Inheritance of Kernel Color in Corn: Explanations & Investigations

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VARIATIONS in the color of corn kernels (*Zea mays* L.) have attracted geneticists since the early 1900s when studies on the inheritance patterns of kernel color helped validate classical genetics. Even now, research scientists investigate the molecular genetics of these characteristics, and students in introductory biology and genetics courses learn more about monohybrid and dihybrid crosses by studying the inheritance of kernel colors. The reasons for introducing corn genetics in the classroom are obvious—a single ear holds a large number of progeny and a variety of ears are available that represent basic inheritance patterns, such as the monohybrid cross (3:1), the dihybrid cross (9:3:3:1), and more complex genetic patterns illustrating gene interactions (12:3:1; 9:3:4, 13:3; 9:7, etc.). In addition, these ears are obtained readily from several biological supply houses and are relatively inexpensive, especially since students can use them for several years.

Students usually confirm different phenotypic ratios of the F_2 generation by first counting kernels, then performing chi-square analyses to test their data. However, their experience can be enriched by introducing explanations about the nature of each gene and its mutations, the related metabolic pathways, and their locations within the kernel. I have included information in this review that would be appropriate at different levels—from high school biology to introductory genetics at the college level. The purpose is the same. By exploring these areas, students more fully understand how these simple phenotypic ratios and variations are derived.

Four Kernel Colors, Four Genes

Three of the four kernel colors—yellow, red and purple—are produced by pigments synthesized from one of two metabolic pathways, the carotenoid (yellow pigments) or the anthocyanin (red and purple pigments) pathway. White, the fourth color, results from the lack of pigments produced from either pathway. The synthesis of each pigment requires numerous genes, including those that are structural (coding for enzymes) and regulatory (called transcription activators and coding for proteins that control the transcription of structural genes). The four genes, *Pr1*, *C1*, *R1* and *Y1*, described in this paper include both types (Table 1). Having been described in 1901, *Y1* was one of the first maize color genes documented in the literature; the other three, all relating to the anthocyanin pathway, were described later in 1911 (see Coe et al. 1988 for review). At that time the role of each gene was unknown.

Although these genes are commonly called ''color genes,'' the names used by maize geneticists, as included in the Maize Genome Database, are more descriptive. For example, Red Aleurone1 (*Pr1*) describes the kernel color associated with the recessive phenotype and identifies a specific structure of the seed involved in its expression, the aleurone. This structural gene codes for the protein flavonoid 3′-hydroxylase, an enzyme that is responsible for the purple color produced in the anthocyanin pathway. Another gene, White1 or (*Y1*), is another structural gene whose name also describes its recessive phenotype. It codes for phytoene synthetase, an enzyme in the carotenoid pathway. Although the names of the other two genes, Colored Aleurone1 (*C1*) and Colored1 (*R1*), are similar, their names and symbols are different. Both encode transcription activators that interact to influence the regulation of several structural genes within the anthocyanin pathway.

The phenotype is affected by many genes, the sum of which is referred to as the ''genetic background.'' In the production of kernel color, genes in both the anthocyanin and carotenoid pathways comprise the genetic background. For example, in addition to the three genes *Pr1*, *C1* and *R1* that affect the production of anthocyanins, other genes are required for the expression of these pigments since this metabolic pathway has multiple steps. When either recessive allele *r1* or *c1* is present as a homozygote, no anthocyanin is produced, and the kernel is either yellow or white, depending on the genes present for the carotenoid pathway. Therefore, in this case genes in both the anthocyanin and carotenoid pathways comprise the genetic background.

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Table 1. Summary of the four genes, *Pr1*, *R1*, *C1* and *Y1*, influencing kernel color.

