Contents

Plant Transposons and Genome Dynamics in Evolution

Editor
NINA V. FEDOROFF
King Abdullah University of Science and Technology
Thuwal, Saudi Arabia
and Pennsylvania State University
Pennsylvania, USA

WILEY-BLACKWELL
A John Wiley & Sons, Inc., Publication

This edition first published 2013 © 2013 by John Wiley & Sons, Inc.

Wiley-Blackwell is an imprint of John Wiley & Sons, formed by the merger of Wiley's global Scientific, Technical and Medical business with Blackwell Publishing.

Editorial offices: 2121 State Avenue, Ames, Iowa 50014-8300, USA
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK
9600 Garsington Road, Oxford, OX4 2DQ, UK

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com/wiley-blackwell.

Contributors

Jeffrey L. Bennetzen	Department of Genetics, University of Georgia, Athens, Georgia, USA
David Botstein	Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey, USA
Vicki L. Chandler	Gordon and Betty Moore Foundation, Palo Alto, California, USA
Hugo K. Dooner	Waksman Institute, Rutgers University, New Jersey, USA
Nina V. Fedoroff	King Abdullah University of Science and Technology, Thuwal, Saudi Arabia and Pennsylvania State University, Pennsylvania, USA
Mary A. Gehring	Whitehead Institute and Massachusetts Institute of Technology, Cambridge, Massachusetts, USA
Avraham A. Levy	Department of Plant Sciences, Weizmann Institute of Science, Rehovot, Israel
Damon R. Lisch	Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, California, USA
Robert A. Martienssen	Howard Hughes Medical Institutes, New York, New York, USA and Gordon and Betty Moore Foundation, Palo Alto, California, USA and Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA
Thomas Peterson	Department of Genetics, Development and Cell Biology and Department of Agronomy, Iowa State University, Ames, Iowa, USA
Alan H. Schulman	MTT Agrifood Research Finland and Institute of Biotechnology, University of Helsinki, Helsinki, Finland
Clifford F. Weil	Department of Agronomy, Purdue University, West Lafayette, Indiana, USA
Thomas Wicker	Institute of Plant Biology, University of Zurich, Zurich, Switzerland
Jianbo Zhang	Department of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa, USA

Foreword

David Botstein

Science is both a rational and a social endeavor. The basic paradigm for scientific progress, comprising a progression from observation to theory and experimentation, has been in place for several hundred years. Mostly science moves in steps small enough so that the introduction of a new idea, theory, or point of view is followed fairly quickly by a consensus among scientists who become convinced by the evidence. Scientists are taught to value new ideas and to evaluate the evidence, even when new ideas are revolutionary and fundamental. Often they have no difficulty doing so: Einstein's astonishingly original ideas were understood and largely accepted by physical scientists within a decade or so of their publication.

Occasionally, however, there are geniuses who make observations, propose theories, and carry out convincing experiments that are somehow so far in advance of contemporary scientific understanding that the general acceptance of their ideas, even by the scientific community, lags for many decades. The work of three well-known giants in the history of biology displayed this kind of intellectual "prematurity": Charles Darwin, Gregor Mendel, and Barbara McClintock.

This book is a compendium of what is known and accepted today about transposons and genomic dynamics in plants, a field whose basic ideas manifestly derive from the work and insights of Barbara McClintock to a degree similar to the influence of Mendel on genetics and Darwin on evolution. As with Darwin and Mendel, acceptance of McClintock's ideas

has taken many decades. But unlike Darwin and Mendel, the depth of her insight is still to be fully appreciated.

What stood in the way? Some of McClintock's discoveries, such as the relationship between chromosomes and linkage groups, achieved immediate acceptance. Transposition, by contrast, took decades and repeated rediscovery in organisms other than maize. Still others, such as the concept that genomes sense and respond to external stimuli, are just beginning to find experimental support and intellectual acceptance.

