexes after they became mixed or overlapped in time during their introduction into a new territory (e.g., separation of the large knob complex of Venezuela); b) for showing how different the knob constitutions are among races within and between two distinct geographical territories, even though both have germplasm with common origin (e.g., the role of germplasm from the highlands of Guatemala within Venezuela and between this country and western South America). These cases probably are good examples for explaining the causes of the differences, based on the idea that chromosome knobs have a modulating function on gene expression without necessarily changing the gene structure, as mentioned in the introductory section.

Chromosome knob distributions in maize of the Americas shows that certain knob patterns are found in races of specific geographic regions and other patterns in different groups of races growing in other regions, but with completely different climatic conditions. For example, races from the highlands, with a cooler climate (Central Mexico, Northwestern Guatemala, and the Andean mountains) and regions of higher latitudes, with cold winters and hot summers (the Knobless Flints in the northeastern United States and annual teosintes in northern Mexico) tend to have small knobbed or knobless chromosomes. On the other hand, those races adapted to the lowlands tend to contain medium and large knobs (both coasts of Mexico, lowlands of Central America and South America). However, in the highlands of Mexico and higher latitudes of North America, with the exception of the northeastern United States (where maize arrived late) and the northern Mexican extreme for teosinte (Nobogame and Durango), there are maize races with medium and large knobs. Based on these facts, the general conclusions are: a) chromosome knobs appear to have variable selective values, so that different combinations of them can contribute specific adaptability to races or their varieties; b) as knob size increases, genetic modulating capacity appears to be higher, so maize populations with larger knobs might have wider adaptation.

Even though the basic general information about maize origins and domestication has been determined and some ideas about the origin of specific races were described, part of the story needs more detailed studies in the future. The recommendation is that a reasonable number of collections of each race should be selected within the territory where the particular race is cultivated and, for each collection a goodly number of plants should be analyzed. These studies possibly would give information on the origin of particular races and collections or groups of them. The study should detect presence or absence of specific knobs, and their knob frequencies. This information should be of interest for breeders and evolutionists, as Wellhausen stated in the preface of McClintock et al. (1981), “For a fuller and more efficient exploitation of these vast germplasm complexes, we need to know more about their origin, migration and relationships and about the natural heterosis patterns that exist.”

It is necessary to clarify several aspects of this discussion about the origin and diversification of maize, analyzed from chromosome knob data and obtained from many races of maize. First the origin and initial domestication of maize has been estimated to have occurred some 8,000 to 9,000 years ago, based on still incomplete archaeological remnants of maize kernels, ears, vegetative tissues, pollen grains and phytoliths, including some of teosinte. Since these remnants are known only from limited places, it is clear that there is not enough information about how teosinte and initial maize plants appeared morphologically, or how similar their physiology and genetics were with respect to present day plants, and even under what conditions humans were living at that time, as well as many other ecological aspects. It seems almost impossible to know and demonstrate with certainty, what happened at the time that maize originated and was domesticated. Any proposal about the occurrence of these events necessarily would be hypothetical, and nothing more than interpretations of sets of indirect data, like those of the maize racial chromosome knob constitutions mentioned here. Secondly, as new data are obtained for clarifying aspects of the hypothesis or for filling gaps therein, they may strengthen the hypothesis, or the new information may make the hypothesis weaker so that it could be rejected and a new hypothesis developed to start a new cycle. Science usually advances by this procedure, refuting and rejecting hypotheses or by strengthening hypotheses. The case of the origin and spread of maize as given here is no exception and certainly it is not final, but it must be subjected to criticisms like any other hypothesis before it is rejected or accepted, possibly eventually becoming a theory.

Though much has been advanced on the knowledge about the origin and diversification of maize, no current theory gives an adequate explanation for how the small distichous female inflorescence of teosinte was changed into the large polystichous female inflorescence of maize. Many recent studies are still incomplete. Therefore, comparative
studies on morphology and genetics of teosinte and maize plants, such as those conducted by Galinat (1956, 1963, 1970, 1985, 2001), Doebley and Stec (1991, 1993), Doebley et al., (1990), Dorweiler et al. (1993) and others, should be continued, extended and deepened in order to better understand the changes hypothesized in these reports. As proposed by Wilkes (2004), it is necessary and important to make more archaeological explorations to search for new macrofossils of both plants, especially in Mexico. Thus far, there are few reports of archeologically significant discoveries of teosinte or its crosses with maize (both of which should be well preserved relative to the soft-cobbed early maize recovered from sites like Tehuacan).

Acknowledgments

The author is very much indebted to Dr. Lee B. Kass of Cornell University for having invited him to contribute to this Companion Volume of perspectives accompanying McClintock’s publications. He is also deeply grateful to Dr. Major M. Goodman of North Carolina State University for having read several drafts of this paper and contributed invaluable comments, which made it more readable and understandable.

Literature Cited

invasion into heterochromatin. Genetics 149: 2025-2037.

fold-back DNA segment: Are chromosome knobs megatransposons? Proceedings of the National Academy of
Sciences, USA 95: 10785-10790.

Doebley, J. F. and A. Stec. 1991. Genetic analysis of the morphological differences between maize and teosinte. Genetics 129:
285-295.

Doebley, J. F. and A. Stec. 1993. Inheritance of the morphological differences between maize and teosinte: comparison of
results of two F2 populations. Genetics 134: 559-570.

Doebley, J. F., A. Stec, J. Wendel, and M. Edwards. 1990. Genetical and morphological analysis of a maize-teosinte F2 pop-
ulation: Implications for the origin of maize. Proceedings of the National Academy of Sciences, USA 87: 9888-
9892.


Galinat, W. C. 1956. Evolution leading to the formation of the cupulate fruit case in the American Maydeae. Botanical Mu-
seum Leaflets, Harvard University 17(8): 217-239.

Economic Botany 17: 51-59.

Galinat, W. C. 1970. The cupule and its role in the origin and evolution of maize. University of Massachusetts Agricultural
Experiment Station Bulletin 585. 22 pp.


Galinat, W. C. 2001. A reconstruction of the possible role of critical observations leading to a rapid domestic transformation
of wild teosinte into the first maize. Economic Botany 55: 570-574.

to their origin and evolution. University of Massachusetts Agricultural Experiment Station Bulletin 635. 253 pp.


Castillo (eds.). Gene Flow Among Maize Landraces, Improved Maize Varieties, and Teosinte: Implications for
Transgenic Maize. CIMMYT, Mexico, D. F.


The title and sub-title of this book accurately reflect its contents. Most New World maize races have been examined, except for those in Peru, most of those in Colombia, and from certain areas of the Amazon Basin, the United States and Argentina. Over 1,200 races, strains and varieties of maize were analyzed for the presence and/or absence of small, medium or large chromosome knobs, the occurrence of abnormal chromosome 10 and the number of B-chromosomes. The knob forming regions are at specific positions on particular chromosome arms. The state at each knob forming position, e.g., large or knobless, is highly heritable. Thus, pachytene/diplotene analyses reveal the heterozygous or homozygous condition of a knob at any one of the knob forming regions in maize and teosinte.

The book is a superior contribution of encyclopedic proportions. Well over half its pages are devoted to tables and distribution maps of individual chromosome constituents. From this vast amount of complex data, the distribution and frequency of selected individual knobs or groups of the chromosome components are interpreted in terms of where maize developed initially and when, where, and how it was introduced into other parts of the Americas and its fate following introduction. The focus of this study developed as it became evident during the investigations, initiated in the late 1950’s, that the degree of likeness and difference in chromosome composition among the accessions could demonstrate differences of racial and/or geographical relationships.