Biological Supply[1]		*Maize Database*[2]		*Biochemical*	*Metabolic*			*Chromosomal*
Gene Name	*Symbol*	*Gene Name*	*Symbol*	*Phenotype*	*Pathway*	*Protein Role*	*Protein Name*	*Location*[3]
Color gene	*Pr*	Red	*Pr1*	Purple aleurone[4]	Anthocyanin	Structural	Flavonoid 3′-hydroxylase	5L
	pr	Aleurone1	*pr1*	Red aleurone				
Color gene	*R*	Colored1	*R1 (R-r)*	Colored aleurone[4]	Anthocyanin	Regulatory	Transcription factor	10L
	r		*r1 (r-r)*	Colorless aleurone[5]				
Color gene	*C*[I]	Colored	*C1-I*	Colorless aleurone[5]	Anthocyanin	Regulatory	Transcription factor	9S
	C	Aleurone1	*C1*	Colored aleurone[4]				
	c		*c1*	Colorless aleurone[5]				
Color gene	*Y*	White1	*Y1*	Yellow endosperm[6]	Carotenoid	Structural	Phytoene synthetase	6L
	y		*y1*	White endosperm[6]				

[1]Carolina Biological Supply.
[2]The Maize Genome Database Project.
[3]Chromosome arm designations: S = short arm; L = long arm.
[4]Kernel color depends on the genetic background or the sum total of all genes affecting the anthocyanin pathway (e.g *R1*, *C1* and *Pr*).
[5]Kernel color depends on the color of starchy endosperm (white or yellow).
[6]Yellow and white kernels have a colorless aleurone.

Kernel Structure, Gene Dosage & Imprinting

Although the embryo and endosperm represent the same generation within the kernel, it is the color of the endosperm rather than the embryo that is usually described (Figure 1). This is because the embryo is small—about 15% of the kernel weight (Poethig 1982), located on the broad side of the kernel, and is, therefore, not easily seen without removing the kernels from the cob. Within the endosperm the carotenoids and anthocyanins are synthesized in different regions. The carotenoids are produced in the starchy endosperm, the inner region of the endosperm, while anthocyanins are found only in the aleurone, the outermost layer surrounding the starchy endosperm. Because anthocyanins produce an intense color in the aleurone, the yellow or white colors of the starchy endosperm are seen only when the aleurone layer is colorless.

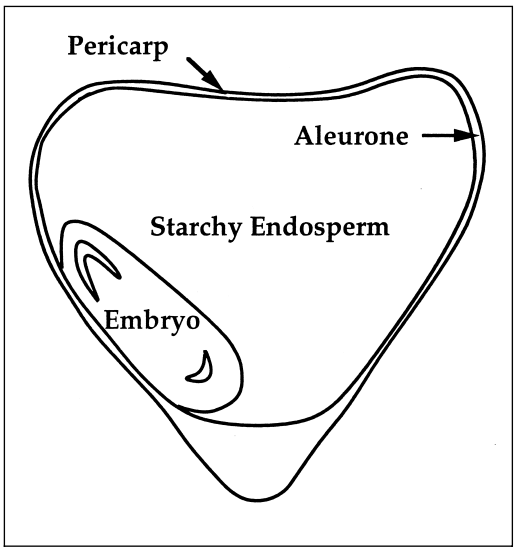

Figure 1. Kernel structure.

The endosperm and the embryo have different genetic compositions—the embryo is diploid and the endosperm is triploid. This difference results from fusion of the two polar nuclei in the embryo sac (female gametophyte) and one sperm nucleus from the pollen (male gametophyte). Therefore, in the heterozygote, the endosperm has one of two genotypes—either two dominant or two recessive alleles—depending on the allele contributed by the maternal parent (Table 2). These genetic dissimilarities may result in phenotypic differences. For example, in the monohybrid cross for *Y1*, the endosperm can be one of four possible genotypes that produce variation the intensity of yellow (Simcox et al. 1987). These color differences correspond to the amount of carotene in the endosperm and reflect the concentration of phytoene synthetase within this tissue. This phenomenon of equating gene product with the number of alleles in the cells is called the gene dosage effect. Of the four genes discussed in this review, only *Y1* exhibits the effect of gene dosage.

Another variation observed in kernel coloration of heterozygotes is purple mottling; that is, the seeds

Table 2. Phenotype and gene dosage of *Y1* in the starchy endosperm.

