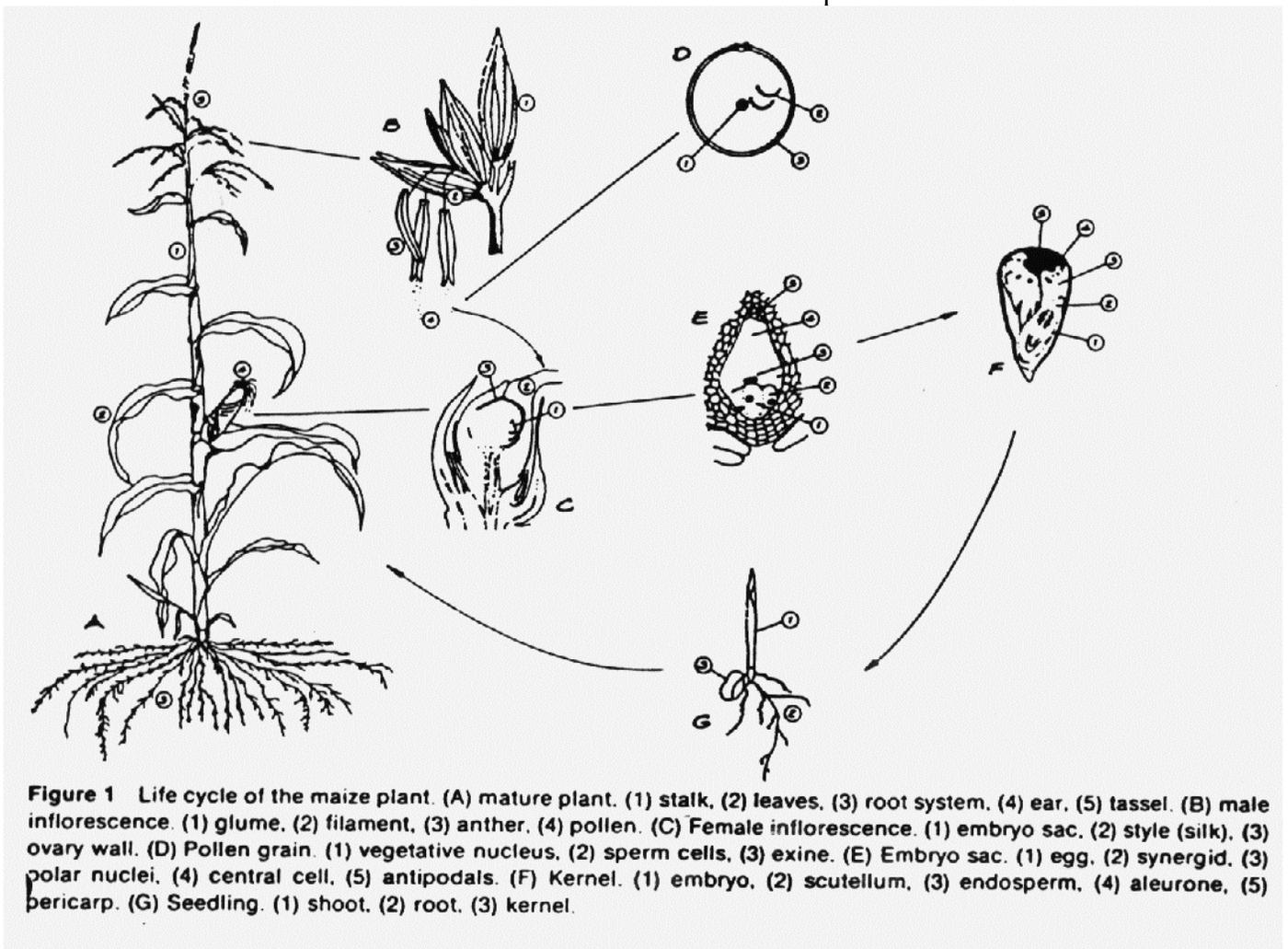


Transposable elements: Barbara McClintock and early experiments in maize

In 1938 Marcus Rhodes provided the first genetic description of an unstable mutation, an allele of a gene required for the production of pigment in maize. This instability resulted in a variegated phenotype. Variegation describes irregular patterns of pigmentation and reflects changes in the expression of genes that control pigment production. Rhodes defined two genes required for this instability: the unstable allele defined one gene, and a dominant mutation called Dotted that controlled this instability mapped to a separate locus, defining a second gene. In the 1940s and 1950s Barbara McClintock showed that this type of instability was caused by genetic elements that moved or transposed from one locus to another. It was not until the description of transposable elements in bacteria two decades later that McClintock's work was truly appreciated. McClintock won the Nobel Prize for her work on transposable elements in 1983.

