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Control of Anthocyanin Synthesis by the *C* Locus in Maize[1]

Shu-Mei Chen[2] and E. H. Coe, Jr.[3,4]

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Alleles at the C *locus in maize include* C, *one of the complementary genes required for anthocyanin pigmentation in the aleurone tissue of the mature kernel;* C-I, *dominant inhibitor of pigmentation; and* c, *recessive colorless. The recessive colorless alleles can be differentiated into two distinct forms*: c-p (p *for positive*), *conditional colored, develops pigment in the light during germination;* c-n (n *for negative*), *colorless, fails to develop pigment at any time. Four-point linkage data support the differentiation of* c-p (*in the alleles derived from either W22 or K55 inbreds*) *from* c-n. *Light and germination conditions are both required for anthocyanin synthesis in* c-p *tissue, but light "induction" and germination "induction" are two separable events inasmuch as the light stimulus can be stored. The ratio between the two major pigments, cyanidin and pelargonidin, is lowered in* c-p *tissue relative to that in dominant colored* (C) *tissue for the W22 allele and background but not for the K55 allele and background. Segregation tests reveal that the difference in the cyanidin-to-pelargonidin ratio in the* C *and the* c-p *tissue of W22 background is associated with the* C *locus. Several properties of the* C *locus, including anomalous allelic functions, stage- and condition-dependent anthocyanin synthesis controlled by its multiple allelic*

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[2] Formerly Graduate Assistant, University of Missouri, Columbia, Missouri; Present address: 2237 Cedar Road, York, Pennsylvania 17404.

[3] Geneticist, Agricultural Research Service, U.S. Department of Agriculture, and Professor of Agronomy.

[4] Address reprint requests to E. H. Coe, Jr., Curtis Hall, University of Missouri, Columbia, Missouri 65201.

series, and tissue-specific function, suggest that C *may be a regulatory locus in the control of anthocyanin synthesis in aleurone tissue.*

KEY WORDS: corn, *Zea mays* L.; regulation; induction; light; aleurone; cyanidin; pelargonidin; flavonoids; germination.

INTRODUCTION

The *C* factor on chromosome 9 of maize is one of the complementary genes controlling anthocyanin pigmentation in the aleurone tissue of the kernel and the cotyledon (scutellum) of the embryo. Emerson *et al.* (1935) and Coe (1962) have reviewed the inheritance and interactions of the allelic series at this locus. The series includes the dominant inhibitor, *C-I*, and the recessive colorless, *c*, with effects divergent from the colored, *C*. Structural compoundness at the locus has been suggested, but extensive allelism and recombinational tests have failed to support this suggestion (Hutchison, 1922; Coe, 1962, 1964).

The phenomenon in which kernels of certain *c* testers (homozygous *c* strains carrying all required factors except *C*) develop pigment during germination under light has been known for some time. Kirby and Styles (1970) have suggested that light can substitute for the action of *C* under certain conditions and that light may "force" the whole biosynthetic system. Certain *c* tester strains, however, do not develop pigment at all (Hsu, 1970). Genetic analysis has been carried out in the present study to identify the factors controlling pigmentation during germination. Conditional colored alleles, *c-p* (*p* stands for positive), have been distinguished from *C* and *c-n* (negative): aleurone tissue with *c-p* develops pigment during germination under light, tissue with dominant *C* develops pigment during normal seed maturation without light, and tissue with recessive *c-n* develops no pigment at all. These characterizations raise some interesting problems, including how the gene is activated to direct the biosynthetic pathway in *c-p* tissue during germination under light and where the light requirement for anthocyanin synthesis fits into the gene action sequence and the biosynthetic pathway. These problems are difficult to approach directly with the present state of knowledge of this system. We have compared the genetic, biochemical, and physiological properties of the conditional colored type, *c-p*, to those of the dominant colored, *C*, in an attempt to understand the role and function of the *C* locus in anthocyanin biosynthesis.

MATERIALS AND METHODS

Stocks

The sources and pedigrees of the strains have been described previously

(Chen, 1973). J. L. Kermicle and C. C. Chang supplied *c* tester strains of W22 and KYS backgrounds, respectively; testers derived in K55 and N1 (a *C* genetic strain) backgrounds and mixed backgrounds were employed as well. Evidently, the *c* alleles of the W22 and K55 backgrounds were derived from the respective inbreds themselves.