Phenotype	*Endosperm Genotype*	*Maternal Contribution*	*Paternal Contribution*
Yellow	*Y1Y1Y1*	*Y1Y1*	*Y1*
Light Yellow	*Y1Y1y1*	*Y1Y1*	*y1*
Pale Yellow	*y1y1Y1*	*y1y1*	*Y1*
White	*y1y1y1*	*y1y1*	*y1*

exhibit an irregular distribution of seed pigmentation. The mottled kernels are *r1r1R1*; whereas kernels for the other hybrid, *R1R1r1*, are solid. Further investigations using the genotypes *R1R1r1r1* and *r1r1R1R1*, ones with the same gene dosage but with the alleles originating from opposite parents, did not produce similar phenotypes, suggesting that mottling is associated when the recessive allele comes from the maternal parent. In this case the maternal allele acts as a dominant allele; hence, the kernels are mottled. Imprinting, as this phenomenon is called, describes a difference in the activity of a gene based on its parental origin (Kermicle 1978). It is attributed to the loss of the activity of a gene from a parent because of methylation of the bases, and thus a gene is changed from an active state to an inactive state (Lewin 1997).

Classical Inheritance Patterns, Kernel Color & Epistasis

In the classroom a discussion of monohybrid cross—purple and yellow kernels—provides an introduction to explain the influence of genetic background on phenotype (Table 3). For example, the phenotypic ratio of 3 purple: 1 yellow for the segregation of *R1* depends on the presence of *Pr1*, *C1* and *Y1* in addition to *R1*. Since the kernels are homozygous for the dominant alleles for the other two genes (*Pr1* and *C1*) in the anthocyanin pathway, the purple pigment is synthesized only when *R1* is present. Otherwise, the aleurone is colorless and the starchy endosperm's yellow pigment, which is present in all kernels including the purple ones, is observed. Since the yellow endosperm is present whether or not the anthocyanin is produced, the phenotypic ratio for this cross is expressed more precisely as 3 colored: 1 noncolored aleurone.

Crosses with more than one gene pair, dihybrid and trihybrid, provide inheritance patterns that are challenging to explain. Because these four genes are nonlinked or located on different chromosomes, the phenotypic ratios illustrating gene interactions for the dihybrid crosses (e.g. 9:7, 9:3:4, 13:3 and 12:3:1) are variations of the 9:3:3:1 ratio and the trihybrid cross (9:3:3:1) represents a variation of the 27:9:9:9:3:3:3:1:1:1 ratio. The five dihybrid crosses listed in Table 3 include examples of both recessive and dominant epistasis as well as more complex patterns of duplicate recessive epistasis and dominant epistasis coupled with recessive epistasis. The trihybrid cross is a case of simple recessive epistasis.

Table 3. Phenotypic ratios of eight crosses illustrating inheritance of kernel color in corn including the genetic background and type of interaction.

Category of Cross	*F_2 Ratio*	*Segregating Alleles*	*Genetic Background*[1]	*Type of Interaction*
Monohybrid	3 purple 1 yellow	*R1* and *r1*	*Pr1Pr1/Y1Y1/C1C1*	None
	3 purple 1 yellow	*C1* and *c1*	*Pr1Pr1/Y1Y1/R1R1*	None
Dihybrid	9 red 7 white	*C1* and *c1* *R1* and *r1*	*pr1pr1/y1y1*	Duplicate recessive epistasis
	9 purple 3 red 4 white	*Pr1* and *pr1* *R1* and *r1*	*y1y1/C1C1*	Recessive epistasis
	9 yellow 3 purple 4 white	*C1-I* and *C1* *Y1* and *y1*	*Pr1Pr1/R1R1*	Dominant epistasis
	12 purple 3 yellow 1 white	*Y1* and *y1* *R1* and *r1*	*Pr1Pr1/C1C1*	Recessive epistasis
	13 yellow 3 purple	*C1-I* and *C1* *R1* and *r1*	*Pr1Pr1/Y1Y1*	Dominant & recessive epistasis
Trihybrid	9 purple 3 red 3 yellow 1 white	*C1* and *c1* *Pr1* and *pr1* *Y1* and *y1*	*R1R1*	Recessive epistasis

[1]The genetic background includes other genes that influence the phenotypic ratio.

Epistasis in these examples can be attributed to the blocking of the biochemical pathway for a particular pigment because enzymes critical to the pathway are either missing or nonfunctional.