The barrier was not obscurity or even gender. McClintock achieved a high status early in her career on the basis of her achievements. Unlike Mendel, she and her ideas were well known and widely accepted in her community. Despite her eminence, other scientists appear simply to have failed to understand some of her ideas or the evidence on which they were based. Unlike the case of Darwin, there were no religious or ideological barriers to the acceptance of McClintock's ideas. Hers seems to have been purely a matter of having been ahead of her time.

But I also believe that two commonly held convictions had to change to make way for full acceptance of McClintock's ideas about genome dynamics in evolution, which are the subject of this book. The first was the generalization that only proteins and their regulation are really important in understanding biology and evolution. The second was the conviction that the mechanisms of evolution could not themselves evolve.

The focus on proteins was entirely understandable: it was at the heart of the molecular biology revolution. Molecular biologists explicitly taught the "central dogma" that information flows from DNA to RNA to protein and only thence to phenotype and fitness. No surprise, then, that the biological community sought to understand everything in terms of proteins and their regulation. This led to an

unwanted and, and surely unintended consequence: dismissal of noncoding DNA as “junk.” Of course, this “junk” included all the transposons and transposon remnants that, as readers of this book will see, are what make the genome dynamic and are the drivers of genome evolution.

The misunderstanding about evolution of evolutionary mechanisms arose from the long-running debate about Darwinism, not only among scientists, but also in society more generally. Countering religious rejection of Darwinism in favor of divine intention and teleological arguments, scientists rather vehemently rejected the legitimacy of teleology in scientific reasoning. This rejection led, perhaps unwittingly, to the dismissal of “evolvability” as a property that could be selected in evolution. For most of the twentieth century, the scientific community treated the concept of evolvability as requiring something like intention or, at least, precognition.

The idea that one genome is more fit than another because it is more mutable was an idea that, at best, was hard to imagine. The dismissal of transposons as “junk” DNA and as “parasites” whose destructiveness genomes must rigidly control also interfered with perception of their contribution to evolvability. Thus in order for McClintock’s ideas about genome dynamism to be accepted, it was first necessary for the scientific community to assimilate the existence and sheer genomic abundance of transposons and then to appreciate their agency in the mechanisms of chromosome mechanics and functional evolution.

This book is a thorough examination of the current state of knowledge about the numbers and nature of transposons and retrotransposons and how they have shaped plant genomes. Progress of this more incremental variety has come through the invention and application of rapid techniques for genome and transcript analysis. The results have led to a renewed appreciation that Barbara McClintock

understood much more than the basic ideas of chromosome mechanics and transposition, both of which were already widely accepted by the time of her death in 1992.

Indeed, McClintock discovered and recognized the significance of what we now call “epigenetics” – the heritable, reversible regulation of gene activity. The study of epigenetics and epigenomics has only recently become one of the hottest research fields of our time. The larger community is just now beginning to assimilate fully the notion that phenotypes reflect not only genotypes but also the epigenetic consequences of both development and response to the environment. Finally, and perhaps most importantly, McClintock understood, as the rest of us are only beginning to figure out, that there are well-orchestrated genomic stress responses that can rapidly restructure genomes – the quintessence of evolvability.

Introduction

Nina V. Fedoroff

McClintock's discovery of transposition in the middle of the twentieth century was roughly contemporaneous with Watson and Crick's landmark elucidation of DNA structure. But although Watson and Crick were recognized with a Nobel Prize within a decade, several more decades elapsed before the importance of McClintock's work on transposons was recognized with the award of an unshared Nobel Prize. The mystery of why it took so long for transposable genetic elements to be recognized as something more than a genetic oddity is dissipating as we increasingly appreciate the role of epigenetic mechanisms in silencing transposons and maintaining chromosome stability. Given the current recognition of their importance, it is curious that the study of DNA methylation and other epigenetic mechanisms has only recently advanced from the status of mildly disreputable to the cutting edge of investigation.