Germination

Seeds were germinated in plastic petri dishes, on one layer of moistened and sterilized Whatman No. 3 filter paper, at room temperature under fluorescent lights throughout the experiments unless otherwise specified. Sterile water was added at intervals to keep the seeds moistened. Aleurone color classifications were made at the end of 3 days.

Genetic Analysis

C Locus Dependence

To determine whether positive and negative tester strains differ in *c* alleles or in modifiers distinct from the *C* locus, positive strains were crossed with negative strains carrying markers around the *C* locus on chromosome 9: *yg2* (yellow-green seedling), *sh* (shrunken endosperm), and *wx* (waxy endosperm). For linkage data, F_2 and test cross kernels were classified for *sh* and *wx*, germinated under light for 3 days, classified for *c-p* and *c-n*, and then transplanted to a sandbench for classification of *yg2*.

Heritability of Variations in Intensity

Because variations in intensity were often observed among *c-p* kernels in ear progenies segregating *c-p:c-n*, progeny tests were carried out to study whether the variation was caused by a gene dosage effect at the *C* locus, by other modifiers, or by nonheritable interactions between the physiology of individual kernels and environmental factors. Fifty kernels from each of two such ear progenies were germinated under light, classified for shrunken and for color intensity, and planted according to color intensity. The plants were self-fertilized, and the resulting kernels were classified for *c-p:c-n* and *Sh:sh*.

Other Anthocyanin Factors and Cryptic Factors

To study whether factors complementary to *C* in the aleurone tissue are also complementary to *c-p* during germination, doubly recessive strains for *c-p* with *a*, *a2*, *bz*, *bz2*, *c2*, and *r* were derived and tested for pigment formation during germination in the light. For possible effects of plant color factors, progenies segregating for *R-r:R-g*, *B:b*, and *Pl:pl* with *c-p:c-n* were studied. If cryptic factors affecting the *c-p* expression were present in some strains,

they could be revealed in crosses such as those used to generate the segregations for plant color factors; negative strains and negative derivatives from diverse sources were routinely tested for complementary relationships that would identify cryptic factors.

Effects of Light and Developmental Stage

Relationships between the onset and loss of responsiveness to light and the duration of light exposure during germination were explored in 25-seed lots of *c-p(W22)* germinated in the dark. After 4, 12, 20, 31, 37, or 44 hr, the seeds were illuminated with a 100-watt tungsten lamp at 30 cm for 1 hr, 4 hr, or continuously from the light step to classification at 72 hr.

To test whether *c-p* tissue can synthesize pigment during normal seed development if light is provided, husks of *c-p(W22)* ears were removed to expose aleurone tissue to light from around 15 days after fertilization until maturity. For study of the interaction of germination and pigmentation, ears segregating *c-p:c-n*, *Vp:vp* were dehusked to expose the aleurone tissue to light. Homozygous viviparous seeds (*vp*, premature germination) do not become dormant but germinate on the cob before reaching complete maturity. Dependence of pigment formation on the embryo was studied in *c-p* kernels by dissecting out the embryo axis or the entire scutellum plus the embryo axis before germination.

Anthocyanin Constituents in *C* and *c-p* Tissues

Materials used for chemical analysis included (1) *A C R* and *A c-p R* in W22 background, (2) *A C R* and *A c-p R* in K55 background, (3) F_1 and F_2 from crosses between *A C R* and *A c-p R* within W22 background, and (4) F_1, F_2, and backcrosses of *A c-p R* (W22) with *A c-p R* (K55).

Aleurone tissues of *c-p* were obtained by peeling 50 germinated kernels by hand; *C* aleurone tissues in these strains were extremely difficult to remove after germination and were collected instead by scraping dry seeds with a blade. The tissues so collected were repeatedly extracted with ice-cold 0.1% HCl in methanol in the refrigerator and then concentrated in vacuum in a rotary evaporator with gentle heating. These crude extracts of *A C R* and *A c-p R* were applied on TLC plates (Brinkman Avicel SF microcrystalline cellulose glass plates, 20 by 20 cm) for two-dimensional chromatography (in the first direction, BAW, consisting of *n*-butanol, CH_3COOH, and water, 4:1:5; in the second, Formic, consisting of HCOOH, concentrated HCl, and water, 5:2:3) to compare the qualitative differences in pigments in these two types of tissues. For quantitative measurement, crude extracts were hydrolyzed for 45 min in 4 N HCl; the resulting aglycones were extracted with