Each allele can be assigned a mechanism of action for its epistasis effect, either dominant or recessive. The two mutant alleles of *C1* (*C1-I* and *c1*) exhibit different types of epistasis yet produce the same phenotype (colorless aleurone). The *C1-I* allele, illustrating dominant epistasis, is dominant to *C1* because the *C1-I* mutant protein competes with the *C1* wildtype protein for the regulatory sites of structural genes associated with the pathway, thereby blocking synthesis of the corresponding enzymes required for pigment production synthesis (Paz-Ares et al. 1990). In contrast, the gene (*c1*) corresponding to recessive epistasis is not transcribed (Scheffler et al. 1994), and in heterozygotes (*C1c1*), one copy of the wildtype gene is sufficient for producing enough regulatory protein to produce the purple color.

Anthocyanin Structure, Synthesis & Roles of Pr, C1 & R1

Anthocyanins are phenolic compounds having a common structural unit of the flavone C15 skeleton (Figure 2). These water-soluble pigments accumulate in the vacuoles of aleurone cells late during development of the kernel after abscisic acid, a plant hormone, activates this biosynthetic pathway. Many genes and their associated proteins required for synthesis of these pigments are well understood (see Figure 3 for a simplified pathway). Besides the three genes described in this paper, more than 20 genes, which comprise the genetic background and include structural and regulatory ones, must be present in at least one dose of the dominant allele for synthesis of these pigments (Coe et al. 1988).

Pr1: Red Aleurone1

The *Pr1* allele encodes the enzyme, flavonoid 3′-hydroxylase, which is required for production of cyanidin-glycoside, the purple pigment (Donner et al. 1991). This enzyme functions early in the flavonoid pathway by adding a hydroxyl group to the ring structure. When this enzyme is absent (i.e. in the presence of the *pr1* allele), the red pigment, pelargonidin-glycoside, is produced. One copy of the *Pr1* allele is sufficient to produce purple kernels, thus heterozygotes (e.g. *Pr1Pr1pr1* or *pr1pr1Pr1*) are purple. At this time the mutation within the *pr1* allele is not known. However, it is a common marker for maize geneticists and often used in tester stocks, ones that are used to analyze mutations, including deletions and transpositions.

C1: Colored Aleurone1

Unlike *Pr1* that is active in tissues of the plant other than the seed, *C1* is restricted to the kernel. *C1*, a member of the *C1/P1* family of genes, codes for a nuclear transcriptional activator that, along with the gene product of R1, regulates structural genes in the anthocyanin pathway (Styles & Ceska 1977). Of the three control points within the anthocyanin pathway, one occurs in a gene required at the beginning of the pathway and two are for genes whose enzymes occur near the end of the pathway, thus insuring no anthocyanins are produced. To initiate transcription of the target genes, the *C1* protein together with *R1* protein bind to the promoter region of target genes allowing transcription to occur.

Both the promoter and coding regions of the *C1* locus are well characterized (Figure 4). It encodes a protein, 273 amino acids in length, having regions that are homologous to the DNA-binding domains found in many transcriptional regulatory proteins (Paz-Ares et al. 1990).

The allele, *C1-I*, has two mutations—one in the promoter region that increases the rate of transcription and a second in the coding sequence that produces a site for premature termination of translation that produces the shorter polypeptide. The faulty protein is 252 amino acids in length and is present in the cells at a higher concentration than that of the wildtype protein; thus the allele is dominant. The mutant protein is believed to bind to the DNA but lacks the region to bind to the RNA polymerase. With the *c1* allele, no protein is produced because of a mutation within the promoter region that prevents transcription.

R1: Colored1

Like *C1*, *R1* belongs to a small multigene family of genes (*R1/B1*) producing similar proteins that are tissue-specific activators of transcription. The locus for *R1* is compound, consisting of two similar functional sequences positioned in an inverted head-to-head orientation with a common promoter (Figure 5) (Walker et al. 1995). The loss of seed color associated with the recessive allele has been attributed to a deletion in this shared promoter region, thereby blocking transcription from both coding regions.