The manner in which the data are used to determine relationships is clearly demonstrated. In this review, I have condensed and freely used the interpretations and conclusions of the authors without repeated reference to them. This was done to call attention to (1) the kinds of information contained in the book, and (2) how the authors combined individual segments of data to develop a coherent theme on the evolution of maize races. The distribution and frequency, for example, of five knobs (the large and medium knobs on the short arm of chromosome 4; and the large, medium and small knobs on the short arm of chromosome 5) link the Mexican races Zapalote Chico to Zapalote Grande and to Palomero Toluqueño, Peptillita, Chapalote, Reventador and Harinoso de Ocho. This same group of knobs is used to substantiate the relationship of these germplasm components to Nal Tel and the Venezuelan race, Guaribero. Similarly, the distributions of the large, medium or small knobs on the short arm of chromosome 7 are used to trace the migration routes by which maize from one area was carried to another, the probable role of that material in introgression and its influence on subsequent racial germplasms. The large knob links Zapalote Chico and Nal Tel to much of the Venezuelan maize. Maize with that knob is not found in the United States, eastern or central Mexico, or the western Mexican coast unless it is related to those two races. Maize with both the large and medium knob developed in southwestern Mexico. It was taken then to Guatemala and subsequently carried by introgression in the lowlands and was transported along the Pacific Coast from Oaxaca and Chiapas to Sonora. These components generally did not enter the highlands, the Mayan territory, or Central America south of Guatemala. However, maize having these two knobs may have arrived in Venezuela before other maize did, and, after Tuson and Coastal Tropical Flint developed, had extensive influence in the Caribbean and Guianas. In contrast, the small knob did enter the Mexican Maya area, many races of Guatemala, other Central American countries, or Cuba. In Central and North America, six other groups of special knobs, or combinations of them, were used as additional examples to illustrate how the data reveal the relationship of maize to teosinte, the manner of maize racial formation and contributing germplasm, and geographical areas in which some of the early diverse racial types developed.

The concept of knob complexes is developed from the co-occurrence of specific knobs or knob sizes in certain regions. The origins of knob complexes and their significance in association with migration pathways, introductions and introgressions are discussed. One example illustrates how the Andean Complex, in the highland Andean chain from Ecuador through Chile, may represent an early introduction into South America, perhaps from highland Guatemala where small knobs and knobless chromosomes are concentrated. Subsequent long isolation and selection resulted in the many distinctive Andean races prior to the influence on or from later germplasm introductions and introgressions. Examples are also given relative to several complexes in a single area and how these represent different formative germplasms and their sequential infusions.

The highly patterned geographical distribution of chromosome constituents is also convincingly demonstrated in South America and discussed under six broad areas: Northern Territory, Western Highlands, West Coast, Central Region, East Coast and Central Matto Grosso. Here, the types of maize that were transported from one region to another, the times of these transports and the routes taken are discussed with the same logic as that used in North and Central America. These phenomena seem more vividly demonstrated in South America, however, because of the broader geographical scale and greater isolation from original germplasms.

The early interchange of germplasm between maize of the Andean Highlands and Guatemalan Highlands is clearly documented, as is the influence of South Mexican maize on that of northwestern Venezuela. Subsequent migration and introduction to and from these and other areas are also made evident.

The book is an essential reference for students of maize or others having interest in maize races and their origins. Typographical errors are few. Unfortunately, many of the maps are overly reduced, making it difficult to study the relative frequencies and distribution of specific knobs plotted by identifying symbols. Clarity in distinguishing classes of symbols can sometimes be achieved by using a magnifying lens. In some cases, the use of other maps and tabular material in the book is required to reconstruct the distributions and relative frequencies in which one has particular interest. Overall, however, this is an excellent book containing voluminous data, critical analyses and insightful discussions.

D. H. Timothy, Raleigh
Part V: CYTOGENETIC STUDIES OF Neurospora crassa (1945-1954)

McClintock first assisted researchers with Neurospora chromosome studies in 1940, during her employment as Assistant Professor of Botany (1936-1942) at the University of Missouri, Columbia (Kass 2005).

During the fall of 1944 McClintock took a break from studies of maize cytogenetics. George Beadle had invited her to the Biological Laboratories of Stanford University to collaborate with members of his research group on chromosome studies of the fungus Neurospora (red bread mold) (McClintock 1945a, b). This research was necessary to correlate cytologically what Beadle’s group was observing genetically. Just as she had done with maize chromosomes, McClintock confirmed the haploid number of seven chromosomes in Neurospora crassa as distinguished by relative length, position of centromeres, and internal organization (Beadle 1945). Fincham (1949) soon found other species of Neurospora with the same chromosome numbers.

McClintock (1947) continued investigations of Neurospora chromosomes during the fall and winter of 1946 at the California Institute of Technology (Caltech). There, she collaborated with graduate student Jesse Singleton, who published their results upon completing his doctoral research (Singleton 1953). Continued collaborations at Caltech (McClintock 1954) with Mary Mitchell (formerly Houlahan) afforded new insights into Neurospora translocations and recombination. Neurospora investigations soon provided the first proof of gene conversion by M. Mitchell in 1955 (Perkins 1992).

In this section we reprint McClintock’s preliminary American Journal of Botany report on the chromosomes of Neurospora crassa (McClintock 1945), where readers will find the generous acknowledgment to her collaborators, Mary B. Houlahan, Herschel K. Mitchell and Lottie Steinitz.

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

edited by Lee B. Kass

Perspective:¹

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¹ Perspective solicited.
² For cross-reference purposes, this is the publication number in the annotated, chronological list of McClintock’s publications (Table I) in the Front Matter.
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NEUROSPORA. I. PRELIMINARY OBSERVATIONS OF THE CHROMOSOMES OF NEUROSPORA CRASSA

Barbara McClintock

The present report on the chromosomes of Neurospora crassa represents the results of observations which were confined to a period of ten weeks in the biological laboratories of Stanford University. The purpose of this study was to obtain some knowledge of nuclear and chromosome behavior in normal and mutant strains. The author realizes that no single phase of these investigations could be adequately studied in so short a period of time. Because of the interest in Neurospora as genetic material, a summary of some of these observations will be given at this time.

The observations were confined to the nuclei and chromosomes in the ascus, from fertilization to spore formation. Union of two haploid nuclei occurs in the young ascus. This is followed by a simultaneous enlargement of the ascus and fusion nucleus. During this growth period, the chromosomes in the fusion nucleus enter into meiotic prophase activities, including homologous association of chromosomes, elongation of chromosomes, chiasmata formation and contraction until typical metaphase I bivalents are produced. Although the consequences of this meiotic prophase activity are essentially similar to those observed in many other organisms, the timing of chromosome synopsis and elongation is dissimilar and is of some theoretical interest. The two meiotic mitoses follow in rapid succession leading to the formation of four haploid nuclei. In essential details and accomplishments, the chromosome and nuclear behavior in these two divisions is typical of meiosis in general. Particular details, however, are of interest to the cytologist. Each of the four haploid nuclei in the now greatly enlarged ascus undergoes a typical equational mitosis resulting in a row of eight haploid nuclei. Associated with each nucleus is a centriole which has become greatly enlarged during the meiotic and first post-meiotic mitoses. Fibers emerging from each centriole extend and encircle the cytoplasm surrounding each nucleus. This process initiates wall formation and the cutting out of eight independent ascospores. Shortly after the spore walls are differentiated, the nucleus in each spore undergoes an equational mitosis. The ascospore continues to maturity with the two resulting nuclei.