a few drops of *n*-amyl alcohol and then were separated by paper chromatography (using Formic as the solvent system). The separated aglycones were cut from the paper and eluted in 0.01% HCl in methanol for spectral measurements in a Beckman DB-G spectrophotometer. The relative concentrations of the two major aglycones from the same tissue were expressed as the ratio of two optical densities, OD cyanidin (cy, λ_{max} = 538 nm)/OD pelargonidin (pg, λ_{max}= 524 nm).

RESULTS

Among *c* tester stocks, those in W22 and K55 backgrounds developed uniform and intense pigmentation in the light during germination. Those in N1, KYS, and many mixed backgrounds did not give any color at all, while a few in mixed backgrounds developed very faint or small patches of pigment. The *c* tester stocks from W22 and K55 backgrounds, which developed intense and uniform pigments, were chosen for linkage tests and chemical analysis. Intermediately colored strains were ignored because of the extreme difficulty involved in classification.

Genetic Anyalsis

C Locus Dependence

All F_1 crosses of *c-p* with *c-n* developed pigment no matter what the crossing direction. The intensity of *c-p c-p c-n* endosperms was much higher than that of *c-n c-n c-p*, presumably because of dosage effects at this locus. Linkage data from test cross and F_2 progenies (Table I) indicate that the major factor controlling pigment formation during germination is at the *C* locus. Recessive *c* alleles can therefore be differentiated into two forms: *c-p*, which develops pigment in the light during germination, and *c-n*, which fails to develop pigment at any time.

Table I. Recombination Data for F_2 and Test Crosses from *+c-p++/yg2 c-n sh wx* Showing Association of Light-Inducible Pigment Formation with the *c* Locus

Allele	Progeny	Population	Recombination percentage		
			yg2 c	*c sh*	*sh wx*
c-p(W22)	F_2	2579	21.4±1.86	4.4±1.96	20.3±1.86
	Test cross	5584	20.6±0.54	3.5±0.25	17.8±0.51
c-p(K55)	F_2	1805	18.9±2.33	4.8±2.34	21.2±2.23
	Test cross	3161	17.1±0.67	5.4±0.40	20.3±0.72
Typical values (Emerson *et al.*, 1935)			21	3	21

Table II. Distributions of Genotypes of F_2 Kernels of Various Intensity Levels from *c-p Sh/c-n sh*

Progeny No. and intensity class	Genotype						
	c-p Sh / *c-p Sh*	*c-p Sh* / *c-p sh*[a]	*c-p Sh* / *c-n sh*	*c-p Sh* / *c-n Sh*[a]	*c-p sh*[a] / *c-n sh*	*c-n Sh*[a] / *c-n sh*	*c-n sh* / *c-n sh*
H264-10							
++++ *Sh*	1		1				
+++ *Sh*	3	2					
++ *Sh*	3	1	6				
+ *Sh*	1		6				
− *Sh*			1			1	
+ *sh*					2		1
− *sh*							5
H265-5							
+++ *Sh*	5		3				
++ *Sh*	1	1	6				
+ *Sh*			6	1			
− *Sh*			1			1	
± *sh*			2				
− *sh*							7

[a] Recombinant gametes 9/136 = 6.6%.

Heritability of Variations in Intensity

Variations in color intensity in progenies segregating *c-p*:*c-n* were not heritable except for the *c-p* dosage effect itself (Table II). Kernels having higher intensity tended to be homozygous *c-p c-p* more frequently than those having lower intensity. The color expressions of ears segregating *c-p c-n* whose parental kernels had been classified as intensely colored, intermediate, or slightly colored were indistinguishable, although they again showed some degree of variation within an ear. The variation is either physiological or environmental but not heritable except for the *c-p* dosage effect.

