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*Univ. of Wisconsin
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"Organization of the R Chromosome Region in Maize"

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1. RESEARCH ACTIVITIES

Anthocyanin pigmentation in maize is strain and tissue specific. The primary source of variation is represented in maize races indigenous to widely separated geographic regions of North and South America. Secondary sources include variants which have appeared spontaneously in culture, arose following mutagenic treatment, or were incited through paramutation or by means of controlling elements. Much of the observed variation is attributable to a narrowly restricted segment of chromosome 10, designated the R region. The studies on R organization seek to analyze this variation in terms of the number, kind and arrangement of the components involved.

Early studies related R's pigmenting effects to discrete units of function, defined by independent mutation and recombinational separability. L.J. Stadler termed these units "genic elements". An element may occur singly in a chromosome or in combination with other elements as an allelic complex. At least seven different elements have been identified. The number of combinations and arrangements of these elements found among maize races is surprisingly large.

Allelic complexes originate by chromosome segment duplication. In past years we gave attention to defining genetically the end points, the position of R, and the orientation of such duplication segments. Alleles differ not only in genic element composition but in the chromosome segments which subtend the elements. Even alleles of similar phenotype often differ in these respects.

Our previous studies were directed largely at levels of organization higher than the genic element. Only recently, using alleles characterized as carrying a single element, has it been feasible to focus sharply on the component makeup of individual elements. The first clear break came

through analyzing instances in which expression of the seed color element (Sc) had been inhibited following insertion of the transposable unit Dissociation (Ds) at the locus. The point of insertion proved not to be at the site that distinguishes (Sc)'s action from that of other elements but slightly removed. Thus in heterozygotes involving (Sc) Ds in combination with (P), an element governing plant pigmentation, crossing over separated (Sc) from Ds thereby restoring (Sc)'s activity. (P) evidently furnished components that substituted for ones affected by Ds. By implication, (Sc) and (P) share components that are tissue nonspecific. A brief manuscript describing the critical experiments has been accepted for publication in Science. Because this finding has a major bearing on the future direction of these investigations, the manuscript is appended to this report.

Details of our other studies are recounted in part in published reports, manuscripts, and in an accompanying annual report. It will suffice here to enumerate a number of specific findings, emphasizing those that are not discussed elsewhere.

1. Two colored-seed, red-plant (R-r) accessions from Venezuela mutate and recombine as though composed of a single genic element. Homozygotes as well as heterozygous combinations involving the colored-seed, green-plant element (S), yielded colorless-seed variants only infrequently. Without exception, such cases also had lost the potential for plant coloration. Their occurrence was not associated with recombination of flanking markers. The phenotypic effects of this element resemble those of the (Sa) component of R-r:cherry.

2. Analysis of a spontaneous variant of R-r:cherry (colored seed, red plant, colored pericarp) provided new insight into the structure of this complex allele. The variant retains only seed coloration and that in pale

form. The variant reverts sporadically to full R-r:cherry expression (see annual progress report). More important for understanding R-r:cherry structure, heterozygotes of the variant with a single element plant color allele (P) yield a recombinant product having the seed and plant characteristics of parental R-r:cherry but lacking pericarp coloration. It is this product which resembles the naturally occurring R-r's of Venezuelan origin. By inference, a separate element governs pericarp coloration although it has yet to be isolated directly as R-r:cherry.

3. A collection of R-r:cherry derivatives was tested for effect on striate-2. Expression of this chlorophyll striping gene is modified in a dosage dependent manner by a gene very near R. The modifier locus is included in R duplication alleles such as R-r:standard and R-r:Ecuador 1172. Although R-r:cherry undergoes unequal crossing over, a characteristic of direct duplications, it tests as having but one dose of the modifier. The same proved true of its various derivatives. The sr2 modifier locus evidently is not represented in the segment duplicated in R-r:cherry.

4. Six genic elements, including two that occurred as separate alleles and four that were extracted from allelic complexes, are being analyzed for tissue-specific site differences by recombination. Seven pairwise combinations have given no indication of site separability. An eighth case, namely (Sc)/(P) heterozygotes, yielded two gametes having neither (Sc) nor (P) function but which were recombinant for markers flanking R. More extensive tests of the (Sc)/(P) combination are underway.