METHODS.—Approximately seven days (at 25°C.) after inoculation of an agar slant with the two sex strains, A and a, perithecia were present containing numerous asci in various stages of pre-fertilization, fertilization, meiosis and spore formation and development. These perithecia were removed from the slant and placed in a drop of staining solution. With the bent end of a needle, pressure was applied to the perithecial wall. When this pressure was properly exerted, the asci within the peritheciun were forced out through the ostiole. They usually emerged as a single mass. The perithecial wall was removed and a cover slip placed over the drop. The slide was then gently heated. Several methods of staining were attempted such as aceto-orcein, aceto-carmine, propionic-orcein, lacto-orcein and acetic-lactic-orcein combinations. After many trials, it was realized that the genetic strain being utilized had much to do with the success of the staining procedure. A cross of two particular wild-type strains always gave excellent results, whereas other strains gave moderate or consistently poor results. In general, aceto-orcein was the most reliable chromosome stain but the nucleoli were not differentiated. When, in any particular aceto-orcein preparation, it was necessary to observe the nucleoli, aceto-carmine was subsequently run under the cover slip. The nucleoli, taking up the carmine stain, were then clearly visible.

CHROMOSOME NUMBER.—The haploid chromosome number in all the examined strains of N. crassa was seven. This number does not agree with that given by Lindgren and Rumann (1938) for N. crassa (six to nine chromosomes) nor that given by Colson (1934) for N. tetrasperma (six chromosomes). Seven haploid chromosomes had previously been observed (Dr. E. A. Weaver and author, unpublished) in a strain of N. tetrasperma supplied by Dr. B. O. Dodge. The author is indebted to Dr. G. W. Bohn, a former graduate student of the University of Missouri, for calling her attention to the Neurospora chromosomes. He observed seven haploid chromosomes in his excellent aceto-carmine preparations of Neurospora sp.

CHROMOSOME SIZE.—The lengths of the chromo-
some were measured at various stages from presynapsis in the zygote nucleus to the metaphase of the division in the ascospore. The longest chromosome is approximately 2.7 times the length of the meiotic prophase nuclei, the relative lengths of the chromosomes were consistent within each nucleus. Figure 1 illustrates the relative lengths of the seven chromosomes as computed from these measurements.

Morphology of the Chromosomes.—Centromere positions.—The centromere position was adequately determined only for the two longest chromosomes. The analysis of centromere positions was suspended temporarily because it was thought that one of the smaller pairs of chromosomes might be heteromorphic. If this were true, two sets of chromosome morphologies with respect to centromere positions, would have to be considered. The presence of a heteromorphic pair was not confirmed in subsequent examinations which were confined mainly to a cross between two particular wild-type strains. Whether a heteromorphic pair is present or could be identified in crosses of other wild-type strains remains to be determined. Due to the pressure for other determinations, no time was taken to renew the studies of centromere positions. In order to convey some idea of centromere positions in the complement as a whole, the tentative positions that had been assigned to chromosomes 3 to 7 before this analysis was suspended, are included in figure 1.

The nucleolus chromosome.—The second longest chromosome (chromosome 2) possesses a nucleolus organizer close to the end of its short arm. Consequently, there is a very minute satellite. The nucleolus organizer functions in the usual manner and develops a nucleolus in each telephase nucleus.

Chromomere patterns.—At late pachytene, each chromosome shows a distinct chromomere pattern. The pattern for any one chromosome is constant. The chromomeres have various sizes and shapes. They are separated by thinner strands of chromatin but are not spaced equally along the chromosome. The smaller chromosomes have only a few distinct chromomeres (five to six or seven), whereas the longer chromosomes have correspondingly more. No attempt was made in this preliminary study to map the chromomeres of each chromosome. However, these distinctive chromomere patterns could be useful in identifying individual chromosomes at pachytene. No knobs were recognized in these chromosomes. Centromeres could not be identified with certainty in the orcein stained preparations of pachytene.

Heterochromatin.—Heterochromatic segments of chromosomes were not recognized as such in the pachytene chromosomes. However, the presence of heterochromatin was detected in the telophase nuclei following the second meiotic mitosis and in the resting nuclei of the one- and two-nucleated ascospores. It could also be observed in the hyphal nuclei. There are two main segments of heterochromatin. They are located adjacent to a centromere. It has not been determined whether these two recognized segments lie adjacent to the centromere on opposite arms of one chromosome or whether they are parts of two separate chromosomes. Congression of the centromeres in late anaphase of division III,
and in the spore division, results in the formation of a somewhat pear-shaped resting nucleus. The centromeres of all seven chromosones lie in the apex of this pear-shaped nucleus. Here, also, are found the two heterochromatic bodies lying so close together that they suggest a single dumb-bell shaped structure. It is believed, however, that they have not fused to form a single chromocenter but are forced close to one another by the intimate spacial association of all seven centromeres. Extensive observations have not been made of these two heterochromatic bodies nor has an attempt been made to identify the chromosome or chromosomes involved.

**Nuclear fusion, chromosome synapsis and the subsequent elongation of the synapsed chromosomes.**—Fusion of two haploid nuclei to form the zygote occurs in the very young ascus. Illustrations of the appearance of the ascus at this stage are given by Colson (1934). At the time of fusion, the chromosomes of each nucleus appear to be in a resting stage and a nucleus is present in each. Following nuclear fusion, the chromosomes contributed by each nucleus undergo what appears to be a typical prophase contraction until, in some strains, the chromosomes may be almost as short as those of the metaphase of the third division in the ascus. No obvious doubling of the chromosomes was observed, however. During this period, fusion usually occurs between the nuclei contributed by each nucleus. At the end of the contraction period, the two haploid sets of chromosomes lie, roughly, at opposite sides of the zygote nucleus. In this highly contracted state, the homologous chromosomes enter into the synaptonomic phase of the meiotic cycle. In the early synaptic phase, many nuclei were observed with some homologous chromosomes lying adjacent to one another but not in actual physical contact. It is not clear whether this early stage in the association process is the consequence of a directed migration of homologues toward one another or whether this stage is reached following random movements of the chromosomes within the nucleus. Possibly the movements of the chromosomes could be followed in tissue cultures of the living asci. It is of considerable theoretical interest to determine the range of the force of synaptonomic attraction. The actual physical association of the chromosomes usually begins at one or both ends and continues along them. In many zygote nuclei, synapsis is completed for some pairs of chromosomes before the members of other pairs have come in contact. Soon, many nuclei show seven short, but completely synapsed, bivalent chromosomes. (Most of the detailed observations of the synaptic phase were confined to ascis resulting from the cross of two wild-type strains (Emerson 5256A × Chilton-a). In crosses of some other strains, synapsis appears to occur when the chromosomes are less contracted.) Following completion of synapsis and possibly during this period, the chromosomes commence their elongation. This is possibly an uncoiling process for in some early post-synaptic nuclei, the elongating chromosomes appeared to possess compressed gyres. This elongation process continues until the chromosomes have reached their full extension. At this stage, the chromosomes are essentially similar in appearance to the pachytene chromosomes of many other organisms. The term “pachytene” has therefore been used. Although homologous chromosomes lie side by side at late pachytene, they are often not closely appressed. Often, there is little or no relational coiling of the two homologues around one another. During the period from zygote formation to late pachytene, the volume of the nucleus and nucleolus increases steadily. In all post-synaptic stages, the volume of the nucleus is much greater than that of the chromosomes. Consequently, the chromosomes are widely spaced within the nucleus. During all these stages, the chromosome 2 bivalent remains attached to the nucleolus by the organizer regions. At pachytene, the organizer regions of the two homologues may diverge slightly from one another; the satellites may be some distance removed from them.