Other Anthocyanin Factors and Cryptic Factors

None of the doubly recessive types, *c-p a*, *c-p a2*, *c-p bz*, *c-p bz2*, *c-p c2*, and *c-p r*, developed purple or red pigment during germination in the light. Apparently the presence of the complementary dominants *A*, *A2*, *Bz*, *Bz2*, *C2*, and *R* is required for the *c-p* kernels to develop pigment. Pigmentation due to *c-p* was found to have some properties in common with *C*, such as mottled expression with *R r r*, inhibition by *C-I*, faint color with *bz*, and effects on scutellum color in the embryo.

Observations in many families that segregated for *R-r R-g*, *B b*, and *Pl pl*

led to the conclusion that plant color factors do not have obvious effects on the pigmentation of aleurone tissue during germination. The segregation of plant color factors had no influence on the pigmentation of *c-p* and was independent of the segregation of *c-p* and *c-n*.

Progeny from crosses between various negative strains were negative, displaying no complementation. All F_1s between negative strains and positive strains developed pigment during germination under light, and most F_2s segregated 3 colored to 1 colorless: a few exceptional cases segregating at peculiar ratios were noted. These tests neither identified any cryptic genes nor suggested that any are likely to be present.

Effects of Light and Developmental Stage

Table III shows the effects of several light schedules on anthocyanin formation in *c-p* tissue at different stages of germination. The data indicate the following: (1) Light is necessary for pigment development in *c-p* tissue, since kernels germinated in the dark remained colorless. (2) Low-energy, brief illumination is sufficient to induce pigment formation, but longer illumination can enhance the pigment intensity. (3) Illumination is most effective in the early stages of germination. Possibly at late stages aleurone tissue becomes physiologically inactive, or it may have a specific period of responsiveness to light.

On the ear, *c-p* kernels remained colorless through maturation even after husks were removed to expose the aleurone tissue to light. During germination, these light-exposed *c-p* kernels developed fairly intense pigment in the dark, indicating storage of the light stimulus.

In ears segregating for vivipary, many of the germinated *c-p vp* kernels were colored in light-exposed ears, whereas all of the nonviviparous remained colorless. Anthocyanin in kernels of colored strains is known to be

Table III. Effect of Illumination of *c-p* Tissue for Different Durations at Different Stages of Germination[a]

Light duration	Germination time preceding illumination					
	4 hr	12 hr	20 hr	31 hr	37 hr	44 hr
1 hr	7	7	6	2	2	1
4 hr	8	8	7	3	2	1
Step to end	8	8	7	5	2	1

[a] The amount of pigment induced was graded artificially from 0 to 8 at 72 hr. Checks: dark, 0; light, 8.

affected by *vp* (Robertson, 1955) and may range from intensely colored to pale or colorless. Similar variability and range of pigment intensity were observed on *c-p vp* kernels.

In partially or completely de-embryonated kernels, stronger pigment than in intact control kernels formed upon germination in the light. Germination "induction" is thus independent of the embryo itself.

Anthocyanin Constituents in *C* and *c-p* Tissues

Two-dimensional TLC plates of crude extracts from *A C R* and *A c-p R* of K55 background as well as *A c-p R* of W22 background showed qualitatively very similar patterns. They all had six well-defined colored spots: spots 1 and 2 were magenta and migrated close together; spots 3 and 4 were orange-red and were also very close to each other; spot 5 was pink, with high R_f values in both solvent systems; and spot 6 was orange, with the highest R_f values. These six spots, based on their color and their aglycone products, were three cyanidin (cy) derivatives (1, 2, 5) and three pelargonidin (pg) derivatives (3, 4, 6).

The relative concentration of the two aglycones varied among genotypes (Table IV). The ratio of cy/pg in *C* (W22 background) was 15.53, while that in *c-p*(*W22*) was 1.84. The F_1 between *C* and *c-p* within W22 background had a cy/pg ratio similar to that of the *C* parent. In the F_2, the *C* (colored) class had a cy/pg ratio resembling that for the *C* parent, while the homozygous *c-p* class had a ratio the same as that for the *c-p* parent. These data do not favor the hypothesis that an independent modifier other than *C* causes the observed low cy/pg ratio in *c-p*(*W22*) tissue. The cy/pg ratio may be due either to an allele-specific effect at the *C* locus or to the differences between the two types in their specific physiological conditions during anthocyanin synthesis. The