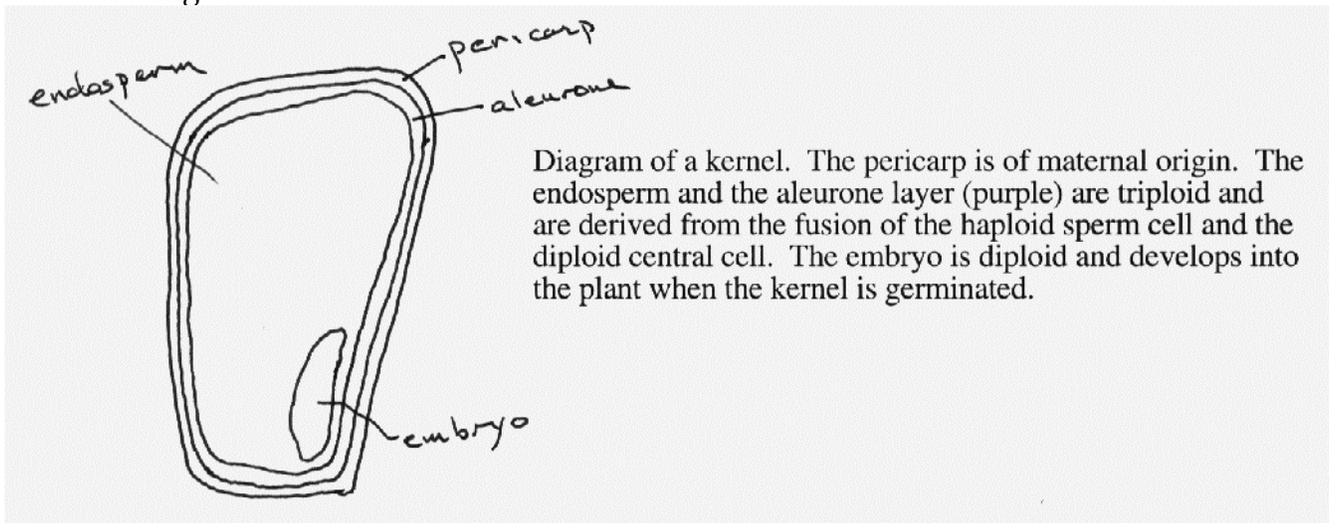


The maize life cycle and kernel development

A single corn plant contains both male and female germlines and is capable of self or cross fertilization. Fertilization begins when a pollen grain lands and germinates on a silk, a long structure connected to the ovule that entraps pollen.

Each pollen grain contains several nuclei. The diploid male pollen precursor cell undergoes meiosis, giving rise to four haploid microspores. Each microspore divides mitotically several times to generate one pollen grain. Two of the products of these mitotic divisions are the two genetically identical sperm nuclei. The diploid female macrospore mother cell undergoes meiosis to generate four haploid macrospores, three of which degenerate. The remaining macrospore divides mitotically three times to generate the embryo sac, which includes one egg nucleus and two haploid nuclei in the central cell.

When a pollen grain lands on a silk, it produces a pollen tube that runs down the silk to the ovule. The two pollen sperm cells migrate down the tube and are deposited in an embryo sac. Fertilization is actually a double fertilization event. Upon reaching the embryo sac, one sperm cell fuses with the egg cell to produce the diploid zygote, and the second sperm cell fuses with the two primary endosperm nuclei in the central cell to produce the triploid endosperm (and aleurone). Thus the embryo that develops from the fertilized egg cell and the endosperm that develops from the fertilized central cell are genetically similar except that the endosperm is triploid, containing two copies of the maternal genome. The kernel consists of an internal diploid embryo, a triploid starchy endosperm, a triploid pigmented aleurone layer that surrounds the endosperm, and a hard pericarp that is of maternal origin. Each kernel represents the product of a single cross and many phenotypic traits can be scored in the triploid endosperm and aleurone layers of the kernel before it is grown into a plant. A single ear of corn contains the products of multiple matings.



The genetic and cytological markers used by McClintock

In the crosses we will describe here, McClintock was working on the short arm of chromosome 9, where the order of genes and chromosomal structural elements that we will consider are: telomere - Colorless (C^+) - Bronze (Bz^+) - Waxy (Wx^+) - centromere. The Colorless gene is required for pigment production in the aleurone layer and is assayed visually: C^+ results in a purple colored aleurone layer; the $C-I$ mutation is dominant to C^+ and results in a colorless phenotype; c mutations are recessive and also result in a colorless phenotype. Bronze is also required for pigment production in the aleurone layer

and assayed visually. Bronze encodes an enzyme that modifies the brown of bronze colored product of the C^+ gene, changing it to a purple pigment. Thus, Bz^+ results in a purple colored aleurone layer, and bz mutations are recessive and result in a brownish aleurone layer. If both the Colorless and Bronze genes are mutant, for example, in a $C-I\ bx/C^+ bz$ or a $c\ bz/c\ bz$ kernel, the kernel is colorless because the C^+ pigment is not produced and the genotype at the Bronze locus is irrelevant.

