The discovery that genes move, or transpose, can be dated quite precisely. The discoverer was Barbara McClintock and the time was more than half a century ago. McClintock was studying an unstable mutation, one that reverts to the wild-type during development, resulting in a variegated pattern of mutant and normal tissue. The mutation she was studying was caused by insertion of a genetic element she had named the Dissociation (Ds) locus for its ability to cause chromosome breakage and dissociation. McClintock wrote: "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." Inkerd comments in McClintock's hand on the unpublished, but neatly typed manuscript from January of 1949, identify the spring as that of 1948 and the greenhouse crop as that of "winter 1947-1948."

Studies on variegation

As with most discoveries, 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" varieties. These are plants which show a speckled or variegated pattern of expression of a given trait, such as flower pigmentation or the green pigmentation in green leaves. And even though these earlier studies did not lead directly to McClintock's discovery, she was undoubtedly aware of them, particularly the work of Emerson (1469, 1447, 1448) and Rhoades (1459, 1460, 1461). These geneticists studied a particular kind of mutation in maize (corn), which causes variegation patterns such as the familiar "Indian corn" pattern shown in Figure 1. Figure 2 shows the size of the variegated patch depends upon the time at which the somatic mutation occurs.

Years before the studies of Emerson and Rhoades, the geneticist De Vries developed a general concept of "ever-sporting" varieties, as variegated plants were called, from studies in Antirrhinum, the snap dragon. He concluded that the inheritance of variegation and the occasional fully colored mutations or "sports" arising from them most often do not show Mendelian inheritance (1464). In the same vein, Emerson opened his first important paper on the genetics of variegation, with the following striking statement: "Variegation is distinguished from other color patterns by its incorrigible irregularity" (1469). He went on to elegantly refute his own statement.

Emerson explained that variegation "...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." (See Figure 1) He notes that there are some completely red or colorless kernels and that there are also occasional "freak" ears with large patches of red kernels.

Emerson commenced his study using a few such "freak" ears. He asked whether there is a relationship between the amount of red-pigmented tissue in a given kernel and the number of red ears produced 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 (1469). 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 variegated 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."

What Emerson proposed was that something was in some way attaching to a gene for red color and inactivating it, but only temporarily. That is, the change from colorless to red must be happening occasionally in some of the flowers on a plant that produces striped kernels so that some of its progeny produce ears with all red kernels. Effectively Emerson interpreted variegation in Mendelian terms by adding the important insight that a somatic change could occur in a Mendelian factor. When this happened, the heritable change 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...." "

Mutable genes

During the decade that followed, much work was done on mutable genes in both Drosophila and plants, including Zea mays, and a debate arose over Emerson's view that variegation was caused by ordinary mutations that reversed themselves at a high frequency. In 1936 Rhoades made a seminal observation that has withstood the test of controversy and time: that a stable mutation could become unstable in the presence of a particular gene. He was working with a mutation at the maize A locus, which is necessary for the production of pigment in the kernels. This mutant form of the gene, called the a allele, was thought to be an ordinary kind of mutation. What he discovered was another gene, which he called the Dotted (Dt) gene, that changed the ordinary mutation into an unstable one (1459)! The result was that the a locus displayed variegation. He further showed that the Dt locus was not even on the same chromosome as the A locus and that the mutant a allele only showed variegation if the Dt locus was present. Thus Rhoades made the argument that the difference between stable and unstable mutations was, at least in some cases, dependent on the genetic environment and was not intrinsic to the a locus itself (1459). But Dt had a very specific effect. It neither caused a general increase in mutation frequency nor affected variegation of the pericarp color allele Emerson had studied (1460). So it was a special kind of gene that made the difference between stability and instability at the a locus.

McClintock's studies on chromosome breakage

McClintock's discovery of transposition had its origins in her studies on the behavior of broken chromosomes. Her objective was to determine the behavior of a chromosome with a broken end during mitotic divisions. The results of her experiments led to the insight that chromosomes lacking ends (telomeres), do not separate during replication. As a result, they produce chromosomes with two centers (called dicentric chromosomes). The dicentric chromosomes break, regenerating chromosomes with broken ends, establishing what McClintock referred to as the "breakage-fusion-bridge" cycle (McClintock, 1468, 1453, 1454, 1454, 1455) (see Genes VII: 15.11 Controlling elements in maize cause breakage and rearrangements). The product chromosomes therefore have terminal deletions.