Table IV. Relative Concentrations of Anthocyanidins (cy, cyanidin; pg, pelargonidin) from *C* and *c-p* Tissues in Two Different Backgrounds

	W22 background			K55 background		
Genotype	cy OD[a]	pg OD[a]	cy/pg ratio	cy OD[a]	pg OD[a]	cy/pg ratio
A C R	2.64	0.17	15.53	1.60	0.39	4.10
A c-p R	1.69	0.92	1.84	0.64	0.14	4.57
F_1 (*C C* × *c-p c-p*)	1.54	0.10	15.40			
F_2 *C* - -	1.64	0.14	11.71			
F_2 *c-p c-p c-p*	0.59	0.32	1.84[b]			

[a] Average optical density of four replicates.
[b] Average of two replicates only.

Table V. Relative Concentrations of Anthocyanidins from F_1 *c-p(W22)* × *c-p(K55)*, F_2, and Backcrosses

	cy OD	pg OD	cy/pg ratio
F_1	1.64	0.70	2.35
F_2	0.71	0.38	1.87
$F_1 \times$ *c-p(W22)*	1.50	0.74	2.03
$F_1 \times$ *c-p(K55)*	1.12	0.44	2.54

cy/pg ratio for *C* tissue in K55 background, however, was 4.10 and that for *c-p* in the same background was 4.57. To study whether the effect of *c-p(W22)* on the cy/pg ratio is different from that of *c-p(K55)*, anthocyanidin constituents of F_1, F_2, and backcrosses between them were examined (Table V). The ratio for the *c-p(K55)* parent, 4.57, was not recovered in backcrosses, and the data were more consistent with multiple-factor (quantitative) inheritance than with single-gene inheritance. The data indicate that the two *c-p* alleles present in W22 and K55 have essentially the same effects. The materials used for *c-p(K55)* were from weak inbred ears, which may be a cause for the high ratio of 4.57 in Table IV in contrast to the ratios for the crosses. On the other hand, it could be argued that *c-p(K55)* is different from *c-p(W22)* in quantity and quality of pigments. The intensity of *c-p(K55)* pigments was much less than that of *c-p(W22)* visually. The data in Table IV do not indicate, however, that germination of *c-p(K55)* kernels has an effect of lowering the cy/pg ratio compared to *C* tissue.

DISCUSSION

The *c-p* allele is the factor determining the conditional pigmentation. The complementary aleurone color genes *A*, *A2*, *Bz*, *Bz2*, *C2*, and *R* are required for *c-p* kernels to develop pigments, but plant color factors (*R-r R-g*, *B b*, and *Pl pl*) have no effect. The present study has not identified any cryptic genes for the expression. Four-point linkage data from both coupling selfs and test crosses support the differentiation of the well-known recessive *c* allele into two distinct forms: *c-p*, the conditional colored allele, which develops pigment in the light during germination and *c-n*, the colorless, which fails to form pigment at any time. The origin of this conditional colored allele is not known through experimental observation; it was found to be present in the *c* testers of W22 and K55 background. Many of the common *c* testers, on the other hand, are *c-n*.

The anthocyanins in *c-p(W22)* are different from those in *c-p(K55)* strains in relative concentration. Kirby and Styles (1970) reported that the

relative concentration of cyanidin to pelargonidin was much lower in *c-p* germinated seeds than in normally pigmented *C* seeds. In the present study, the cy/pg ratio in W22 background was 15.53 in *C* and 1.84 in *c-p* tissue, while in K55 the ratio was 4.10 in *C* and 4.57 in *c-p* tissue. The segregation test in Table IV indicates that the difference in the cy/pg ratio between the *C* and the *c-p* tissue of W22 background is associated with the *C* locus and that it is not caused by modifier genes other than *C*. The reason for this difference is possibly physiological (under light and germination conditions) or allele-specific. It has been observed in other organisms that 3′-hydroxylation of flavonoid compounds is somewhat regulated by light. For example, in *Pisum sativum* seedlings both kaempferol and quercetin derivatives are formed in light-grown tissue, but only kaempferol derivatives in dark-grown tissue (Furuya and Galston, 1965; Bottomley *et al.*, 1965, 1966). In rye seedlings, both cyanidin and pelargonidin are found in light-grown seedlings but only cyanidin in dark-grown ones (Metche and Gay, 1964). In the case of maize aleurone tissue in the present study, the cy/pg ratio is much lower in *c-p* tissue (in which light is essential for anthocyanin formation) than in *C* tissue (in which synthesis proceeds without direct light exposure). Because the general physiology of aleurone tissue under the light and germination conditions can differ considerably from that of the *C* tissue during normal seed development, the observed difference in the cy/pg ratio in *c-p(W22)* and *C* (W22 background) may be caused by some basic change in the biosynthetic pathway before or after the 3′-hydroxylation step as a result of differences in general physiology. F_1, F_2, and backcrosses between two conditional colored alleles, *c-p(W22)* and *c-p(K55)*, show a multiple quantitative effect with respect to the cy/pg ratio rather than a clear-cut segregation of two distinct alleles.