The term "transposable element" (TE) is generally used here to refer to both transposons that move through a DNA intermediate and retrotransposons that move through an RNA intermediate. The present volume seeks to capture and distill the veritable mountain of information that has now accumulated on the many flavors of plant TEs, their genetics, genomics, and epigenetics. It also provides an opportunity to indulge in a bit of hindsight, with its extraordinary acuity, and to reassess the larger picture of transposons in gene structure and regulation, as well as in genome and organismal evolution.

Although she is best known for her discovery of transposable genetic elements, recounted in Chapter 1,

McClintock's contributions went well beyond transposition. Her seminal work on epigenetic regulation, described in Chapter 4, remains largely unrecognized, as do her insights into genome restructuring. Indeed, it became fashionable to discredit McClintock's view that transposons are gene regulators. And yet, although she did not get everything exactly right, her early insights seem remarkably prescient from a contemporary vantage point, compelling a rethinking of both regulation and the relationships among the genome's indigenous gene populations.

Early in her work on transposons, McClintock came to the conclusion that they were unmoored gene regulatory systems that had become associated with different genes by virtue of their ability to move. This view was reinforced by her growing appreciation that a single active transposon could promote excision of transposition-defective elements belonging to the same family from insertion sites in several genes simultaneously. This hierarchical relationship, in turn, reinforced her conviction that transposons were integral parts of the developmental regulatory machinery and she therefore named them "controlling elements."

She viewed transposons as bits of heterochromatin by analogy to the connection between heterochromatin and certain types of variegated gene expression in *Drosophila.* In a 1950 paper published in the *Proceedings of the National Academy of Sciences*, she wrote:

> Changes in quantity, quality or structural organization of heterochromatic elements may well alter the kind and/or degree of particular exchanges that occur, and in this way control the chromosome organization and the kind and the relative effectiveness of genic action.

This has turned out to quite close to the contemporary recognition that large blocks of silenced and recombinationally inert retrotransposons separate small

“islands” of genes in many plant genomes, as discussed in detail in Chapters 2 and 10.

McClintock’s intense study of the *Suppressor-mutator* (*Spm*) transposon, described in Chapter 4, produced the first detailed genetic characterization of an epigenetic regulatory system, further expanding the range of transposon regulatory attributes that could influence expression of a gene into which a transposon had inserted.

Contemporary concepts of gene regulation are rooted in the pioneering work of Jacob and Monod on bacterial genes. Although McClintock’s assessment of the parallels between the prokaryotic and eukaryotic regulatory systems appears quite accurate in retrospect, her views gained little traction at the time, perhaps because the gene regulatory story was confounded by transposition, a phenomenon that had not yet been discovered in bacteria. But writing in the *American Naturalist* in 1961, McClintock said:

> It should be emphasized that, although transposition of controlling elements in maize made it possible to recognize their presence in the chromosome complement and to study the mode of operation of the component elements of a system, transposition does not necessarily characterize the behavior of a controlling element. An element previously exhibiting transposition may become fixed in a location. If it is the gene-associated element that becomes fixed, the action of the gene will then be permanently under the control of the system to which the element belongs.

We now know that precisely such regulatory captures underlie the various phenotypes of *Spm* insertion mutations (Chapter 4). More that that, we know that such captures are a regular feature of gene evolution in plants, so much so that it has been proposed that all epigenetic regulation of plant genes derives from transposons (Chapters 6–8).

By the time McClintock wrote the 1961 *American Naturalist* article comparing bacterial and maize gene regulatory systems, her understanding of the *Spm* transposon's genetic regulatory mechanism had advanced well beyond anything that had then been described in either prokaryotes or eukaryotes. In retrospect, it is clear that the complexity of McClintock's description arose primarily from the fact that the *Spm* element is regulated by both epigenetic modification and the transposon's regulatory system. As a result, insertions of different transposition-defective elements at different positions in the gene and promoter regions of the pigment biosynthetic genes that served her as reporter genes gave alleles with a remarkable variety of phenotypes (Chapter 4).