Carotenes, Pathway of Synthesis & Role of Y1

Carotenes are the most diverse and widespread group of pigments in plants (Bartley & Scolnik 1994). B-carotenes are the most abundant carotene in the kernel (Figure 2). These lipid-soluble pigments are synthesized early in the development of the embryo and endosperm, giving kernels their yellow color

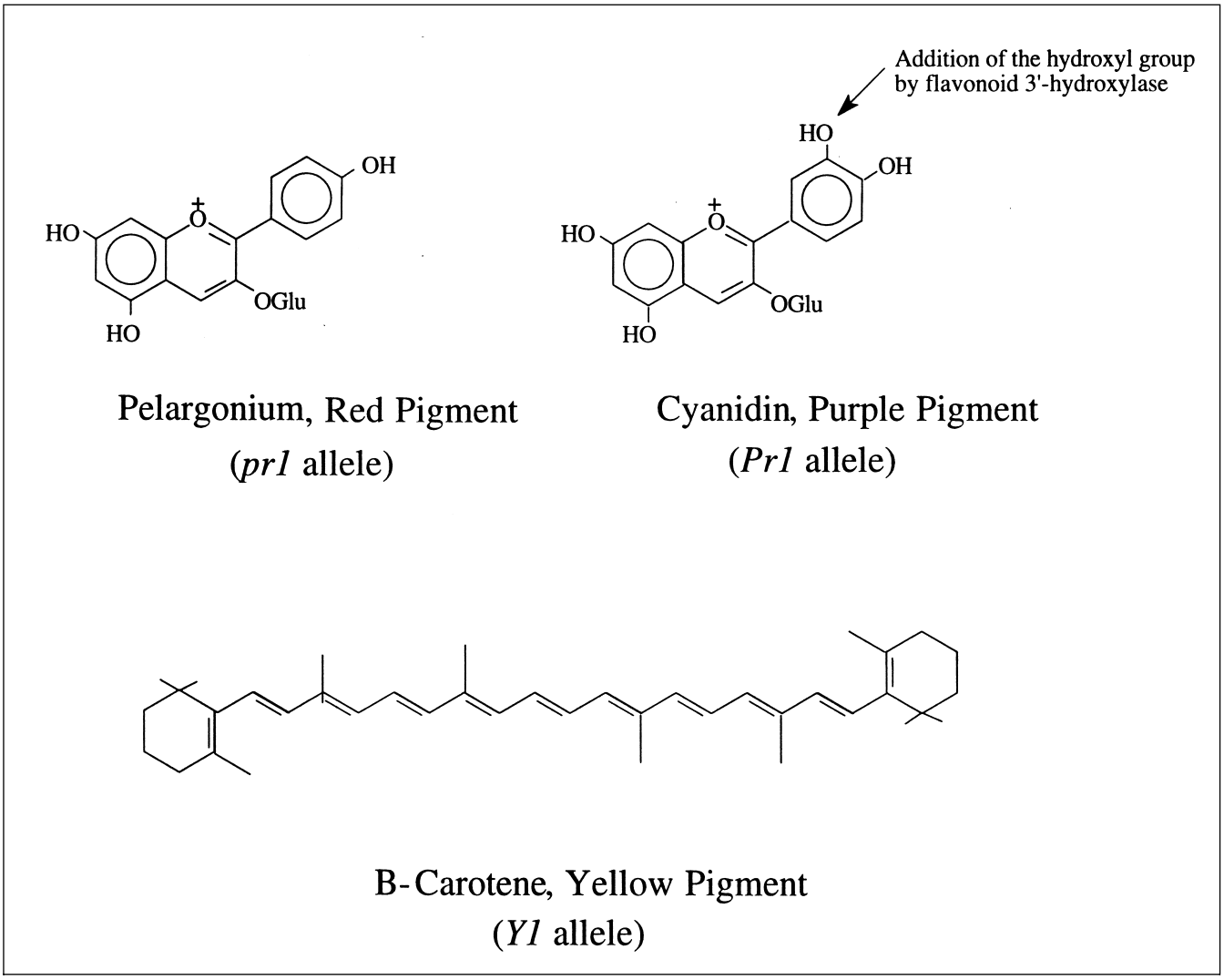

Figure 2. Structure of pigments. Pelargonium and cyanidin are the red and purple glycosylated anthocyanins found in the aleurone layer of the endosperm. B-carotene, a major carotenoid, is synthesized in the starchy endosperm.

and functioning, in part, as the precursor for the synthesis of abscisic acid.

The carotenoid biosynthetic pathway begins in the cytoplasm with the formation of 6-carbon precursors from three molecules of acetyl-Coenzyme A, intermediates in the respiratory breakdown of carbohydrate and fatty acid metabolism. These precursors are transported into chromoplasts, where, through successive condensations, carotenes and xanthophylls are synthesized (see Figure 6 for a simplified pathway).