Chromatographic studies did not reveal qualitative differences in the nature of the pigments of *C* in K55 background, *c-p(K55)*, and *c-p(W22)*. Each contained three cyanidin derivatives and three pelargonidin derivatives. Peonidin derivatives, which were found to be present in purple husks in fairly large amount (Chen, 1973), were not detected in aleurone tissue. The concentration of this pigment must be very low, if it is present in aleurone tissue at all.

Where does the light requirement for anthocyanin synthesis fit into the gene action sequence and the biosynthetic pathway? Many stages in the flavonoid biosynthetic pathway have been reported to be light dependent in varying degrees in different species. Light induction of pigment formation has been attributed in some cases to the buildup of precursors such as malonate, giving rise to ring A (Harraschain and Mohr, 1963; Hendricks and Borthwick, 1965), and in others to cinnamic acid, giving rise to ring B (Scherf and Zenk, 1967*a,b*). Lange *et al.* (1971) have shown that light can induce phenylalaine ammonia lyase (PAL), which catalyzes the formation of *trans-*

cinnamic acid from phenylalanine, and *trans*-cinnamic acid 4-hydroxylase, which catalyzes the formation of *p*-coumaric acid from *trans*-cinnamic acid. The effect of light on anthocyanin synthesis seems to be at diverse sites in various organisms. For *c-p* tissue in maize, the information so far obtained is too vague to assign a specific role of light in pigment biosynthesis. There is an obligatory light-induction phase for biosynthesis (e.g., before or at the beginning of germination); after that, pigments can be formed in the dark. According to the data given in Table III, the time of competence for induction appears to be rather specific. Kernels that have been exposed to light during development remain colorless at maturation but can form pigment in the dark during germination. Therefore, between the light induction and pigment formation there can be a delay of several weeks or longer. In order to induce pigment formation in *c-p* tissue, light can be received long before or during, but not after, germination. The tentative conclusion that can be drawn from these observations is that light induction and germination induction are two separable events and that the light-requiring process proceeds before the germination-induced process:

$$\text{State A} \xrightarrow{\text{light}} \text{state B} \overset{\text{(storage)}}{\cdots\cdots\cdots\rightarrow} \xrightarrow{\text{germination}} \text{anthocyanin}$$

The light-catalyzed process in *c-p* tissue is presumed to be at an early stage in anthocyanin biosynthesis. The nature of precursors, if any, accumulated (at B) in dehusked *c-p* kernels was not studied. Kirby and Styles' (1970) study indicated that neither flavonol nor leucoanthocyanin accumulated in dark-grown *c-p* tissue. The nature of precursor B in the above scheme could be that of an intermediate preceding the completion of the C_{15} skeleton or a signal carrier which is not an intermediate itself. The indication is that it is a stable compound or state that does not decay rapidly with the storage duration of mature seeds. In order to locate the light induction in a biosynthetic sense, it would be necessary to study the accumulated substances before germination in light-exposed and in dark-grown *c-p* aleurone tissue.

The physiological conditions of germination are apparently required for the *c-p* tissue to develop pigments: first, *c-p* kernels do not form pigment during normal seed development even if light is provided, and, second, *c-p* kernels can be induced to form pigments on the ears before harvest if light and vivipary are provided. The interaction between viviparous embryo and anthocyanin formation in *c-p* aleurone tissue is intricate and may be indirect; the growth of viviparous embryos has been shown to be independent of endosperm genotype (Robertson, 1955). The induction of anthocyanin synthesis in *c-p* kernels on the ear by the viviparous condition plus light is difficult to understand in view of the embryo independence of the pigmentation and in view of the anthocyanin-reducing effect of vivipary in *C* kernels. Further studies on the interaction of viviparous embryo and nonviviparous

endosperm, or *vice versa*, would help in understanding how the premature germination of *vp* affects anthocyanin pigmentation in *c-p* aleurone tissue.