What is extraordinary is her insight that the *trans*-acting *Spm* transposon itself could undergo changes in expression that were heritable, but reversible – what we now call epigenetic. In the same *American Naturalist* article, she wrote:

> One of the most interesting and theoretically important types of expression of *Spm* consists in the sequentially occurring reversals in phase of its activity – from active to inactive and back to active. …Following such a reversal of phase, the duration of the particular phase may be long, continuing unaltered through many cell or even plant generations, or it may be short, reversal occurring again in a number of cells only a relatively few cell generations removed from that which initiated the preceding phase. Control of duration of a particular phase appears to be associated with the event that produces the particular reversal of phase. By selective methods, it has been possible to isolate *Spm* displaying either a long duration of an active phase or a long duration of an inactive phase.

More than that, she reported that an active *Spm* transposon could activate an epigenetically inactive one,

suggesting that the transposon encodes its own epigenetic activator, subsequently identified as the transposon-encoded TnpA protein and shown to function precisely as predicted from the genetic interactions (Chapter 4).

We now know that epigenetic silencing is accomplished by a complex of mechanisms that includes histone modification, RNA interference, and RNA-directed DNA methylation. DNA methylation is reversed by both passive and active mechanisms, and reactivation of silent transposons occurs under a variety of conditions, as described in Chapter 5. Although recent experiments suggest that there is gene- and transposon-specificity in epigenetic silencing and reactivation, the *Spm* transposon's epigenetic regulatory mechanism remains among the very few that have been extensively investigated to date, either genetically or at the molecular level.

The invention and perfection of DNA sequencing techniques in the late 1970s set the stage for the subsequent rapid expansion of knowledge about the structure, gene content, and organization of genomes. During the debates that took place at the time about whether it was worth sequencing complete genomes in view of the suspicion that much of the DNA was repetitive and did not code for either proteins or the then-known structural RNAs, transposons were lumped with other kinds of repetitive sequences and given Ohno's and Dawkins labels of "junk" and "selfish" DNA based on the view that they existed solely to propagate themselves and made no contribution to genome structure or function.

Angiosperm genomes, like the genomes of other higher eukaryotes, vary widely in their haploid DNA content, even within a single species, a phenomenon long known as the C-value paradox. We now know that, indeed, this wide C-value disparity is attributable to the differential accumulation of transposons and retrotransposons, predominantly the latter

(Chapters 2 and 10). At the same time, it has become increasingly clear that the organization and evolution of higher plant genomes are driven largely by transposons and retrotransposons.

Astonishingly, the vast majority of the DNA in higher plants comprises transposons and retrotransposons: 85% of the maize genome, for example, consists of TEs, predominantly retrotransposons. The typical large angiosperm genome exhibits small “islands” of genes in a “sea” of repetitive DNA, primarily consisting of retrotransposons (Chapter 10). Although there is significant constancy of total gene numbers and retention of gene complements, the colinearity of homologous genes declines with evolutionary distance and intergenic regions change rapidly (Chapter 10). Comparisons even among inbred strains of maize reveal substantial differences in gene organization and even larger differences in both the length of intergenic regions and their content of transposons and retrotransposons (Chapter 10). Whole genome comparisons across species suggest that both the movement of genes and the intergenic churn are caused by transposons and retrotransposons. Whether examining the results of transposition events involving a single transposon (Chapter 3) or viewing the contribution of transposons to the evolution of chromosomes (Chapter 10), the centrality of transposons to contemporary genome organization is inescapable.

Transposons make many subtle contributions to gene and genome evolution, as well. Transposons create genes, modify them, and program and reprogram their expression (Chapters 7–10). The traffic in genes and regulatory sequences is bidirectional: transposons pick up genes that code for proteins other than transposases and transposase genes are pressed into services other than transposition. Transposons are central to the epigenetic phenomenon of

“imprinting” that imbues genes with different expression patterns depending on whether they were transmitted through male or female gametes (Chapter 7), differences that arise during the major epigenetic reprogramming that takes place during gametogenesis (Chapter 5).

