COMPOUND VERSUS BIFUNCTIONAL NATURE OF THE *C* LOCUS IN MAIZE¹

E. H. COE, JR.²

*U.S. Department of Agriculture and Department of Field Crops,
University of Missouri, Columbia*

Received April 27, 1964

LOCI whose multiple alleles include both dominants and recessives divergent from the wild type are commonly suspected to be compound, because two different functions by alternative templates in one functional region are otherwise required. A compound region can provide for the occurrence of two functions; in fact, compoundness has been demonstrated for several such anomalous loci (reviews in LEWIS 1951; CARLSON 1959). The recognition of duplications in these and other compound regions has emphasized the importance of duplication in gene evolution (LEWIS 1951; STEPHENS 1951). A duplicate segment would be required for maintenance of an essential function when a new function arises (LEWIS 1951; JUDD 1961), but a locus might also be expected to assume a new function in the absence of duplication. Examples of bifunctional loci might best be sought in nonessential systems, where duplication would not be required for maintenance of the alternative functions.

The *C* locus in maize, which is concerned with anthocyanin synthesis in the kernel, was recognized as anomalous by HUTCHISON (1922): "If *C* and *I* are allelomorphs a rather interesting situation occurs, in that, of the three allelomorphs of this series, one (*C*) is necessary for color, another (*C'*) is a dominant 'inhibitor' of color, and the third (*c*) is a recessive 'absence' of color." HUTCHISON carried out a provisional test for allelism by examining 12,000 progeny from the cross of *C' c* × *c*, but found no colored exceptions. This test rested on the hope that *C'*, if not allelic to *c*, carried *C* in coupling with *I* but masked by the inhibitor. Since there was no assurance that this was the case, however, as HUTCHISON recognized, failure to obtain exceptions did not constitute an adequate test for allelism of *C'* with *C* and *c*.

Genetic studies by McCLINTOCK (1948, 1950, 1951a, b) have suggested that the *C* locus may be concerned with two or more reactions in the synthesis of pigment and that two or more components may be needed to explain dosage effects and the behavior of different mutable alleles.

This paper presents experiments designed to provide a more direct test for allelism and compoundness through repetition of the HUTCHISON test, in a procedure designed to produce the coupled or *cis* relationship of *C* and *I*. Extensive data

¹ Contribution from Crops Research Division, ARS, USDA, and the Missouri Agricultural Experiment Station. Journal Series No. 2716. Aided by a grant from the National Science Foundation.

² Geneticist, Agricultural Research Service, U.S. Department of Agriculture.

on recombination in flanking regions also have been obtained to test for synaptic homology among the alleles. Preceding papers (COE 1961, 1962) discuss expression and mutation of the alleles and some aspects of the structure and function of this locus.

MATERIALS AND METHODS

Markers in this region of the short arm of chromosome 9 are arranged as follows: Yg_2 -20- C -4- Sh -centromere. For the allelism-compoundness test, constitutions and orders are considered to be as follows, with the reservation that not every symbol need represent an actual element:

$$\begin{aligned} C^I &: IC, CI, Ic, \text{ or } cI \\ C &: \overline{ic} \text{ or } \overline{Ci} \\ c &: \underline{ic} \text{ or } \underline{ci} \end{aligned}$$

If the locus is compound and the two elements retain synaptic homology, the cross of $C^I c \times c$ should yield colored exceptions (C) by crossing over between the components of IC or CI constitutions but not of Ic or cI , which lack C and consequently cannot yield it. An adequate test requires a design that will seek cis arrangements of C and I , derived from Ic or cI . For this purpose, progeny from marked crosses of $C^I C \times c$ can be selected for exchange between flanking markers, hypothetically including some of constitution IC or CI as a result of exchange between I and c ; backcrosses of the selected individuals ($C^I c \times c$) should then yield colored exceptions through crossing over if the desired type has occurred.