Figure 3 shows how successive cycles remove additional material from the ends. Based on her knowledge of the behavior of broken chromosomes, she developed a method for producing small terminal and subterminal deletions, using plants that developed from zygotes receiving a broken chromosome 9 from each parent (1455, 1470).

Having identified and studied several recurring mutations, McClintock began to search for new mutations in plants that received a broken chromosome 9 from each parent (1471). Initially she observed that one of the broken chromosome 9s was continually lost from cells during development. Following up this observation she found that: "In the cultures that were grown to observe the mutations produced as a consequence of the breakage cycles of chromosome 9, an unusual and unexpected series of new mutants appeared, characterized by types of instability known in the genetic literature as mutable genes, variegation, or mosaicism."

This type of instability was characterized when McClintock carried out a particularly instructive cross to a strain that carried several recessive markers spanning most of the short arm of chromosome 9 and found that all of the dominant markers on the breaking chromosome were being lost simultaneously. This can be seen in Figure 4, which shows a picture of a kernel that has the chromosome 9 genetic constitution C-I Bz/C bz. C-I is a dominant inhibitory colorless allele of the C locus required for pigmentation (C-I gives colorless tissue and C gives purple tissue in the appropriate genetic background) and Bz is the wild-type allele at the bronze locus (the Bz allele gives purple pigmentation with C, while the bz allele gives a bronze color). (The purple borders between the colorless and bronze areas in Figure 4 are due to complementation of the C and Bz alleles at the interface between the sectors which are C-I Bz and sectors which are C bz, because it is necessary to have both C and Bz to get color). But there were no large pigmented sectors that had lost C-I, but not Bz. This suggested to her that the chromosome broke in the same place whenever it broke. Figure 5 explains how the chromosome breaks were correlated with the presence or absence of sectors of each color. The result also suggested that she should be able to see both the broken chromosome and the piece that was being lost if she examined the chromosomes of these special plants. She did the experiment and found precisely what she expected: the distal part of the chromosome disconnected from the rest of the chromosome and was subsequently lost.

By the following year, McClintock was confident enough that the chromosome breakage in the new
strain was happening at a single site to name it the Dissociation or Ds locus "...because the most readily recognizable consequence of its action is this dissociation." She had determined the position of breakage to be on the short arm of chromosome 9, near the centromere (1473). McClintock also recognized that a second locus was required for chromosome dissociation at the Ds locus and she named it Activator or Ac, for its ability to activate chromosome breakage at the Ds locus.

Turning to the investigation of the unstable mutations that had surfaced in her cultures, McClintock soon figured out that although some did not require a second locus for instability, others were unstable only in the presence of Ac (1473). This convinced her that she had been right in her earlier guess that the chromosome breakage and mutability were related in some way.

Recognition that Ds transposes

It was at about this time that McClintock became aware that Ds could move. In her Carnegie Yearbook report for 1947-48 (1474), the simple statement appears: "It is now known that the Ds locus may change its position in the chromosome after such coincidental [chromosome] breaks have occurred." This statement is followed by a brief summary of the observations leading to this startling conclusion. McClintock had previously observed that when Ac was present and caused breaks at Ds, the dominant markers C-I and Bz were lost simultaneously, as illustrated in Figure 5. What provided her with the first clue that Ds could move was noticing two exceptional kernels that did not lose all of the dominant markers distal to Ds simultaneously. Among many kernels that looked like that in Figure 4, she observed a small number that had breaks between the C-I locus and the Bz locus (giving purple sectors), as well as breaks that led to losses of the Bz locus (giving bronze sectors). These observations led to an intense investigation, summarized in a detailed description nearly 50 pages in length, accompanied by more than 40 tables and diagrams, of the genetic and cytological experiments that led McClintock to the conclusion that the Ds element had changed its chromosomal location. Figure 6 summarizes the first part of her explanation of the observations, showing how the transposition of Ds to the region between C-I and Bz resulted in the loss of C-I and the subsequent appearance of purple sectors. Figure 7 shows the second part of her conclusion, that the breakage-fusion-bridge cycle was initiated after Ds broke at its new location, resulting in the loss of Bz and the appearance of bronze sectors.