Although ideas about junk DNA have evolved substantially over the past two decades, the transposon monikers have stuck. Transposons are still referred to as “selfish” DNA, “invaders” and “parasites,” with the subtext that they are largely dangerous and destructive, hence in need of taming. The idea that epigenetic mechanisms evolved precisely to “control” the destructive potential of such “parasites,” advanced a decade and a half ago, has also persisted.

The value of these notions diminishes as we learn more about epigenetic mechanisms and gain insight into how transposons shape genomes. The real puzzle is that transposons accumulate in large numbers in eukaryotes, but not in prokaryotes. But this is also true of other categories of sequences, both coding and noncoding. That is, what distinguishes eukaryotic genome organization from that of prokaryotes is the ability to retain and manage large amounts of duplicated DNA. How did eukaryotes tip the balance between duplication and deletion that keeps genome size small in organisms in which homology-dependent recombination mechanisms predominate?

The answer to these questions lies precisely in the epigenetic mechanisms that eukaryotes have elaborated to a much greater extent than prokaryotes. Plants have a more complex and redundant array of epigenetic mechanisms even than animals (Chapter 5); importantly, however, transposons are not its only targets. A common denominator triggering silencing is the repetitive character of the sequence, not its identity as a transposon. Repeats are readily eliminated by unequal crossing over by homologous recombination and it is precisely homologous

crossing over that is subject to increasingly stringent control in eukaryotic evolution.

The capacity to keep duplicated sequences is an essential step in the evolution of multicellular organisms, underpinning the ability to target expression of different subsets of genes to different cells and tissues. Equally key is the ability to program genes for differential expression by a variety of mechanisms, among which are the relatively stable mechanisms involving DNA and histone modification, as well as the more labile small-RNA-mediated mechanisms.

Because homology-dependent illegitimate recombination events between transposons in different locations have the potential to disrupt genomes, the very ability to suppress illegitimate recombination must inevitably favor the accumulation of transposons, the results of whose antics might otherwise be relegated to the evolutionary scrap heap. Thus it was perhaps the elaboration of epigenetic mechanisms originating in prokaryotes to moderate homologous recombination that made it possible for genomes to grow by duplication and for transposons to proliferate. This is precisely the inverse of the “parasite control” hypothesis, which posits that epigenetic mechanisms arose to control transposons.

And yet, even though epigenetic silencing mechanisms effectively minimize transposable element activity, they do not eliminate it, and the fingerprints of transposon activity are evident at every level of genome organization. That brings me to the final piece of the puzzle McClintock left us. McClintock described the suite of nuclear events, including transposon activation and various chromosome aberrations and rearrangements, that unfold in the wake of what she called genomic “shocks,” such as irradiation or mutagenesis, or, as in her experiments, the introduction of two broken chromosomes by a genetic cross. It has, by now, been amply documented that plant transposons are

activated in response to a variety of genomic perturbations and both biotic and abiotic stresses, including pathogen infection, the passage of plant cells through tissue culture, interspecific hybridization and allopolyploidization (Chapters 2, 6, and 9). This appears to be true, as well, in other eukaryotes, from yeast to flies to humans, and the common denominator is, of course, DNA damage. Chapter 9 proposes that dysregulation of the epigenetic machinery underlies responses to such genomic shocks. Experimental evidence has just begun to emerge from the molecular study of hybrid dysgenesis in *Drosophila*. A deeper understanding of how the epigenetic regulatory systems are themselves modulated to facilitate damage control and restore genome integrity remains for future investigations to unravel.