**Chromosome behavior from diplontene to the third division in the ascus.**—At diplontene, a wide separation occurs between parts of a bivalent chromosome but the individual chromatids were difficult to follow. Coiling commences at diplontene and the contraction of the chromosomes is very rapid. At diakinesis, typical chiasmata may be seen. No attempt was made to count chiasmata but it is possible to do so at this stage. The chromosomes continue contraction to form typical metaphase I bivalents with terminal and interstitial chiasmata. Although the nucleolus becomes smaller during the pre-metaphase stage, it does not disappear. Chromosome 2 remains attached to the nucleolus by its organizer region. Anaphase I separation of the chromosomes appears to be essentially typical except for the nucleolus. This may be dragged toward one pole or stretched between the poles because the nucleolus organizers of one or both of the dyad chromatids of chromosome 2 have not been released from their attachment to the nucleolus. The nucleolus becomes detached before telophase sets in and may subsequently be seen in the cytoplasm of the ascus. At telophase I (and likewise telophase II and III) the centromere regions of all the chromosomes form an aggregate that lies at the apex of a distinct protrusion of the nucleolus (the beak: Dodge, 1927). No true resting nucleus is formed. Instead, the chromosomes uncoil and the individual arms of each chromosome extend into an elongated nucleus. A new nucleolus is produced by and remains attached to the nucleolus organizers of chromosome 2. Prophase II proceeds by contraction of these elongated chromosomes until the two dyads of each chromosome form very short, parallel rods, each showing a conspicuous centromere region. Metaphase and anaphase II proceed normally. At telophase II, the chromosomes, whose centromere regions are again aggregated at the apex of the beaked nucleus, uncoil and the two arms of each chromosome extend into the nucleus as individual strands and remain in this con-
dition until the following prophase. The extent of elongation of the chromosomes appears to be similar to that of late pachytene. In each nucleus, a new nucleolus is formed at the position of the nucleolus organizer of chromosome 2. Prophase III proceeds by contraction of the arms of the chromosomes. Because the chromosomes maintain their previous telophase orientation (J's and V's) during this contraction, the prophase of division III is a satisfactory stage for observing the relative lengths of the arms of a chromosome. Metaphase and anaphase of the third division proceed as a typical equatorial mitosis. The telophase of this division is followed by a condition of the nucleus resembling a resting stage. Shortly after spore delimitation, a mitosis occurs in each ascospore. This is likewise a typical equational mitosis. In essential details, divisions I and II are typically meiotic. Division III is essentially a somatic mitosis except that the chromosomes retain their identity from the telophase of division II to the prophase of division III. It would be of interest to determine the time of effective splitting of the chromosomes for this division.

Reciprocal translocations.—In the Stanford laboratory, many mutants have been obtained following x-ray and ultra-violet irradiations. Chromosomal abnormalities could likewise be expected to occur from such treatments. Three irradiation-induced mutants (4637, 44105 and 45502) whose genetic behavior suggested the presence of some chromosomal abnormality, were selected and crossed to normal wild-type strains. The chromosomes were examined in the asci developing from these crosses. In all three cases, the ascus nuclei were heterozygous for a translocation between two non-homologous chromosomes. In the limited time available, it was not possible to make an extensive study of each translocation. Nevertheless, some observations and interpretations based on these studies will be mentioned.

Translocation 4637.—Figure 2 represents an outline drawing of late pachytene chromosomes in an ascus nucleus developing from the cross of the albino mutant strain 4637 by a wild-type strain. There are five normal bivalents and a synaptic configuration of four chromosomes (right). In these nuclei, homologous associations of all parts of the four chromosomes were not always accomplished. Unsynapsed segments, as illustrated in figure 2, were frequently observed. Sometimes, at pachytene, the four chromosomes were present as two "bivalents" with synaptic associations only between their respective homologous parts. At diakinesis and metaphase I, either a ring of four chromosomes, a chain of four chromosomes or two "bivalent" chromosomes were observed.

Translocation 44105.—Relatively few observations were made of the translocation introduced by mutant strain 44105. These were limited to a few figures of diakinesis and metaphase I. A ring of four chromosomes was observed in one metaphase I figure. In several others, one or more of the chromosomes were present as univalents. In two figures, all four chromosomes were present as univalents. No pachytene configurations were observed.

Translocation 45502.—The reciprocal translocation introduced by mutant strain 45502 involved a very unequal exchange of segments of two non-homologous chromosomes. The breaks appear to have occurred close to the end of the long arm of chromosome 1 and close to the centromere in the long arm of one of the chromosomes with a sub-terminal centromere. This translocation could serve several purposes which will be outlined below.

Estimates of the types of disjunction of chromosomes in ascus heterozygous for T 45502.—Because of the small size of the metaphase and anaphase I chromosomes in Neurospora, it would be very laborious to determine by direct observations the modes of disjunction of the four chromosomes involved in translocation configurations. An examination of the eight-spored ascus developing from asci whose fusion nuclei were heterozygous for translocation 45502 has suggested a possible method of estimating these disjunctions. In most organisms, a two-by-two disjunction of the four chromosomes of an interchange complex usually occurs at anaphase I. In organisms having the Oenothera type of disjunction, alternate chromosomes in a ring or chain of four or more chromosomes go to the same pole at anaphase I. In maize, Pisum, etc., the four chromosomes of a ring usually disjoin so that two members go to one pole and two to the opposite pole. In these forms, alternate disjunctions occur in some cells. In other cells, however, two adjacent members of the ring or chain of four chromosomes may go to the same pole. When a heterozygous translocation is present in Neurospora, do the chromosomes disjoin according to the Oenothera pattern or do disjunctions follow the maize and Pisum pattern? The analysis given below suggests that the disjunctions in Neurospora are similar to those observed in maize and Pisum.

Although the exact position of breakage in the two chromosomes has not been determined, a diagram illustrating the type of synaptic configuration to be expected in asci heterozygous for T 45502 is given in figure 3. If no crossing over occurs in either re-
region a or b, figure 3, alternate disjunctions (1+4 : 2+3, fig. 3) of the four chromosomes at anaphase I when a ring or a chain is present, or the counterpart type of disjunction when two "bivalents" are present, should produce an ascus with eight normal spores (Type I ascus, fig. 3). In this case every spore would receive a full genomic complement, four with the normal chromosomes (1+4, fig. 3), and four with the translocation chromosomes (2+3, fig. 3). When two adjacent chromosomes of this complex pass to the same pole at anaphase I, all eight of the resulting spores in an ascus would be deficient for some part of the genomic complement. There are two possible types of adjacent disjunctions, those which result from disjunctions of homologous centromeres (1+2 : 3+4) and those which result from non-disjunction of homologous centromeres (1+3 : 2+4). The former will be called adjacent I disjunction, the latter, adjacent II disjunction. Following adjacent I disjunctions, four of the spores (with 1+2) would be deficient for nearly all of the long arm of one chromosome. In contrast, the four spores with 3+4 would be deficient only for a small segment of the genomic complement. Comparable studies in maize have shown that spores with deficiencies of large segments of the genomic complement are defective in appearance, whereas spores with small deficiencies may be normal in appearance, especially in the early developmental stages. If the response in Neurospora is similar, it could be expected that the spores with 1+2 would be defective in appearance, whereas those with 3+4 may be normal in appearance, especially in the young eight-spored asci. If this occurs, adjacent I disjunctions would give rise to asci with four adjacent defective spores and four adjacent spores which appear to be normal (Type III ascus, fig. 3). If adjacent II disjunctions occurred, all eight spores would be deficient for relatively large segments of the genomic complement. All eight spores could be expected to show visible evidence of the deficiencies (Type IV ascus, fig. 3).

On the supposition that the spores with 1+2 are defective in appearance and those with 3+4 are normal in appearance, a fourth type of eight-spored ascus could be anticipated. This would be formed whenever a crossover had occurred between the centromere and the position of break (regions a or b, fig. 3). In this Neurospora translocation, such a crossover is probably confined almost entirely to the long segment of region a. Few crossovers would be expected to occur in the very short b segment. Studies of the disjunction of the four chromosomes involved in a heterozygous translocation in maize have revealed that whenever a crossover has taken place between the centromere and the position of breakage, homologous centromeres will pass to opposite poles at anaphase I (McClintock, unpublished). If this crossover-disjunction relationship likewise applies to Neurospora, the resulting eight-spored asci should possess four spores with normal genomic complements (two with 1+4 and two with 2+3), two normal appearing spores with the short deficiency (3+4) and two adjacent defective spores with the longer deficiency (1+2) (Type II ascus, fig. 3).