Y:1 Yellow1

Kernels with white endosperms (*y1y1y1*) lack phytoene synthase, an enzyme required early in the biosynthetic pathway for the synthesis of phytoene. The precursors that accumulate in these kernels are colorless, so the endosperms appear white. Surprisingly, the embryo is still yellow, having the same carotenoid content as in kernels having a dominant *Y1*, and the photosynthetic regions of the plant also produce carotenes and xanthophylls, accessory pigments that prevent the photooxidation of chlorophyll. This difference in pigment production within the kernel suggests that *y1* is developmentally regulated or that tissue-specific isozymes of *Y1* are present (Buckner et al. 1996). The mutation of *y1* in the promoter region blocks transcription; hence, no enzyme is present in the endosperm tissue.

Suggested Projects: Lab Exercises & Questions for Critical Thinking

I have listed several laboratory projects that allow students to explore the basis of genetic interactions in corn kernel color. These projects are appropriate to students at different levels, some for high school and introductory college biology (A, B, C, D) and some for introductory college genetics (E). Most are

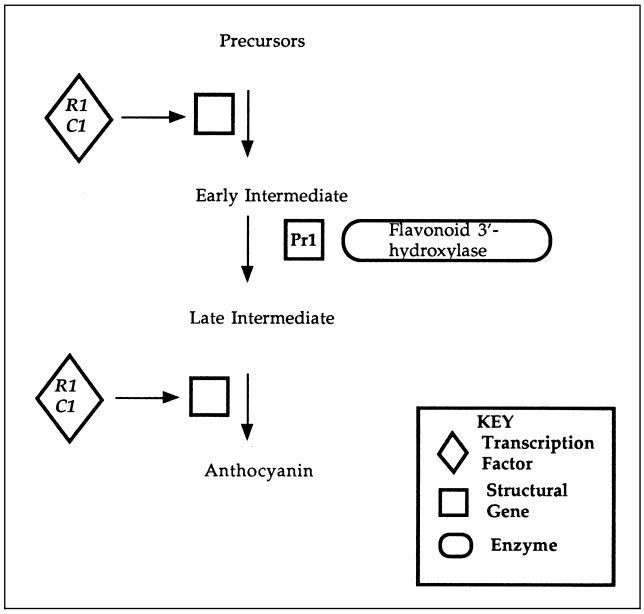

Figure 3. Simplified pathway for synthesis of anthocyanin.

hands-on projects (A, B, C and D); one is a "thinking" project in which students work in small groups to propose solutions to the questions (E).

A. Masking of the starchy endosperm by the aleurone.

This is a simple experiment to illustrate epistasis attributed to the colored aleurone masking the starchy endosperm. Colorful ears of corn, known as Indian corn, are available at minimal cost from grocery stores and other stores during the fall. These kernels can be dissected to observe the difference in color of the aleurone and the starchy endosperm. The following procedure can be used to dissect these kernels:

1. Soak the kernels in water for about 5 minutes.
2. Using a scalpel, remove the colorless seed coat, leaving the endosperm and embryo. Because the aleurone layer is only one layer deep and rehydrates quickly, it is easily removed simply by scraping off the purple layer. The lightly