In sum, then, the present volume provides a rich picture of the role that TEs have played in sculpting the genomic landscape of plants at multiple levels of organization and on time scales from the generational to the evolutionary. Given their abundance in most higher-eukaryotic genomes, the ancient origins of the DNA resecting enzymes that they encode, and the clear evidence of their impact on gene and genome structure and regulation, there seems little value in continuing to view them as “parasites.” As well, given the ubiquity and variety of epigenetic regulatory mechanisms, it seems increasingly implausible that they were invented to control TEs. On the contrary, it seems more probable that TEs proliferated and came to drive eukaryotic genome evolution because of and not despite epigenetic regulation. The present volume documents the many ways that transposons have contributed to the evolution of plant genes and genomes, arguably explaining their extraordinary plasticity – indeed, their very evolvability.

Finally, there are many questions to be addressed once we accept TEs as legitimate – indeed, dominant – inhabitants of

the eukaryotic genome. The extraordinary size of many plant genomes suggests that the accumulation of vast numbers of TEs and other kinds of repetitive DNAs is tolerable. Whether the large and rapidly evolving blocks of retrotransposons actually confer a selective advantage is not known. How might the transposon landscape of chromosomes influence the stability and the participation of chromosomes in the mechanics of mitosis and meiosis? The observations that retrotransposon blocks are recombinationally inert and that disruptions in epigenetic regulation disrupt meiosis may well be hints that will lead us to a deeper understanding of the architecture and dynamics of contemporary genomes.

1 The Discovery of Transposition[1]

Nina V. Fedoroff

Introduction

The discovery of transposition can be dated quite precisely. Writing about the first case of an unstable mutation caused by insertion of the *Dissociation* (*Ds*) locus, which she had earlier identified and named for its ability to cause chromosome breakage and dissociation, McClintock observes: “At the time, I did not know that *Ds* could change its location. Realization of this did not enter my consciousness until late this spring, following the harvest of the greenhouse crop.” Inked corrections in McClintock’s hand on a typed manuscript, never published, from January of 1949 identify the spring as that of 1948 and the greenhouse crop as that of winter 1947–1948.