As table 1 indicates, four main types of ascus corresponding to types I to IV, figure 3, were observed. The eight-spored asci were all relatively young, as the counts were made from slides prepared for chromosome studies. In each count, the relative frequencies of the types of asci are similar. Observations of the spore appearances in mature asci were made by Mrs. Mary B. Houahan. She found that the asci with two very defective spores had, in addition, two immature appearing spores plus four normal appearing spores. These should be type II asci; the spores with the short deficiency, (3+4), not distinguishable in the young stage from spores having a normal genomic complement, are now detectable because of their slower rate of maturity.

It should be stated that in ascus type II, the two adjacent defective spores occupied any one of the four possible positions in the ascus, with approxi-
Table 1. Frequencies of asci with normal and defective spores in six preparations. The zygote nuclei were heterozygous for a translocation associated with mutant strain 45502.∗

<table>
<thead>
<tr>
<th>Type I ascus</th>
<th>Type II ascus</th>
<th>Type III ascus</th>
<th>Type IV ascus</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 defective</td>
<td>4 adjacent</td>
<td>All 8 spores</td>
<td></td>
</tr>
<tr>
<td>sister spores</td>
<td>defective spores</td>
<td>normal</td>
<td>defective</td>
</tr>
<tr>
<td>6 normal spores</td>
<td>4 normal spores</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>All 8 spores</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>normal</td>
<td></td>
<td>defectives</td>
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<td>50</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>24</td>
<td>41</td>
<td>34</td>
<td>8</td>
</tr>
<tr>
<td>37</td>
<td>85</td>
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<td>37</td>
<td>58</td>
<td>30</td>
<td>17</td>
</tr>
<tr>
<td>54</td>
<td>97</td>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td>25</td>
<td>50</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td>Totals</td>
<td>197</td>
<td>198</td>
<td>93</td>
</tr>
</tbody>
</table>

∗ Record was made of 17 asci with normal and defective spore orientations other than types II and III of this table. See text for description.

In making these slides for chromosome studies, many of the asci of types I to III were broken and their spores scattered. Only non-broken asci were scored. Type IV asci were not so readily broken. Thus, the figure for type IV probably is relatively too high.

Ultimately equal frequencies. This is to be expected if the orientation of the chromosomes at metaphase I and II is at random with respect to the long axis of the asci. Likewise, in ascus type III, the four adjacent defective spores occupied positions either at the base or the tip of the ascus.

On the basis of the explanation of the types of eight-spored asci given above, the following conclusions may be drawn: (1) When no crossing over occurs between the centromere and the point of interchage, alternate and adjacent I disjunctions will occur equally frequently (types I and III, table 1). (2) Adjacent II disjunctions are relatively infrequent (type IV, table 1; see accompanying footnote). (3) A crossover occurs in the longer chromosome between the centromere and the position of breakage in approximately half of the ascus nuclei (type II, table 1). It is fully realized that these studies are only preliminary and require further investigation. Nevertheless, the author wishes to emphasize the possible usefulness of this type of analysis as a complement to the cytological observations.

A possible method for determining the frequency of transposition of spores.—In many genetic analyses, the order of the spores in an ascus is of prime importance. The eight spores in an ascus are linearly arranged and are assumed to reflect the orientation of the nuclei and spindles in the three preceding divisions in the ascus. Following division I, the two resulting nuclei are some distance apart in the ascus cytoplasm. The spindles they form are parallel to the long axis of the ascus. Thus, following the second division, four nuclei are present, the upper two derived from one nucleus, the lower two derived from the second nucleus. Maintaining their respective positions in the ascus cytoplasm, each nucleus again divides and a row of eight free nuclei are formed. It is not until then that walls appear cutting out the eight spores. If no disturbances have occurred in the arrangement of the nuclei and spindles during the free-nucleated stage, the position of each spore reflects its origin with respect to the three preceding divisions. Lack of wall formation following divisions I and II in the ascus is a distinct disadvantage. Irregularities in spindle orientation or transposition of the usual order of two or more of the free nuclei will lead to linear arrangements of spores which do not reflect their origin in the previous spindles. Irregularities of this sort are known to occur and it is important for some investigations to determine their frequencies.

The reciprocal translocation in mutant strain 45502 or a chromosomal abnormality giving similar types of recognizable defective spores, might be useful for estimating the frequency of occurrence of aberrant alignments of some of the spores in an ascus. In addition to the asci types recorded in table 1, there were 17 asci with normal and defective spore orientation other than types II and III. If, after the second meiotic mitosis following an adjacent I disjunction described above, the two inner nuclei (with 1+2 and 3+4, respectively) exchanged positions, the spore alignment would not be type III. Instead, two adjacent normal appearing spores (with 3+4) would be inserted between the two sets of sister defective spores (with 1+2). Seven of the 17 aberrant asci were of this type. If, following division III in an ascus destined to be of type II, two non-sister nuclei exchanged positions, a spore alignment other than type II could appear. This would occur if one of these nuclei possessed the long deficiency (1+2) which gives rise to the defective appearing spores. In these asci, the two defective appearing spores would now be separated by a normal appearing spore. Five such asci were observed among the 17 aberrant asci mentioned in the footnote to table 1. These observations are not considered adequate for estimating the frequency of nuclear displacements. More study needs to be given to the aberrant asci to determine whether displacement of spores may occur after spore delimitation through rough handling, or whether additional disturbances, such as aberrant chromosomal behavior, are contributing factors. Because of the significance of aberrant alignment of spores in genetic investigations, it was considered worth while to mention a possible rapid method of estimating their frequencies.

Conclusions.—The usefulness of fungi as genetic material has been well demonstrated in recent years. To interpret properly the results of many genetic investigations, it is either advantageous or necessary to know the accompanying chromosomal conditions. On the basis of this brief study of Neurospora chromosomes, the author anticipates that some fungi may prove to be adequate and in some respects superior cytogenetic material. A review of the literature sug-
gests that some forms may be distinctly superior to *Neurospora* for studies of chromosome behavior, particularly of those stages from fertilization to the first meiotic metaphase. Forms with two haploid chromosomes, one of which is associated with the nucleolus, might prove to be very satisfactory in following the stages and motions of the chromosomes during synapsis, in studying the consequences of various chromosomal rearrangements and for other studies involving the meiotic prophase periods. In ascomyces, the ease of isolation of the asci, the abundance of asci and the relation of size to stage in meiosis should recommend this material for tissue cultures when it is desired to observe the chromosomes during the meiotic stages in living nuclei.

The haploid chromosomal complement of *Neurospora crassa* is similar in its organization to that observed in many organisms. Each of the seven chromosomes may be identified not only by its relative length, the position of its centromere, but also by the constancy of its internal organization as exhibited by chromomere patterns in the meiotic prophase. One chromosome of the haploid complement possesses a nucleolus organizer which functions just as it does in other organisms. Because of the location of the nucleolus organizer near the end of one arm of this chromosome, there is a minute satellite. Even the coiling and uncoiling processes leading to contraction and expansion of the chromomera appear to be similar to that observed in many other organisms. No distinctively unique features of chromosomal organization were recognized. The presence of translocations between non-homologous chromosomes following irradiation treatment and the behavior of these translocated chromosomes in the meiotic stages of heterozygous asci likewise are indicative of the orthodox organization of the *Neurospora* nuclei and chromosomes.