The complete series of tests was carried out in one set of crosses, performed in two steps, as follows:

1. $\frac{+ C^I +}{Yg_2 C sh} \times Yg_2 c sh$
2. Select $\frac{+ C^I sh}{Yg_2 c sh}$ and $\frac{Yg_2 C^I +}{Yg_2 c sh}$, backcross to $Yg_2 c sh$.

Progeny from the final backcross were obtained in contamination-marked crosses (su, γ, wx) by mass hand-pollination and by mass crossing in detassel plots, where isolated culture fields consisting of rows of the hybrid plants, with tassels removed, were interspersed with rows of pollinator, $Yg_2 c sh$. Colored exceptions were progeny-tested against contamination and classification errors.

In a more specialized test, a long terminal deficiency with a breakpoint 1 to 2 map-units distal to C^I was used as the distal marker in the cross of $Yg_2 c sh \times Def C^I +/+ C sh$. Since the deficiency is not transmitted through the male (see McCLINTOCK 1943, for this technique), selection of C^I kernels from this cross selects for an exchange in a very short region adjacent to and at the C locus. The resulting $C^I c$ individuals were crossed by c to seek colored exceptions. This highly efficient test, as devised, is applicable to the $\underline{cI}$ constitution but not to Ic , since the exchange-selected kernels will hypothetically include the $cis \underline{CI}$ type derived from $\underline{cI}$ but not from Ic .

Maximum distances between the postulated c and I components were calculated as follows: For IC and CI , half the observed number (N) in the final backcross are test gametes, so if one case had been observed in N , $1/N \times 100 =$ map distance. For Ic , the number of plants of $+ C^I sh/Yg_2 c sh$ constitution tested (n), times the enrichment due to selection for crossing over in the C - Sh region (4 map units), gives $25n$ as the equivalent number of plants tested for the $cis IC$ crossover, and if one case had been observed then $1/25n = ap^2$, where a is half the average number of second level gametes observed per plant, and p is map-distance/100. For $\underline{cI}$, $1/5n = ap^2$, but the calculated number of plants tested by the deficiency procedure, at least $50n$, is additive with the $5n$ of the standard test for this constitution, yielding a sum equal to ap^2 .

To test for subtle differences in synaptic homology between the alleles, recombination frequencies for Yg_2 - C and C - Sh were obtained in heterozygotes for $C^I C$, $C^I c$, and $C c$. The marker

stocks used were closely similar in background but were not isogenic. The testcross parents were $\gamma g_2 C sh$ or $\gamma g_2 c sh$, as appropriate.

RESULTS

In the terminal backcrosses, six colored, one pale, and two variegated kernels were found. The six colored exceptions proved to be derived from contamination, the pale exception proved to be nonheritable, and the two variegated kernels failed to germinate; no crossovers were established. Data in Table 1 include the calculated map distance between the postulated components on the assumption that one case had been found.

Recombination data in Table 2 show marked differences between heterozygotes in both recombination and coincidence for regions adjacent to the *C* locus. The *C'* segment depresses recombination in both regions, most strongly in the longer Yg_2 -*C* region, and causes an unexpected increase in coincidence. The recombination frequencies obtained are somewhat different from the standard values used in the calculations described in METHODS. Although the present data constitute a much larger sample than has previously been obtained for these regions (EMERSON, BEADLE and FRASER 1935) and are a basis for revision of the standard map, the standard values have been used in these calculations because they give more conservative estimates of maximum distances between the postulated components and avoid a problematical choice among alternative values.

DISCUSSION

No recombination has been detected that would demonstrate compoundness at the *C* locus. There are, however, possible circumstances that could result in a negative outcome even if compoundness existed: The size of the test might be inadequate, or the postulated constitutions may be inaccurate.