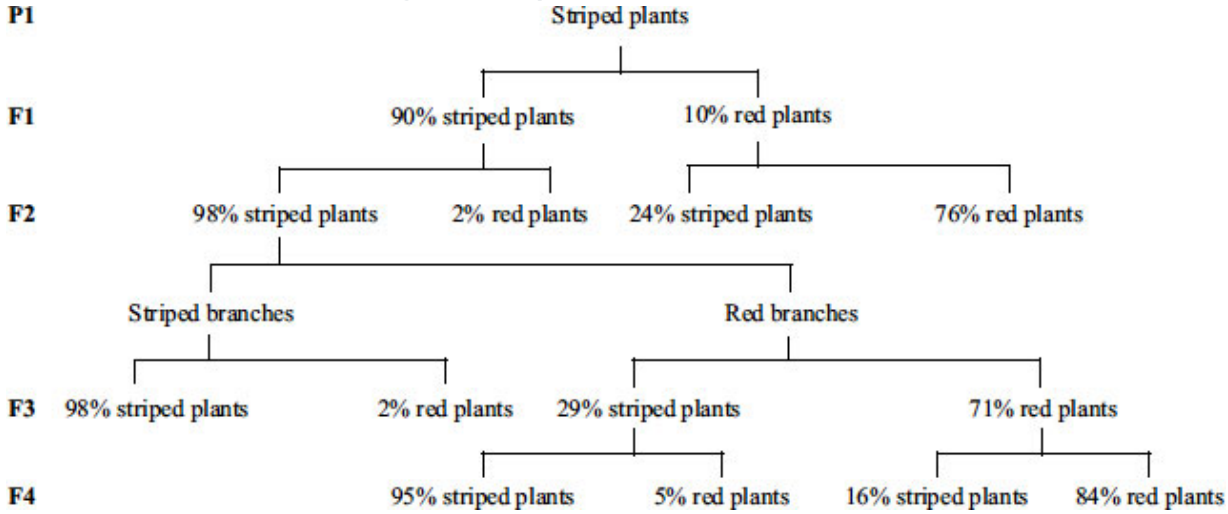
Studies on Variegation

Not surprisingly, the discovery of transposition is embedded in a larger context, both in McClintock’s work and in earlier studies on what were initially called “mutable” or “unstable” genes and “ever-sporting” plant varieties that exhibit variegation for flower and leaf color. While these studies did not lead directly to McClintock’s discovery of transposable elements in the sense that she was working with such materials, she was undoubtedly aware of the earlier work, particularly that of Emerson and Rhoades. Both of these maize geneticists had carried out systematic genetic studies

on mutable genes in maize and contributed substantial insights into their nature and behavior. Thus, it is with the work of these authors that the discussion begins, although it is important to note as preamble even earlier mention in the literature of the peculiar behavior of mutable genes.

De Vries, for example, developed a general concept of “ever-sporting” varieties from studies in *Antirrhinum* (de Vries, 1905). He concluded that the inheritance of variegation and the occasional fully colored mutations or “sports” arising from them generally do not show what we now call Mendelian inheritance, although he did report instances of the inheritance of somatic mutations to full color. Correns, working with *Mirabilis jalapa*, and East and Hays, studying variegation in *Zea mays*, similarly noted that somatic mutations from a variegated to a fully colored phenotype showed Mendelian inheritance. To set the stage, it is worthwhile reproducing a diagram from de Vries experiments on *Antirrhinum* (Figure 1.1) (de Vries, 1905).

FIGURE 1.1 Diagram adapted from de Vries showing the inheritance of variegation patterns in *Antirrhinum*.



De Vries concludes (p. 161):

> From these figures it is manifest that the red and striped types differ from one another not only in their visible attributes, but also in the degree of their heredity. The

> striped individuals repeat their peculiarity in 90–98 percent of their progeny, 2–10 percent sporting into the uniform red color. On the other hand, the red individuals are constant in 71–84 percent of their offspring, while 16–29 percent go over to the striped type. Or in one word: both types are inherited to a high degree, but the striped type is more strictly inherited than the red one.

In the same vein, Emerson commences his first important paper on the genetics of variegation, with the following striking statement, which he thereafter elegantly refutes (Emerson, 1914): “Variegation is distinguished from other color patterns by its incorrigible irregularity.”

Emerson describes his experimental system:

> It is characteristic of the ears of certain varieties of maize known, at least in the Middle West, as “calico” corn. In these varieties, the pericarp of most of the grains has few to many narrow stripes of dark red, the remaining area being colorless or showing a sort of washed-out red. Often broad red stripes appear on some grains, a single stripe covering from perhaps one tenth to nine tenths of the grain. Not uncommonly there are entirely colorless grains (so far as pericarp is concerned) and also solid red grains scattered over the ear. Much more rarely there is found a “freak” ear with a large patch of self-red or nearly self-red grains. Or sometimes an ear is composed largely of red or almost red grains with a small patch of striped or nearly colorless grains. In such cases it is not uncommon for the margin of the red area to cut across a grain so that one side—always the side toward the red patch—is red and the other side colorless or striped. Ears that are colorless throughout, except for a single striped grain, are not unknown and there are even known ears that are red except for a single striped grain. Very rarely a plant has one self-red ear and one variegated ear on the same stalk.

Emerson commenced his study using a few “freak” ears obtained from local and national corn expositions and had no information about their parentage. He asked a different question than de Vries had asked, inquiring whether there was a relationship between the amount of red-pigmented tissue in a given kernel and the number of red ears produced upon self-pollination in subsequent generations. The clear answer emerged that the more red there was in the kernels planted, the larger the fraction of red ears in the progeny. He further found by analyzing the progeny of plants producing red ears that red kernels produced plants that were commonly heterozygous for the red and variegating traits. Emerson concludes: “The development of red in the pericarp is evidently associated with and perhaps due to a modification of some Mendelian factor for pericarp color in the somatic cells.”