The Waxy gene is required for the production of a specific starch in the endosperm that is assayed by a staining reaction: Wx^+ confers a starchy phenotype in which the endosperm stains dark blue with an I_2 -KI solution; recessive wx mutations confer a waxy phenotype in which the endosperm stains reddish brown with I_2 -KI.

In addition to the genetic markers, McClintock used a cytological marker, a chromosome with a knob at the telomere. The normal chromosome lacks this knob.

McClintock's analysis of chromosomal breakage in maize led to the discovery that two elements are involved, which she termed Dissociation (Ds) and Activator (Ac). Ds is located at the site of chromosome breakage and can be defined both genetically and cytologically. Ac is dominant and is required for chromosome breakage at Ds.

With an understanding of these genetic and cytological tools, there are four experiments that we will discuss. These experiments showed that: (1) Ds is a genetic element that results in chromosome breakage; (2) Ds can move; (3) Ac controls Ds breakage and can also move; and (4) Ds can move into a gene to produce an unstable allele.

Dissociation (Ds) is a genetic element that results in chromosomal breakage

Kernels from a cross between $C-I Bz^+ Wx^+ / C-I Bz^+ Wx^+$ pollen and $C^+ bz\ wx / C^+ bz\ wx$ ovules were of the genotype $C-I Bz^+ Wx^+ / C^+ bz\ wx$. Most were colorless and starchy (the Bz^+ phenotype is not expressed in a $C-I$ or c/c background). McClintock noted some kernels had colored (bronze), waxy sectors; i.e., all three dominant traits derived from the male parent were lost. McClintock proposed that chromosomal breakage occurred at a hotspot between Wx^+ and the centromere, and she called this hotspot Dissociation (Ds). She predicted that breakage at Ds resulted in an acentric fragment containing the $C-I$, Bz^+ and Wx^+ genes and that this fragment was lost during development of the kernel.

The variegation was heritable, and McClintock mapped Ds 1 cM from Wx . She also provided cytological evidence for breakage at Ds to confirm her hypothesis. The telomere of the $C-I Bz^+ Wx^+$ chromosome was marked with the knob discussed above, while the $C^+ bz\ wx$ telomere was cytologically normal. Loss of the dominant markers coincided with the loss of the telomeric knob.

Ds can move

McClintock also discovered that the position of Ds is unstable and would move to different positions on chromosome 9. For example, when she started with the genotype described above, most variegated kernels had bronze, waxy sectors ($C^+ bz\ wx$). However, an occasional variegated kernel displayed purple sectors, but were uniformly starchy ($C^+ Bz^+ Wx^+$). This new pattern was heritable, and

Ds now mapped to a new position between C and Bz. Cytological analysis confirmed that breakage was now occurring closer to the telomere between the C and Bz genes.

Ac controls Ds breakage and can also move

During these experiments, McClintock mapped a trait that she called activator (Ac), which was required for breakage at Ds. Ac segregates as a single dominant trait unlinked to Ds.

Parental: $c Ds^+ / c Ds^+; Ac^+ / Ac^+$ female X $C^+ Ds / C^+ Ds; Ac / Ac^+$ male

F1: $C^+ Ds / c Ds^+; Ac^+ / Ac^+$ colored, nonvariegating and $C^+ Ds / c Ds^+; Ac / Ac^+$ colored, with variegated colorless sectors

However, Ac mapped to various positions in different crosses. McClintock proposed that both Ac and Ds were mobile genetic elements and Ac was required for the activity of Ds.

Ds can move into a gene, generating an unstable allele

Some of the most interesting observations were those involving unstable alleles of the genes that were generated in a genetic background containing Ds and Ac. For example an unstable mutant allele of C (c^u) was found in one of 4000 kernels analyzed from a cross between a $c wx Ds^+ / c wx Ds^+; Ac^+ / Ac^+$ female and a $C^+ Wx^+ Ds / C^+ Wx^+ Ds; Ac / Ac^+$ male. Unlike other variegated kernels, this kernel displayed pigmented spots on a colorless background, as if C^+ had mutated to c but could revert spontaneously to C^+ during aleurone development. The plant generated from this kernel carried the c^u allele, and spontaneous reversion to wild-type was dependent on the presence of Ac. The c^u chromosome also contained Ds at a new location, and Ds now mapped to the C gene. McClintock argued that Ds had originally transposed from its original position to the C gene, generating the c^u allele. McClintock concluded from these results that Ds was capable of transposing, and when Ds transposed into a gene it was capable of transposing out of the gene to leave a functional gene behind.

McClintock discovered unstable alleles of other genes and some of these alleles had an unusual property. For example, when mapping Ac in a strain that contained an unstable allele of Wx (wx^u), Ac mapped to precisely the same position as wx^u , demonstrating that in addition to functioning in the transposition of Ds, Ac could also function in its own transposition. These results suggested that Ac and Ds were related in some fundamental way. The Ac and Ds alleles of Wx eventually provided the tools for the cloning these transposable elements.