It has been observed that the behavior of the chromosomes in the first two mitoses in the ascus results in the formation of four haploid nuclei whose chromosomes have been subjected to the processes common to meiosis in general: synopsis of homologous chromosomes, chiasma formation, and typical anaphase I and II disjunctions and segregations of chromatids. The synaptic period, however, is distinctly atypical. In many organisms, synopsis is initiated in the meiotic prophase when the chromosomes are much extended. In the *Neurospora* strains most extensively studied, this period occurs when the chromosomes are contracted, short rods simulating late prophase chromosomes. Elongation of the chromosomes to their maximum meiotic prophase extension takes place after the chromosomes have become homologously associated throughout their lengths. If the chromatena within each chromosome at the time of synaptic attraction and association is tightly coiled, the homologous associations along the chromosomes cannot be equally intimate. Other cases of synaptic attraction of condensed chromosomes have been described but *Neurospora* offers rather unique opportunities for studying this process.

The centriole has not been considered in previous sections of this report, but it deserves a brief mention because of its steady enlargement during the interphase stages of the divisions in the ascus, its relation to the centromeres during this enlargement, as well as its previously known function in initiating spore wall formation (Harper, 1903; Dodge, 1927; Wilcox, 1928). As mentioned previously, the interkinetic nuclei following divisions I, II and III are somewhat pear-shaped because of a decided protrusion or "beak." The centromeres of all chromosomes form a compact aggregate at the apex of this beak. The centriole begins to enlarge into a rod-shaped structure following division I. It functions as a typical centriole in division II. During the following interkineses, the process of enlargement in contact with the centromeres continues. It again functions as a typical centriole during the third mitosis. (For illustrations, see Plates I and II, Dodge, 1927). Following the third division, the greatly elongated centriole, associated with the beak of each nucleus, comes to lie close to the ascus wall. Fibers emerge from it and encompass a mass of cytoplasm about each nucleus thus initiating spore wall formation. That centromeres, centrioles and blepharoplasts are interchangeable cell organelles has been demonstrated in the classic investigations of Pollister and Pollister (1943). In line with these investigations, it is possible to consider that the centromeres of *Neurospora* may contribute to the substance of the centriole during these periods of enlargement. Centromeres, centrioles and blepharoplasts all have the common function of producing fibers. It is possible that the fibers formed by these three interrelated but morphologically distinct cellular organelles are structurally identical or much alike in that they all possess one particular type of molecular organization which is responsible for their capacity to contract or alternately contract and expand.

**Summary**

A summary report is given of the results obtained from a very brief study of chromosome and nuclear behavior in *Neurospora crassa*. The investigations are admittedly incomplete and possibly some errors have been made. Nevertheless, they have revealed that *Neurospora* offers adequate and in some respects unique opportunities for cytogenetic research. The chromosomes were followed from the nuclear division preceding zygote formation through the division in the ascospore. Chromosome morphology was considered with reference to the absolute and relative sizes of the seven chromosomes in various division cycles, the centromere positions, the nucleolus chromosomes, the pachyten chroomomere morphology and the presence of heterochromatin. Chromosome behavior was followed with reference to the atypical timing of chromosome synopsis, the elongation of the chromosomes during a prolonged "pachytene," chiasma formation and the general behavior of the chromosomes in the two meiotic mitoses and the two subsequent equational mitoses. Several re-
ciprocal translocations were investigated and their usefulness for special studies indicated.

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**Wilcox, M. S.** 1928. The sexuality and arrangement of the spores in the ascus of Neurospora sitophila. Mycologia 20: 3–17.
Perspective:¹


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¹ Perspective solicited.
Appendices

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Appendix A-2. Contributors Biographical Sketches

Dr. James A. Birchler is Curators’ Professor of Biological Sciences at the University of Missouri, Columbia. After obtaining a B.S. degree from Eastern Illinois University in botany and zoology (1972), he attended graduate school at Indiana University (Ph.D. 1977) majoring in genetics with a minor in biochemistry. Postdoctoral work was performed at Oak Ridge National Laboratory, Tennessee, and the University of California, Berkeley. Appointments as assistant and associate professor followed at Harvard University beginning in 1985 in the Department of Organismic and Evolutionary Biology. Dr. Birchler joined the faculty at the University of Missouri in 1991. He has served on the editorial boards of Genetics, The Plant Cell, BioMed Central Plant Biology, Tropical Plant Biology, McGraw-Hill Yearbook of Science and Technology, Genomic Insights, Journal of Genetics and Genomics, Journal of Biomedicine and Biotechnology, Molecular Biotechnology, GM Crops, Maydica, Frontiers in Plant Genetics and Genomics, Annual Reviews of Plant Biology and Annual Reviews of Genetics. Birchler has served on the Maize Genetics Executive Committee and on the Council of the American Genetics Association. He is a fellow of the American Association for the Advancement of Science and a member of the National Academy of Sciences.

Dr. Stephen E. Bloom is Professor Emeritus in the Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University. He received a B.S. degree in Biology from Long Island University (1963), and a Ph.D. in Genetics at The Pennsylvania State University (1968). He joined the Cornell University faculty in 1968 (associate professor 1974, full professor in 1981), held appointments as at-large member of the Division of Biological Sciences (1976-1999), and Associate Director for the Institute for Comparative and Environmental Toxicology (1990-2004), and served as Program Director for a National Institutes of Health (NIH) training grant in Molecular and Environmental Toxicology (1978 – 2004). He did sabbatical leaves at the M.D. Anderson Hospital and Tumor Institute, Houston, TX (1975, collaborating with Dr. T.C. Hsu), at the Worcester Foundation for Experimental Biology (1982, collaboration with Dr. H. Robinson). Supported by grants from the NIH, USDA-NRI, and Cornell Biotechnology Institute, Bloom’s research program has focused on the development and investigation of genetic models of diseases, such as meiotic alterations leading to cytogenetic abnormalities and birth defects; cytogenetic mapping and studies of genes regulating disease resistance, including the major histocompatibility locus (MHC); and genetic alterations involved in development of lymphoid cancers and drug resistance. Bloom taught the first Animal Cytogenetics course at Cornell University, and developed a course in Genetic and Molecular Toxicology. He served as an Associate Editor for the Journal of Heredity (1990-2000) and was on the Executive Council for the American Genetic Association (1997-2000). He is a member of the American Association for Cancer Research, Society of Toxicology, American Genetic Association, and American Association of University Professors.

Dr. Allan M. Campbell graduated from the University of California (Berkeley, 1950), majoring in Chemistry. He received his M.S. (1951) and Ph.D. (1953) under the direction of Sol Spiegelman at the University of Illinois (Urbana) in Bacteriology. He became Instructor in Bacteriology at the University of Michigan Medical School (Ann Arbor). After two years in the U.S. Army (1953-55), he returned to Ann Arbor (through 1957). He spent a year at the Carnegie Institution of Washington in Cold Spring Harbor, NY (1957-58) and at the Institut Pasteur avec Francois Jacob in Paris, France (1958-59). He joined the Biology Department faculty of the University of Rochester (1959-68) then moved to the Biology Department at Stanford University, where he became Professor Emeritus in 2010. His spouse, biochemist Alice del Campillo-Campbell (AB, Columbia University; MS, New York University; PhD, University of Michigan), has been his research collaborator ever since their marriage in 1958. His doctoral research concerned regulation of galactose synthesis in yeast. His best known accomplishments are elucidating the mechanism of specialized transduction by phage lambda and the mode of attachment of lambda prophage to the bacterial chromosome, and the isolation of conditionally lethal phage mutants. His laboratory has also studied the regulation of biosynthesis of the vitamin biotin. He is a Member of the National Academy of Science (1971), Fellow of the American Academy of Arts and Sciences (1971), and Fellow of the American Academy of Microbiology (1986). He received the Abbott/ASM Lifetime Achievement Award in Microbiology in 2004.