5. Only a minority of the colored-seed, green plant (R-g) variants of R-r:standard suppress the expression of sr2 in the manner characteristic of the R-r:standard duplication chromosome. They may be designated as

(p)(S) in comparison with (P)(S) for the parental allele and (S) for those R-g derivatives having lost the duplication. The nature of the mutant (p) element in six (p)(S) derivatives was studied in (S) heterozygotes. All yielded a recombinant, colorless-seed and green-plant (p) class, confirming presence of the duplication. Four of the six, however, also yielded a recombinant, colorless-seed but red-plant (P) class. Retention of the distinctive plant pigmentation effects of (P) suggests mutation at a site other than that conferring tissue specificity. The relationship of flanking markers places the lesions distal to (P), that is, between (P) and (S). This finding parallels that involving instances of Ds inhibition of (Sc) in that a lesion mapping distal to the tissue-specific site can be exchanged for components from a different genic element in the homologous chromosome. A further parallel with variants incited by controlling elements was suggested by the outcome of (p)(S) to (P)(S) reversion studies. Three of the four (p)(S) cases that yielded (P) crossovers in (S) heterozygotes, as well as the two that did not, reverted to (P)(S). It should be noted that the six (p)(S) variants arose spontaneously in our R-r:standard strain, a stock not known to be associated with any controlling element. The six variants are being tested for response to known controlling element regulators.

6. Two instances of inhibited (P) expression were isolated from (Sc)Ds/(P) heterozygotes, where (Sc)Ds represents r-mutable 9. Both occurrences were associated with recombination of flanking markers. The marker combination was that expected if Ds had been transferred from (Sc) to (P) by crossing over, forming (P)Ds. When combined with Modulator, a regulator of Ds activity, plants of the inhibited (P) strains produced

sectors exhibiting strong (P) expression, thereby confirming the constitution (P)Ds. These two strains should prove valuable in placing the site of Ds insertion in a collection of new (Sc)Ds stocks.

7. Stocks representing 39 independent insertions of Ds at (Sc) were recently established. This collection augments a set of four obtained previously. The new stocks constitute the basic materials for studies designed to discover the extent of the cis functioning region that comprises an R genic element.

8. Crossovers closely distal to (S) were selected in an attempt to separate components of paramutational repression from the basic pigmenting function. A stock of (S) carrying an R duplication displaced two map units distal and marked by Leaf color (Lc) was paramutated by two generations of heterozygosity with R-stippled. From the resulting (S)Lc homozygotes, four unequal crossovers between segments closely distal to (S) and (Lc) were isolated. In none were the repressive effects of paramutation relieved. Previously, we had learned that similar crossovers occurring in (S)Lc homozygotes prior to exposure to R-stippled did not significantly alter (S) sensitivity to paramutation. The frequency of unequal crossing over observed, however, was appreciably higher than following paramutation. This finding correlates with Bray and Brink's [*Genetics* 54:137-149 (1966)] observation that crossover separation of (P) from (S) in R-r:standard is significantly reduced following paramutation. In neither instance is the basis for reduced recombination known.

9. Components of paramutagenicity map to both sides of (Nc) in the R-stippled complex. In stippled, the components proximal and distal to (Nc) are present of course in cis linkage phase. To test for interaction

in trans, one derivative representing the proximal region through (Nc) and another carrying the distal portion, including (Nc), were combined with the sensitive test element (S) in trisomic-10 plants. Paramutation was increased relative to the effect of either of the two derivatives tested separately. However, complementation was only partial since the extent of paramutation in the trans combination was less than in R-stippled heterozygotes.

II. PROJECT PERSPECTIVE

In recent years, increasing attention has been given to questions concerning genetic organization in higher organisms. Various approaches have been fruitful: Cytogenetic studies indicate that the eukaryotic gene, although monocistronic, is much larger than its prokaryotic counterpart. Analysis of genomic DNA shows that many sequences are highly reiterated. There is a striking periodicity in the association of histone with DNA, studies on chromatin structure demonstrate. Comparison of coding and translated sequences show that the structural information for a peptide need not be continuous but often is interrupted by non-coding segments. Other surprises undoubtedly are in store.