We suggest that *C* may be a regulatory gene that controls the whole process of anthocyanin synthesis in the aleurone tissue, for the following reasons:

1. The anomalous allelic series at the *C* locus includes an inhibitor, *C-I* (antimorph); a dominant colored, *C*; a conditional colored, *c-p* (hypomorph); and a recessive colorless, *c-n* (amorph); but so far no evidence has been obtained indicating structural compoundness at the locus (Hutchison, 1922; Coe, 1962, 1964). An allelic series including both dominants and recessives divergent from the wild type would commonly require structural compoundness to explain these divergent functions. An alternative explanation of this anomaly may be that the product of the *C* gene is a regulatory unit and that mutation in this region could result in a hyperrepressed state (*C-I*), a "constitutive" (*C*), an inducible (*c-p*), and a null (*c-n*).
2. Anthocyanin synthesis in *C* and *c-p* tissues occurs at different developmental stages, requires different conditions for pigment formation, and results in different anthocyanin ratios, yet occurs in precisely the same cells at the different stages. This is in general agreement with the nature expected of a regulatory gene subject to developmental effects and environmental conditions.
3. No flavonoid compounds have been found to be accumulated in *c-p* tissue in darkness (Kirby and Styles, 1970) and presumably the same is true of *c-n*; however, leucoanthocyanidins are accumulated in *a2* tissue (Coe, 1955), flavonols in *a* (Kirby and Styles, 1970), and brown pigments in *bz* and *bz2*. This implies that *C* may not be a structural gene (at least not after the C_{15} level) in anthocyanin synthesis.
4. The tissue-specific functioning of the *C* locus (it is required only for anthocyanin synthesis in aleurone and scutellum and not in other plant tissues) suggests a regulatory rather than structural role.
5. The results of intertissue complementation studies as well as genetic evidence have placed the *C* gene at the beginning of the gene-action sequence (Reddy and Coe, 1962). This implies that the product of the *C* gene may regulate the very first step or may be required for the function of subsequent genes in the whole process.

Further studies on the correlation of anthocyanin synthesis with such enzyme activities as phenylalanine ammonia lyase, glucosyltransferase, and others, in response to light and during the process of germination in *c-p* tissue and in other allelic forms, may reveal some important information as to the function of the *C* locus in the control of anthocyanin synthesis. Studies on

the mutational spectrum at the *C* locus, especially how the antimorph *C-I* and hypomorph *c-p* arise, and a search for new alleles that respond to germination or light independently would also contribute to the understanding of the structure and the function of the locus. As a working hypothesis to be explored, we propose a tentative model for the role of the *C* locus. The model is based on a few assumptions: (1) The inhibitor allele and dosage effect imply that *C* probably releases some products extrachromosomally (e.g., the activator RNA in the 1969 proposal of Britten and Davidson). (2) There is absence of structural compoundness at the *C* locus. (3) *A*, *A2*, *Bz*, and *Bz2* are probably structural (or producer) genes in anthocyanin synthesis. The proposed role of *C* is a regulatory one via mixed positive or negative control of anthocyanin synthesis in aleurone tissue. A simple model of regulator–operator interaction such as that identified in several microbial systems does not adequately explain regulation in more complex eukaryotic systems. The four obvious alleles at the *C* locus could be acting in the following ways: (1) The dominant colored *C* produces an activator that forms a complex with the operator sites (receptor sites) and activates the structural genes, thus initiating anthocyanin synthesis. (2) The product of *C-I*, the inhibitor allele, has higher affinity for the operator (receptor) but has lost the effect of turning on the structural gene. (3) The product of *c-p* has lost the affinity for the operator and must be activated to regain the affinity by some metabolites or conditions developing in the cell under light during the germination stage. (4) The amorphic *c-n* allele produces either nothing or an activator that has lost the ability to turn on the structural genes and cannot be activated by germination and light.

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