With respect to the size of the test, the two systems in maize that have been demonstrated to involve duplication, *A^b* and *R^r*, both contain components separated by 0.05 to 0.1 map units (LAUGHNAN 1949, 1952; STADLER and NUFFER 1953; STADLER and EMMERLING 1954, 1956). At the *wx* locus, intracistron recombination reaches the same magnitude (NELSON 1962). In *Drosophila*, most of the pseudoallelic systems studied are quantitatively similar, although some much closer components have been demonstrated (GREEN 1959a; CARLSON 1959; WELSHONS, VON HALLE and SCANDLYN 1963; PETERSON and LAUGHNAN 1963). When high-resolution techniques (CHOVNICK, SCHALET, KERNAGHAN and TALSMA 1962) are applied to intracistron analysis in *Drosophila*, the lower limit for recombination between separable sites is found to extend below 10^{-6} . Consequently the present test can be considered to have attained a level at which compoundness, but not necessarily intracistron differences, might well have been detected, even for the least tractable constitutions. The efficiency of the second-level test for *Ic* and *cI* decreases exponentially as the number tested increases, greatly reducing the value of further samples.

The postulated constitutions assume all alleles to be compound with tandem

order and synaptic homology between parts. If only *C'* or only *C* were compound, the tests should still yield recombinants. Since homozygous *C' C'* individuals produce few mutant gametes (COE 1962), a homologous tandem repeat for *C'* is unlikely; oblique synapsis and exchange should produce frequent mutations. However, if synaptic homology were lost, or if the repeats were in reverse order, negative results would be expected in both homozygotes and heterozygotes, and these possibilities cannot be eliminated. Regardless of seriation and homology of a repeat, stepwise mutation to lower alleles can indicate compoundness (LAUGHNAN 1949), but there is no evidence for spontaneous stepwise mutation of *C'* (COE 1962), and even mutator-induced changes of *C'* or *C* form a graded series rather than a discontinuous one (McCLINTOCK 1948, 1951a, 1953). Since *c* is an amorph (McCLINTOCK 1950) it might be a deficiency, interfering with the test, but the recombination data for adjacent regions (Table 2) do not support this interpretation.

The highly sensitive recombination test for differences in homology shows that the *C'* segment, which depresses recombination in adjacent regions in each combination tested, is less nearly equivalent to the *C* and *c* segments than the *C* and *c* segments are to each other. Assuming the background to be isogenic in all other respects we would expect a very local aberration to influence the shorter adjacent segment more than the longer, contrary to observations. A larger aberration extending to the left of *C'* appears probable. The observed increase in coincidence for *C'* heterozygotes suggests that the aberration does not interfere with synaptic behavior but may cause wider separation of exchanging regions through the failure of exchanges in the aberrant segment. Any type of minute aberration might have these effects, but a difference in segment length and homology (insertion, duplication, or deficiency) is less likely than an alteration in the ability to exchange successfully.

The only experimental evidence indicating compoundness at this locus is that of McCLINTOCK (1948, 1950, 1951a), derived from the interrelations among mutable *c* alleles and from dosage effects in artificially produced duplications. These conclusions must be tempered by the two-element concept for mutable systems (McCLINTOCK 1961), which opens mutator-induced alleles to wide latitudes of interaction, and by uncertainties in extrapolating structure and function from visual phenotype alone. Structurally, the present data lead to the same conclusion as that stated by HUTCHISON (1922): "... one is forced to the unsatisfactory conclusion that *C* and *I* are either allelomorphs or very closely linked."

Without appropriate, detailed biochemical analysis, the functions of *C* and *C'* cannot be designated. If they are alleles (in the sense of alternative forms of one template), they would be pictured most simply as eliciting two different enzymes, specific to the same substrate but catalyzing divergent reactions, one (*C*) leading to pigment formation and the other (*C'*) away from it. In this sense *C'* would be both a neomorph and an antimorph relative to *C*. Though there is no precedent for the occurrence of such a change, fundamentally an interfunctional alteration, it is not difficult to conceive of the possibility that a single amino acid replacement might result in an enzyme that retains its original substrate specificity but

catalyzes a different transformation of the substrate. That this simple picture is inadequate is shown by a physiological complementation between c and C' (REDDY and COE 1962), in which live tissues of homozygous c and C' , dissected and placed together, interact to produce anthocyanin in the C' tissue. The parallel interaction does not occur *in vivo*, since $C' c c$ tissues do not show rims of pigment around areas in which C' has been lost through deficiency (REDDY and COE, unpublished observations). Obviously much more precise biochemical information would be necessary before concrete distinctions could be drawn among the numerous metabolic and enzymic alternatives.