Explaining mutable genes

There remained one more piece of the puzzle to figure out. What is the relationship between the Ds element and the new Ac-controlled mutable genes? Recalling the earlier contributions, it is clear that McClintock's newly isolated mutations shared certain characteristics of the mutable genes studied by Emerson and Rhoades. Particularly striking was the parallel with Rhoades' Dt-controlled mutable a1 allele (1459). The solution came only when McClintock realized that the Ds element could move and it emerged from the analysis of one new instance of mutability at the C locus in her cultures. On an ear in which most kernels showed chromosome breakage giving colorless c sectors, she noticed a single kernel that showed variegation from the recessive colorless c to the dominant, pigmented C, unexpected for this genetic constitution. She grew a plant from the exceptional kernel and analyzed its progeny. She realized that there was something odd, because the Ds locus wasn't where she expected it to be. She deduced this from the fact that she didn't get some of the combinations of traits she should have gotten as a result of recombination.

By the time she had finished analyzing the results of these crosses, McClintock knew something was wrong with her initial interpretation and began an intensive study of the new mutant, designated c-m1 (1474, 1490). The subsequent crosses confirmed that chromosome breakage was happening very close to the c-m1 locus. She developed the hypothesis that the c-m1 allele might have arisen by transposition of the Ds element into or nearby the C locus, inactivating it and that it reverted by transposition of the Ds away from the locus. Figure 8 shows the phenotypes that result when Ds transposes in and out of the C locus.

In order to test this hypothesis, she selected a number of fully pigmented C kernels arising on c-m1 ears and asked whether they showed Ds-type chromosome breakage. This allowed her to deduce whether Ds was removed when the allele reverted to the wild-type. Of the 16, only 1 showed Ds-type chromosome breakage to reveal the c allele on the homologue. Thus in the vast majority of cases, reversion was associated with stability, which meant that Ds had been removed. Moreover, she carefully examined fully pigmented (revertant C) sectors on kernels that contained Ac and were heterozygous for c-m1 and c or a deficiency lacking the locus. What she observed was that these sectors almost never showed variegation from C to c as might be anticipated if Ds were still present on the chromosome and the Ac locus was causing chromosome breakage and initiating the breakage-fusion-bridge cycle. Thus it appeared that when the c-m1 allele mutated either germinally or somatically to the wild-
type C allele, all evidence of the presence of Ds disappeared. When the results were in, she said: "The combined observations made me suspect that the Ds action was associated with the presence of Ds in the c-m1 locus and that the Ds locus was usually lost from the chromosome when a mutation to C occurred."

Thus the last piece of the puzzle had fallen into place, explaining the basis of the variegation phenomena that had, by then, been under genetic scrutiny for almost half a century. Unstable mutations of the type analyzed by both Emerson (1469, 1447) and Rhoades (1459) could be understood as the result of transposable element insertions into a locus. The inserted transposon frequently excised both during development, giving variegation, and in the germline, restoring function to the respective genes. McClintock was able to make the connection between transposition of a genetic element, the Ds locus, and the origin of a mutable gene giving a variegated phenotype because the particular Ds element she first isolated had a second property, chromosome breakage, by which she was able to track it.

Many of McClintock's original Ds insertion mutations were not caused by chromosome-breaking Ds elements, which have a special structure (152). Nonetheless, they showed the same relationship to the Actuator element as Rhoades' a1 mutation showed to the Dt locus: the mutations were unstable only in the presence of the second, activating locus. McClintock figured out that Ac could itself transpose and cause insertion mutations that differed from those caused by a Ds element simply because they didn't need a second gene to activate their transposition (1490, 1456).