Dr. Edward H. Coe Jr. earned a Ph.D. (1954) in botany at the University of Illinois (with John Laughnan) and received his M.S. degree (1951) in plant genetics (with Charlie Burnham), and a B.S. degree (1949) in agronomy and plant genetics from the University of Minnesota. Following a postdoc with Ernest G. Anderson at Caltech (1954-1955), Coe joined the Plant Genetics Unit of the U.S. Department of Agriculture-Agricultural Research Service at the University of Missouri, where he is currently Professor Emeritus of Plant Sciences. His research has contributed to an understanding of anthocyanin biosynthesis, gametophyte functions, non-Mendelian inheritance, and extrachromosomal inheritance. He is author of or co-author of over 100 refereed journal articles, and author or co-editor of two books; most well-known is the co-edited Mutants of Maize. Coe is highly appreciated for his 26 years of continuous service as editor of the Maize Genetics Cooperation Newsletter (1974-2000). He played a central role in establishing the Maize Genome Database and in the early planning meetings leading to a plant genome initiative, and to sequencing of the first plant genome, and the maize genome. He is a member of various professional organizations, including the Genetics Society of America, the American Genetic Association, and the Crop Science Society of America. In recognition of his “lifetime contributions to the field of genetics,” Coe was awarded the prestigious Thomas Hunt Morgan Award by the Genetics Society of America in 1992. The award was presented to him in recognition of the importance of his basic research, his mentorship of students and postdocs, and his extensive and outstanding service to the maize genetics community. Dr. Coe was described as “the glue that holds the maize community together.”

Dr. Nina V. Fedoroff received her Ph.D. in Molecular Biology from the Rockefeller University, and has served on the faculties of the Carnegie Institution of Washington, the Johns Hopkins University and the Pennsylvania State University, where she was the Director of the Biotechnology Institute and the founding Director of the Huck Institutes of the Life Sciences. Fedoroff has published three books.
and more than 150 papers in scientific journals. She is a member of several academies, including the U. S. National Academy of Sciences and the American Academy of Arts and Sciences. Among her awards is a 2006 National Medal of Science, the highest honor awarded to US scientists. Fedoroff served as the Science and Technology Adviser to the Secretary of State and to the Administrator of the US Agency for International Development (USAID) from 2007 to 2010. She is Distinguished Professor of Biosciences at the King Abdullah University of Science and Technology (KAUST) in Saudi Arabia, an Evan Pugh Professor at Penn State, and a member of the External Faculty of the Santa Fe Institute. She was President of the American Association for the Advancement of Science (AAAS) in 2011-12 and AAAS Board Chair in 2013.

Dr. Lee B. Kass received her Ph.D. in Botany, and Genetics from Cornell University (1975), and earned a B.S. in biology at The City College of New York (CUNY, 1969). She did Postdoctoral research at The University of Cambridge (UK) and Vanderbilt University. She has served on the faculties of The University of Cambridge (UK), University of Tennessee (Nashville), Elmira College (New York), The College of the Bahamas (Nassau), Cornell University, and West Virginia University (Morgantown). Kass has authored, edited or co-edited nine books, and authored or co-authored more than 80 book chapters, proceedings papers, and articles in scientific journals. She is a member of the Botanical Society of America, The Bahamas National Trust, and a former member of many botanical organizations. Kass was chair of the Historical Section of the Botanical Society of America for many years. She established the Elmiran College Herbarium in 1985, and currently serves on the Science Advisory Committee of the Bahamas National Trust. Among her awards is the Josef Stein Award, for excellence in teaching and scholarly achievement (1985) and a Fulbright Scholar Award (1996), during which time she and her spouse, Dr. Robert E. Hunt, established the National Herbarium of the Bahamas. She is Visiting Professor at Cornell University, and West Virginia University (Morgantown). Her research focuses on history of botany, and biodiversity and reproductive biology of Bahamian plants.

Dr. Takeo Angel Kato-Yamakake received his Ph.D. from the University of Massachusetts (Amherst, 1975), under the mentorship of Walton C. Galatia. He earned an M.S. in genetics from North Carolina State University (Raleigh, 1964, mentored by Barbara McClintock), and received his B.S. (Ingeniero Agrónomo) from Escuela Nacional de Agricultura, Chapingo, Mexico (1961). Kato was Research Assistant (1958-1959) at Escuela Nacional de Agricultura, Chapingo, State of Mexico, and Cytogeneticist (1960-1972) for the Inter-American Maize Program (Rockefeller Foundation) and International Maize and Wheat Improvement Center (CIMMYT) Project on Cytogenetic Studies of Races of Maize in the American Continent, Chapingo and El Batán, State of Mexico. Since 1975 he has been Professor Investigador Titular, at Colegio de Postgraduados, Postgrado en Recursos Genéticos y Productividad, Origenación Genética, Montecillo, State of Mexico. His research focuses on origon and evolution of maize in the Americas. He has many publications in this research area, and in 1981 he co-authored, with Barbara McClintock and A. Blumenschein, Chromosome Constitution of Races of Maize. Its Significance in the Interpretation of Relationships between Races and Varieties in the Americas. His most recent co-authored publication on this subject is Origen y diversificación del maíz: una revisión analítica.

Dr. Wojciech (Wojtek) P. Pawlowski received his undergraduate education at the Agricultural University of Krakow, Poland, Ph.D. at the University of Minnesota, and completed postdoctoral training at the University of California at Berkeley. He joined the faculty of the Department of Plant Breeding and Genetics at Cornell University in 2004, and is now Associate Professor of Plant Genetics. His research focuses on investigating molecular mechanisms of chromosome interactions and recombination in meiosis. His lab uses some of the same maize mutants that Barbara McClintock discovered and studied. He also serves on advisory committees of several US and European research consortia that study chromosome biology.

Dr. Ronald L. Phillips is Regents Professor Emeritus and former McKnight Presidential Chair in Genomics, University of Minnesota (UM). He earned B.S. and M.S. degrees from Purdue University and a Ph.D. from the UM; postdoctoral training was at Cornell University. Dr. Phillips advised 55 graduate students and 23 postdoctoral scientists, and taught plant genetics for over 40 years. He received the prestigious Wolf Prize in Agriculture in Israel (2007) for “ground breaking research in service of mankind.” He was elected a member of the National Academy of Sciences (1991). He served on the Board of Trustees of the premier International Rice Research Institute in the Philippines (2004-2009), on the Palm Oil Research Institute of Malaysia Program Advisory Committee for nine years (1991-1999), and on the Scientific Advisory Board of the Donald Danforth Plant Science Center from its inception through 2008. He is a Fellow of American Association for the Advancement of Science, American Society of Agronomy, and Crop Science Society of America. Among his awards are the Dekalb Genetics Crop Science Distinguished Career Award, the Crop Science Society of America Research Award, and Crop Science Presidential Award (2010), and the Medal for Science from the University of Bologna (Italy, 2010). Phillips served as Chief Scientist of the USDA (1996-1998) in charge of the National Research Initiative Competitive Grants Program and chaired the Interagency Working Group that wrote the plan for the NSF Plant Genome Research Initiative. He was President of the Crop Science Society of America (2000) and Chair of the Council of Scientific Society Presidents (2006). His research was one of the early programs in modern plant biotechnology related to agriculture. He is a founding member and former Director of both the Plant Molecular Genetics Institute UM and the Microbial and Plant Genomics Institute. He has served on numerous editorial boards, edited six books, and published over 150 refereed journal articles, 75 chapters, and 355 abstracts. Phillips conducted research and teaching in plant genetics applied to plant improvement with an attempt to bridge basic and applied aspects. As Regents Professor Emeritus, he participates in addressing University-wide, national, and international issues. He serves on institutional advisory boards, the panel of judges for the Monsanto Beachell-Borlaug International Scholars Program and the World Food Prize Youth Institute faculty.