As a corollary of the one gene-one function concept (BEADLE 1951), the function of a locus is generally envisioned simply as causing catalysis of a single type of metabolic reaction. Consequently a set of alleles having divergent effects on a metabolic pathway must be considered anomalous. Although differentiated duplications can be invoked to provide for divergent functions, the dilemma is not solved, because a duplicated segment must be pictured as mutating to the same anomalous function as that required for an allele. At the C locus, for example, it is no more reasonable to picture C duplicating to CC and then mutating to CI than to picture C mutating to I (i.e., C') without duplication. When an interfunctional mutation occurs, the value of duplication to the organism is the retention of essential functions; the value of a new function may balance or outweigh the need for the previous function if the previous function was non-essential.

Apart from a pool of duplicated loci from which new enzymatic functions can be derived, a pool of innately nonessential loci is also available. At one of these loci, a new alteration coding for a functionally new protein, such as an enzyme specific to a new substrate or catalytic for a new metabolic transformation, could be maintained rather easily. Whether the duplication pool or the nonessential pool is the more significant source of raw material is an important question, though ultimately all increases in number of genic functions must be dependent on increases in number of genes. Genic evolution should thus be considered to include not only changes in kind that require prior changes in number (duplication followed by neomorphic mutation) but also changes in kind that are independent of changes in number (neomorphic mutation followed or not followed by duplication). Phenotypically, the spectrum of possible mutant effects for different site changes suggested by WELSHONS and VON HALLE (1962), ranging from wild-type isoalleles to recessive lethals and dominant visibles, might be limited for particular nonessential loci to isoalleles and recessive visibles or to isoalleles alone (see GREEN 1959b), and could include bifunctional series with visible or subliminal effects. If different loci range in effect from essential to dispensable, then quantitative diversity must be largely a reflection of variants at the more dispensable loci, including bifunctional systems.

Because anthocyanin appears to be only remotely related to fitness, interfunctional mutations with conspicuous visible effects might occur and be retained in loci concerned with synthesis of this pigment. In the absence of mutational evidence for interfunctional change at the C locus (COE 1962) and of biochemical

demonstration of this change, this locus can be considered a possible but not a proven example of a bifunctional locus.

SUMMARY

Extensive tests designed to reveal compoundness at the *C* locus in maize have given none of the expected recombinants. Recombination data for adjacent regions show that there is some genetic difference between allele-associated segments in the region, but since there is a concurrent reduction in exchange and increase in coincidence, no difference in segment length or synaptic homology in the region is indicated. Considered as an allelic series in the sense of alternative templates in one functional region, the *C* locus is a possible example of a bifunctional locus involving nonessential metabolic processes and therefore not requiring duplication for the maintenance of the series.