A contemporary coda

A few explanatory notes may help illuminate some of the more mystifying aspects of McClintock's observations. The Ac element is a small transposon (4.5 kb) and encodes a single protein, its transposase (152). Ds elements are often, although by no means always, internally deleted derivatives of an Ac element (see Genes VII: 15.12 Controlling elements form families of transposons). There are many additional, structurally different transposons that are mobilized by the Ac element. Some share little sequence identity with Ac except the 11 bp terminal inverted repetitions and some subterminal transposase binding sites. By contrast, all Ac elements so far isolated are virtually identical in sequence. The chromosome-breaking Ds originally identified by McClintock has a unique structure: it consists of two short Ds elements that comprise the ends of Ac, one inserted in inverted order almost precisely into the middle of the other (152). The ability of this Ds element to break chromosomes is related to the transposition mechanism, which distinguishes the transposon termini on one newly replicated sister chromatid from those on the other. The structure of this aberrant element contains both termini in both orientations on each sister chromatid, resulting in the cleavage of both, rather than one (1449). Finally, the transposon causing instability of the pericarp locus in Emerson's strains was named Mp by Brink and his colleagues. We now know that it is the same as McClintock's Ac (1491, 152).

Future paradox

Today we know that transposons constitute a large fraction—even a majority—of the DNA in some species of plants and animals (see Genes VII: 3.7 Eukaryotic genomes contain both nonrepetitive and repetitive DNA sequences). This includes mice, men and such agriculturally important plants as corn and wheat. So it is paradoxical that the discovery of transposable elements lagged so far behind the discovery of the basic laws of genetic transmission. And it is equally curious that even when transposons were discovered, acceptance of their generality and recognition of their ubiquity came so slowly. It is perhaps an understatement to say that McClintock's early communications describing transposition were not widely hailed for their explanatory power. Indeed, McClintock commented in the introduction to her collected papers (1493) that the response to her first effort in 1950 to communicate her discovery of transposition in "...a journal with wide readership..."; specifically the Proceedings of the National Academy of Sciences, convinced her that "...the presented thesis, and evidence for it, could not be accepted by the majority of geneticists or by other biologists." By contrast, the explanatory power of Watson and Crick's 1953 Nature paper on the structure and mode of replication of DNA was recognized immediately.

Today computers sift through genomes in search of genes, knee-deep in transposons. How could we not have seen them before? The answer is as straightforward as it is mysterious and worthy of thought. Transposons are all but invisible to the geneticist. Despite our growing awareness of the abundance of plant transposable elements and the role they have played in shaping contemporary chromosome organization, the fact is that they eluded discovery for the first half century of intensive genetic analysis. Thus what is perhaps the most striking observation about transposons is not their instability, but precisely the opposite: their stability. Insertion mutations in genes are relatively infrequent and retrotransposition events are so widely
separated that the time interval between insertions in a particular region of the maize genome can be counted in hundreds of thousands to millions of years. Moreover, chromosomes containing many hundreds of thousands of transposable elements are as stable as chromosomes containing few. The question for the future is this: by what means are such sequences prevented from transposing, recombining, deleting, and rearranging?

Barbara McClintock was born on June 16, 1902, in Hartford, Connecticut. She attended Cornell University, undertaking graduate study in the Cornell Botany Department and earning a Doctor of Philosophy degree in 1927. McClintock continued to work at Cornell for a number of years after receiving her Ph. D., interrupted by visits to other institutions and a period spent in Germany on a Guggenheim Fellowship in 1933. In 1936, McClintock went to the University of Missouri as an assistant professor and in 1941 she left for a period as a visiting scientist in the Genetics Department of the Carnegie Institution of Washington at Cold Spring Harbor, New York. She eventually became a staff scientist and remained with the Carnegie Institution of Washington until and after her retirement in 1967. Although McClintock’s early contributions were recognized by her election to the National Academy of Sciences in 1944, the importance of her discovery of transposition was not immediately appreciated. Transposons were not identified in another organism until more than a decade after their discovery in maize. Their ubiquity became increasingly apparent through the 1970s and their pervasiveness was understood in the 1980s. The Nobel Prize in Physiology or Medicine was belatedly awarded to Barbara McClintock in 1983 for her discovery of transposition almost 4 decades earlier.