Dr. James A. Shapiro, author of the recent book Evolution: A View from the 21st Century, is Professor of Microbiology at the University of Chicago. He has a B.A. in English Literature from Harvard (1964) and a Ph.D. in Genetics from The University of Cambridge (UK, 1968). Shapiro’s thesis, The Structure of the Galactose Operon in Escherichia coli K12, written under the supervision of William Hayes,
contains the first suggestion of transposable elements in bacteria. He confirmed this hypothesis in 1968 during his postdoctoral tenure as a Jane Coffin Childs fellow in the laboratory of François Jacob at the Institut Pasteur in Paris. The following year, as an American Cancer Society fellow in Jonathan Beckwith’s laboratory at Harvard Medical School, he and his colleagues used in vivo genetic manipulations to clone and purify the lac operon of E. coli, an accomplishment that received international attention. In 1979, Prof. Shapiro formulated the first precise molecular model for transposition and replication of phage Mu and other transposons. In 1984, he published the first case study of what is now called “adaptive mutation.” He found that selection stress triggers a tremendous increase in the frequency of Mu-mediated coding sequence fusions. Since 1992, he has been writing about the importance of biologically regulated natural genetic engineering as a fundamental new concept in evolution science. Together with the late Ahmed Bukhari and Sankhar Adhya, Prof. Shapiro organized the first conference on DNA insertion elements in May, 1976, at Cold Spring Harbor laboratory. He is editor of DNA Insertion Elements, Episomes and Plasmids (1977 with Bukhari and Adhya), Mobile Genetic Elements (1983), and Bacteria as Multicellular Organisms (1997 with Martin Dworkin). From 1980 until her death in 1992, Prof. Shapiro maintained a close scientific and personal friendship with Barbara McClintock, whom he credits with opening his eyes to new ways of thinking about science in general and evolution in particular.

**Dr. Mark E. Sorrells** received his Ph.D. in Plant Breeding and Plant Genetics from the University of Wisconsin – Madison in 1978. After a short post-doc he joined the faculty at Cornell University in the Department of Plant Breeding & Biometry. Since 1991 Dr. Sorrells has been Professor and since 2006, Chair of the Department of Plant Breeding & Genetics at Cornell University. The primary focus of Dr. Sorrells’ research program is breeding methodology with application to wheat breeding for the Northeastern region of the United States. He is also involved in several international projects in Africa, South America, and Europe. During his career Dr. Sorrells has actively developed and evaluated new breeding methods and currently he is integrating genomic selection into his breeding program to reduce pre-harvest sprouting, increase disease resistance and improve yield. Dr. Sorrells has published more than 225 papers in peer-reviewed journals. He has been active in teaching and advising students, serving as major advisor to 36 Ph.D. students, 9 M.S. graduate students and minor advisor to 22 students.

**Dr. David B. (Burt) Walden** earned his M.Sc. (1958) and Ph.D. (1959) in the Plant Breeding Department at Cornell University (with Herbert Evert & Ronald Anderson), and a B.A. (1954) degree from Wesleyan University (Middletown, Connecticut). Following an NIH post-doc in Genetics at Indiana University, Walden joined the faculty (Assistant Professor 1961, Professor 1971) in the Department of Botany (now Plant Sciences) at University of Western Ontario, London, ON, Canada, where he is currently Distinguished Research Professor Emeritus. As an undergraduate, Walden worked as a summer assistant to the eminent corn geneticist D.F. Jones at the Connecticut Agricultural Research Station. His research has contributed to an understanding of maize fertilization, chromosome staining and breakage patterns, cytogenetic of maize chromosome replication and aberrations, etc. Walden has edited two books, contributed three book chapters, and has published more than 200 articles in the *Maize Genetics Cooperation Newsletter* and many scientific journals. Highly appreciated for his service to genetics, Walden was elected President of the Biological Council of Canada (1976-1980), President of the Genetic Society of Canada (1980-1984), 17th President of the International Genetics Federation (Canadian, 1988-1993), and served as Secretary General of the 16th International Congress of Genetics (Toronto 1988). He is a member of various professional organizations, including the Genetics Society of Canada. In recognition of his outstanding contributions to teaching and research, Walden was honored as outstanding teacher, Faculty of Science (1967), received the UWO Board of Governors’ Award for Excellence in Teaching (1982), and the Award of Excellence from the Genetics Society of Canada (1996)

**Dr. Randy Wayne** is an Associate Professor in the Department of Plant Biology at Cornell University, where he has taught Plant Cell Biology and Light and Video Microscopy. Wayne completed his undergraduate studies in Botany at the University of Massachusetts. He earned an M.A. in Biology from the University of California at Los Angeles, and a Ph.D. in Plant Cell Biology from the University of Massachusetts (1985) working under Peter K. Hepler. At UM he learned cytogenetics from Carl P. Swanson. He was a post-doc at The University of Texas at Austin and had a Japanese Society for the Promotion of Science Fellowship to work with Masashi Tazawa at the University of Tokyo. Wayne is noted for his work on plant development, establishing the role of calcium in regulating plant growth. He is an acknowledged authority on how plant cells sense light and gravity, on the water permeability of plant membranes and on light microscopy. He has written two textbooks including *Plant Cell Biology: From Astronomy to Zoology and Light and Video Microscopy*, soon to be out in its second edition. He is currently working on both light and gravity and in so doing has published alternatives to Einstein’s theories of special and general relativity.

**Dr. Clifford Weil** is a professor of Genetics in the Agronomy Department at Purdue University and a member of the Whistler Center for Carbohydrate Research. He has a B.S. in Genetics from the University of California, Davis and a Ph.D. in Genetics from Cornell University. His connection to transposons and the work of Barbara McClintock is through a postdoctoral fellowship with Susan Wessler, then at the University of Georgia, working on the Wx-m5 allele and derivatives of it. These studies led to descriptions of the Ds chromosome breakage mechanism and studies of short range transpositions within the wx locus, developmental regulation of Ac/Ds activity and the sequence dependence of Ds excision footprints and DNA repair genes and mechanisms that are involved in the transposition process. In 2000, Weil and his colleague, Reinhard Kunze were the first to demonstrate that plant transposable elements could transpose in yeast cells, introducing a model system that is still in use. He is an elected fellow of the American Association for the Advancement of Science.
Appendix B: Manuscript Reviewers

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Harriet Creighton and Lee B. Kass

at the Wellesley College Greenhouse in 1994


Dr. Lee B. Kass received her Ph.D. in Botany, and Genetics from Cornell University (1975), and earned a B.S. in biology at The City College of New York (CUNY, 1969). She did Postdoctoral research at The University of Cambridge (UK) and Vanderbilt University. She has served on the faculties of The University of Cambridge (UK), University of Tennessee (Nashville), Elmira College (New York), The College of the Bahamas (Nassau), Cornell University, and West Virginia University (Morgantown). Kass has authored, edited or co-edited nine books, and authored or co-authored more than 80 book chapters, proceedings papers, and articles in scientific journals. She is a member of the Botanical Society of America, The Bahamas National Trust, and a former member of many botanical organizations. Kass was chair of the Historical Section of the Botanical Society of America for many years. She established the Elmira College Herbarium in 1985, and currently serves on the Science Advisory Committee of the Bahamas National Trust. Among her awards is the Josef Stein Award, for excellence in teaching and scholarly achievement (1985) and a Fulbright Scholar Award (1996), during which time she and her spouse, Dr. Robert E. Hunt, established the National Herbarium of the Bahamas. She is Visiting Professor at Cornell University, and West Virginia University (Morgantown). Her research focuses on history of botany, and biodiversity and reproductive biology of Bahamian plants.
Emerson Garden Field Laboratory
Fondly known as the “McClintock Shed”

Emerson Garden “McClintock Shed”

(Image used with permission of Robert Dirig).