LITERATURE CITED

- BEADLE, G. W., 1951 Chemical genetics. pp. 221-239. *Genetics in the 20th Century*. Edited by L. C. Dunn. Macmillan, New York.
- CARLSON, E. A., 1959 Comparative genetics of complex loci. *Quart. Rev. Biol.* **34**: 33-67.
- CHOVNICK, A., A. SCHALET, R. P. KERNAGHAN, and J. TALSMA, 1962 The resolving power of genetic fine structure analysis in higher organisms as exemplified by *Drosophila*. *Am. Naturalist* **96**: 281-296.
- COE, E. H., JR., 1961 Allelism at the *C* locus in maize. (Abstr.) *Genetics* **46**: 859. — 1962 Spontaneous mutation of the aleurone color inhibitor in maize. *Genetics* **47**: 779-783.
- EMERSON, R. A., G. W. BEADLE, and A. C. FRASER, 1935 A summary of linkage studies in maize. *Cornell Univ. Agr. Exp. Sta. Mem.* **180**: 1-83.
- GREEN, M. M., 1959a Spatial and functional properties of pseudoalleles at the white locus in *Drosophila melanogaster*. *Heredity* **13**: 302-315. — 1959b The discrimination of wild-type isoalleles at the white locus of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.* **45**: 549-553.
- HUTCHISON, C. B., 1922 The linkage of certain aleurone and endosperm factors in maize, and their relation to other linkage groups. *Cornell Univ. Agr. Exp. Sta. Mem.* **60**: 1421-1473.
- JUDD, B. H., 1961 Formation of duplication-deficiency products by asymmetrical exchange within a complex locus of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.* **47**: 545-550.
- LAUGHNAN, J. R., 1949 The action of allelic forms of the gene *A* in maize. II The relation of crossing over to mutation of *A^b*. *Proc. Natl. Acad. Sci. U.S.* **35**: 167-178. — 1952 The action of allelic forms of the gene *A* in maize. IV. On the compound nature of *A^b* and the occurrence and action of its *A^d* derivatives. *Genetics* **37**: 375-395.
- LEWIS, E. B., 1951 Pseudoallelism and gene evolution. *Cold Spring Harbor Symp. Quant. Biol.* **16**: 159-174.
- MCCCLINTOCK, B., 1943 Maize genetics. *Carnegie Inst. Wash. Yearbook* **42**: 148-152. — 1948 Mutable loci in maize. *Carnegie Inst. Wash. Yearbook* **47**: 155-169. — 1950 The origin and behavior of mutable loci in maize. *Proc. Natl. Acad. Sci. U.S.* **36**: 344-355. — 1951a Mutable loci in maize. *Carnegie Inst. Wash. Yearbook* **50**: 174-181. — 1951b Chromosome organization and genic expression. *Cold Spring Harbor Symp. Quant. Biol.* **16**: 13-47. — 1953 Mutation in maize. *Carnegie Inst. Wash. Yearbook* **52**: 227-237. — 1961 Some parallels between gene control systems in maize and in bacteria. *Am. Naturalist* **95**: 265-277.

- NELSON, O. E., 1962 The waxy locus in maize. I. Intralocus recombination frequency estimates by pollen and by conventional analyses. *Genetics* **47**: 737-742.
- PETERSON, H. M., and J. R. LAUGHNAN, 1963 Intrachromosomal exchange at the Bar locus in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.* **50**: 126-133.
- REDDY, G. M., and E. H. COE, JR., 1962 Inter-tissue complementation: A simple technique for direct analysis of gene-action sequence. *Science* **138**: 149-150.
- STADLER, L. J., and M. H. EMMERLING, 1954 Problems of gene structure: III. Relationship of unequal crossing-over to the interdependence of R^r elements (S) and (P). (Abstr.) *Science* **119**: 585. ——— 1956 Relation of unequal crossing over to the interdependence of R^r elements (P) and (S). *Genetics* **41**: 124-137.
- STADLER, L. J., and M. G. NUFFER, 1953 Problems of gene structure II. Separation of R^r elements (S) and (P) by unequal crossing over. (Abstr.) *Science* **117**: 471-472.
- STEPHENS, S. G., 1951 Possible significance of duplication in evolution. *Advan. Genet.* **4**: 247-265.
- WELSHONS, W. J., and E. S. VON HALLE, 1962 Pseudoallelism at the Notch locus in *Drosophila*. *Genetics* **47**: 743-759.
- WELSHONS, W. J., E. S. VON HALLE, and B. J. SCANDLYN, 1963 Notch pseudoalleles in *Drosophila melanogaster*. (Abstr.) *Proc. 11th Intern. Cong. Genet.* **1**: 1.