The foregoing approaches offer promise for a comprehensive description of genetic organization. Those concerned with the organization of single genes are particularly efficient when applied to loci whose genetic fine structure is already known. In order to relate base sequence to a specific function, for example, genetic variants of the region should be mapped. Similarly, structural and regulatory components should be identified and ordered. Our investigations of the R locus provides such information.

The general title proposed earlier, "Organization of the R Chromosome Region in Maize", still encompasses the major activities of this program of

research. The emphasis, of course, has changed. Earlier we were concerned largely with questions of R locus complexity: identifying separate genic elements, working out their linear order, and relating genic elements to the underlying chromosome segment arrangement. Recently, these questions have given way to ones concerned with the structure of individual genic elements. How many different mutational and recombination sites comprise a genic element? To what extent can the components be substituted between different genic elements? How do the tissue-specific components of genic elements differ? In what ways do the variants induced by controlling elements differ from those induced by other means? Such questions are similar in kind to ones posed in conjunction with other detailed studies of single genes in maize and other eukaryotes.

Two main categories of eukaryotic genes have contributed to our understanding of gene structure. In one category are those whose gene product is well characterized such that allelic variants can be characterized in terms of gene product modification. Representative cases are the rosy gene in Drosophila and the alcohol dehydrogenase-1 gene in maize. Although the structural gene in such systems is well defined, recognized variation in control functions is uncommon (e.g. Chovnick, Gelbart and McCarron, 1977. *Cell* 11:1-10). Comprising the second category are those loci which come to attention because of variation in what in all probability are control functions. Alleles in such systems confer biological uniqueness, such as mating reaction, host range, or tissue specificity. Extensive variation in the control aspect of such phenotypes often overshadows any variation in the structural gene. The gene product often is not identified.

The R locus studies first developed as a system belonging to the second category. It was only when attention was given to variants of reduced phenotypic effect, including the null level, that tissue nonspecific functions were discovered. In the immediate future a major effort is to be directed at

identifying and characterizing the tissue nonspecific components in relation to tissue-specific ones. The R system may serve to bridge a gap in our understanding that has developed from the study of two disparate categories of eukaryotic genes.

III. TRAINING PROGRAM (1976-1979)

<u>Name</u>	<u>Dates</u>	<u>Status</u>
Emenhiser, G.	10/75-6/76	M.S. Candidate
Demopulos, J.	9/77-present	Ph.D. Candidate
Lin, B.Y.	2/76-3/76	Postdoctorate

IV. PUBLICATIONS

Dooner, H.K. and J.L. Kermicle, 1976. Displaced and tandem duplications in the long arm of chromosome 10 in maize. Genetics 82:309-322.

Kermicle, J.L., 1978. Imprinting of gene action in maize endosperm. In Maize Breeding and Genetics, D.B. Walden, ed. John Wiley and Sons, New York.

Dooner, H.K. Identification of an R locus region that controls the tissue specificity of anthocyanin formation in maize. Genetics, in press.

Kermicle, J.L. Probing the component structure of a maize gene with transposable controlling elements. Science, in press.

V. OTHER FINANCIAL SUPPORT

During the period of 1976 through 1979, financial support for genetic investigations on maize was available also from the National Science Foundation and the National Institutes of Health. NIH Program Project Grant GM 15422, administered by the Laboratory of Genetics, included an allocation of about \$18,000 annually for research directed by J.L. Kermicle on gene regulation in maize. This grant terminated July 31, 1977. NSF Grant PCM-7716840 provides \$65,000 for August 15, 1977, through April 30, 1980, for study of "Organization of Histone Genes in Maize". Dr. John T. Stout is co-investigator. An application for renewal of this award beyond April 30, 1980 is pending.

The investigations supported by NSF and earlier by NIH are complementary to those proposed under this contract. A common source of genetic stocks is drawn upon and facilities are shared.