Thus, Emerson had captured variegation within the Mendelian paradigm, adding the important insight that a somatic change could occur in a Mendelian factor, becoming a heritable change that obeyed simple Mendelian principles. But he readily admitted that it was “…utterly impossible at the present time to conceive of the cause or even the nature of this change….” He nonetheless went on to conjecture that *V*, as he designated the factor for variegation, might be “…a sort of temporary inhibitor, an inhibitor that sooner or later loses its power to inhibit color development, a power that once lost is ordinarily never regained.” Even more firmly, he suggests that “…it may be that there is present in variegated maize merely a dominant factor for self-color, *S*, that is temporarily inactive, but that sooner or later becomes permanently active.” Emerson had articulated the concept that variegation was due to the association of some kind of a factor with what is now called a gene and that its loss was what allowed the gene to be re-expressed.

In a subsequent study, Emerson noted that, although the loss of the inhibitory factor showed very high heritability, occasional variegated kernels appeared on otherwise fully pigmented ears (Emerson, 1917). This suggested that, at some low frequency, the inhibitory factor might once again become associated with the gene, then called a “unit factor.” Emerson viewed variegation as a reversible change in an otherwise conventional unit factor, which distinguished itself from other kinds of mutations by its high frequency. Emerson later made some puzzling observations that were to remain unexplained until McClintock’s studies decades later. First, he made the counterintuitive observation that reversion to wild-type was less frequent when the variegating pericarp color gene was homozygous and therefore present in two copies than when it was heterozygous with a stable non-pigmenting allele. Second, he noted that chromosomes carrying a stable recessive non-pigmenting allele of the pericarp color locus recovered by segregation from a variegating heterozygote show some ability to suppress variegation when again used to create a variegating heterozygote. As explanations of the latter, he entertained the radical hypothesis that information is transferred between alleles either as “…a direct contamination of one allelomorph by another …” or by transfer of “…distinct gene elements …” from one allele to another. But he readily admits that he, the writer, “…is wholly unable to devise a consistent working hypothesis to account for his results on any such assumption …” and suggests the alternative hypothesis of distinct modifiers of variegation, which had already been reported in *Drosophila virilis*.

Mutable Genes

Substantial work was done on mutable genes in both *Drosophila* and plants, including *Zea mays*, during the ensuing decade, and a debate arose over Emerson's view that variegation was a gene mutation process distinguishable from other types only by its high frequency. Goldschmidt had proposed that mutations are a consequence of position effects and both he and Correns held that mutable genes are sick or diseased genes and that any conclusions derived from their study were not applicable to other types of mutations (Correns, 1910; Goldschmidt, 1938). By contrast, Rhoades and Demerec shared Emerson's view that there was no clear-cut difference between stable and unstable genes (Demerec, 1935; Rhoades, 1941).

Rhoades reported a seminal observation in 1936, the isolation of a dominant maize gene from an ear of Black Mexican sweet corn that caused instability of a previously stable recessive allele of the *A1* locus (Rhoades, 1936). The original segregation ratio suggested that the "dotted" character, exhibiting somatic sectors of the deep pigmentation characteristic of the *A1* allele, required both the recessive a_1 allele and a second locus. Rhoades went on to provide definitive evidence for such a second locus, designating it the *Dotted* (*Dt*) locus (Rhoades, 1938). He further showed that the a_1 allele recovered from the ear that exhibited the variegated phenotype differed in no way from the standard stable recessive a_1 tester allele originally isolated by Emerson and used for two decades without showing evidence of variegation. Rhoades established that the *Dt* locus was not linked to the *A* locus and that both the standard and newly isolated a_1 alleles exhibited variegation or mutability only in its presence. In addition to *Dt*, whose presence was essential for mutability, Rhoades reported that there were additional genes that modified the timing or frequency of somatic mutation.