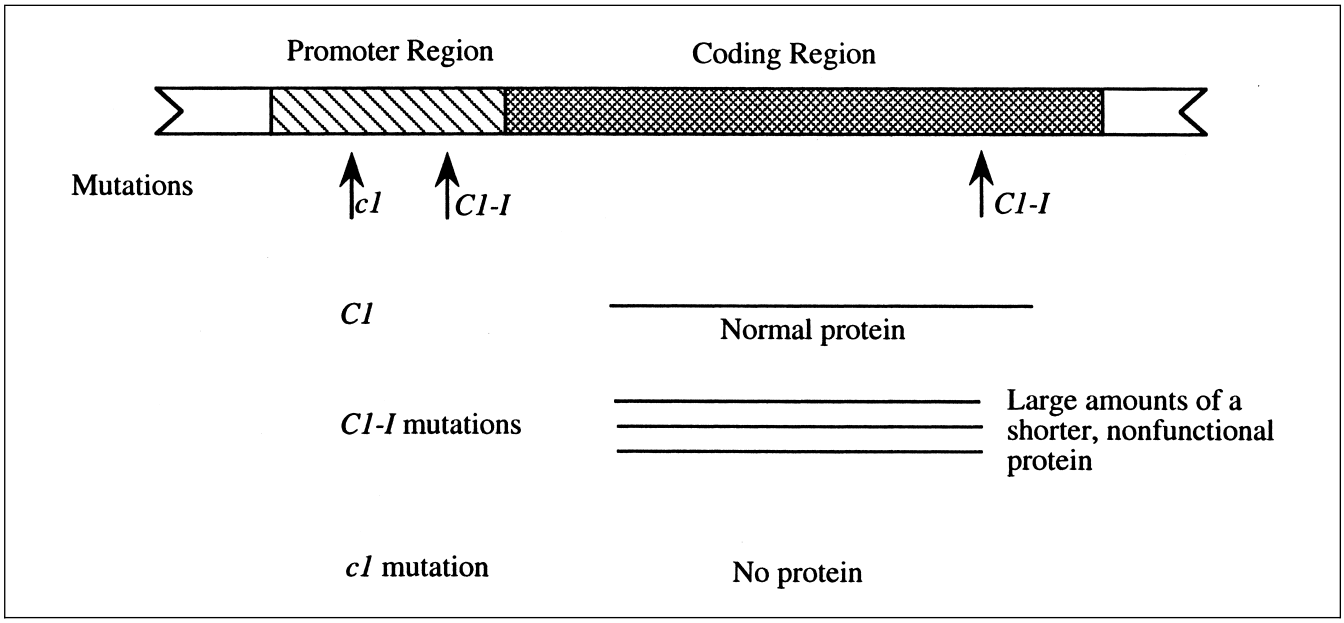

Figure 4. The three alleles of the *C1* gene locus: *C1, C1-I* and *c1.*

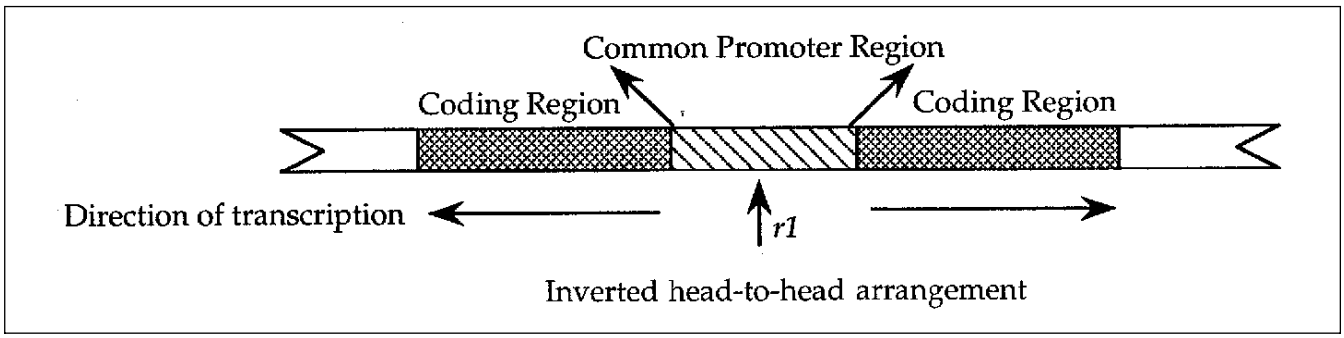

Figure 5. Compound *R1* locus.

colored layer of the starchy endosperm is revealed once the aleurone is removed. The starchy endosperm remains solid and hard during this short hydration period.

B. Predicting phenotypes and genotypes for specific crosses.

Students are provided with the phenotypes and genotypes of the parental generation and are asked to predict the phenotypes and genotypes of the F_1 and F_2 generations. Students must understand the concepts of dominant and recessive epistasis to accurately predict the outcomes. Examples of parental crosses might include:

1. Yellow (*Pr1Pr1/Y1Y1/r1r1/C1C1*) x Purple (*Pr1Pr1/Y1Y1/R1R1/C1C1*)
2. Yellow (*Pr1Pr1/Y1Y1/R1R1/C1-IC1-I*) x Purple (*Pr1Pr1/y1y1/R1R1/C1C1*)
3. Yellow (*Pr1Pr1/Y1Y1/r1r1/C1C1*) x Yellow (*Pr1Pr1/Y1Y1/R1R1/c1c1*)
4. Yellow (*Pr1Pr1/Y1Y1/R1R1/C1-IC1-I*) x Yellow (*Pr1Pr1/Y1Y1/r1r1/C1C1*)

C. Calculating and interpreting chi-square analyses for ears of corn exhibiting gene interactions.

Students perform chi-square analyses on ears illustrating gene interactions, then explain these variations based on the 9:3:3:1 ratios expected from dihybrid crosses demonstrating no gene interactions. Students are given ears with known genes that are segregating and represent gene interaction. The following procedures can be used in class:

1. Categorize kernels on a single cob by placing them in phenotypic classes, then formulate a hypothesis (9:3:4; 13:3; 9:7, etc.) to predict the inheritance pattern observed.
2. Perform the chi-square analysis to test the hypotheses.
3. Explain the gene interaction occurring in each cross using information such as molecular genetics, biochemistry and kernel anatomy.

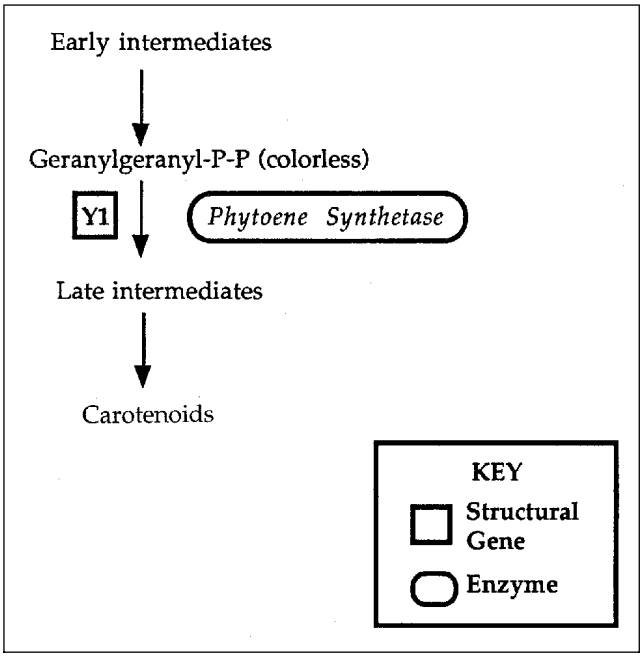

Figure 6. Simplified pathway for synthesis of carotenoids.

D. Researching the color genes using online gene banks.

Students are asked to write reports on the color genes from information obtained from the online gene banks for maize (http://www.agron.missouri.edu).

E. Constructing hypothetical evolutionary events for the C1 and R1 genes.

Students are asked to construct a model that explains the events which may have occurred in the evolution of the *C1* and *R1* genes in corn given the following information:

1. Purple kernels are common in many domestic varieties of corn but unknown in the wild ancestor of maize, teosinte. Yet teosinte carries the dominant alleles for the structural genes for the anthocyanin synthesis (Hanson et al. 1996). Speculate how these regulatory genes evolved to be tissue specific and include production of anthocyanin in the kernel.

Hanson et al. (1996) suggest that the evolution of purple kernels resulted from changes in the regulatory elements of *C1* so that these transcription factors are produced in the late developmental stage of kernels.

2. In addition to the duplicate loci of *R1* on chromosome 10, three others that are similar are located close by. Speculate how these very similar loci evolved.

Walker et al. (1995) suggest that the family of *R1*-like genes on chromosome 10 evolved from an ancestor gene by duplication from unequal crossing over from a synapsis that was not perfectly aligned.

3. Genes in the *R1/B1* family are located on chromosomes 2 and 10 and those of the *C1/P1* family are on chromosomes 6 and 9. These genes are located in large regions that were duplicated—regions that are larger than what would arise from unequal crossing over during meiosis. Speculate how these loci may have originated.

Gene duplications in maize that involve large chromosomal segments are common. The nature of these duplications may be attributed to a hybridization occurring between two species resulting in the ancestor of corn: each ancestor donating one *R1/B1* family and one *C1/P1* family of genes (Helentjaris et al. 1988).

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