Theoretical and Applied Genetics 40, 185–190 (1970)

# Paramutation and Mutation of $R^{ch}$ in Maize

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**Summary.** 1. In maize, $R^{ch}$ conditions anthocyanin pigmentation in aleurone, anthers, silks and reacts with another factor *Pl* to produce cherrycoloured pericarp, thus representing the broadest pigmentation pattern among all the alleles at the *R* locus. The paramutational and mutational behaviour of $R^{ch}$ are reported here.

2. $R^{ch}$ is paramutable with respect to its action in the aleurone, but its pigmenting ability in the pericarp is not sensitive to the paramutagenic action of $R^{st}$.

3. The mutation experiments revealed that $R^{ch}$ is a compound locus containing at least four different components: (*P*) affecting anthocyanin in anthers, (*Si*) conditioning pink silks, (*Ch*) reacting with *Pl* to produce cherry pericarp, and (*S*) affecting aleurone pigmentation.

4. Different interactions of $R^{ch}$ with independent genes support the conclusions drawn from mutation experiments.

5. It is suggested that different components of the $R^{ch}$ locus are unequally sensitive to the action of $R^{st}$.

## Introduction

The discovery and subsequent studies of paramutation by Brink and his collaborators created a new interest in the structure and function of the *R* locus in maize (see review, Brink, Styles and Axtell, 1968). Various *R* alleles in concert with complementary genes characteristically condition anthocyanin production in different tissues of the plant. In the process of paramutation the aleurone pigmenting action of a paramutable allele such as $R^r$ is reduced with great regularity following heterozygosity with a paramutagenic allele like $R^{st}$. The reduction in the pigmenting capacity of paramutated allele is inherited. Styles and Brink (1966) extended the meaning of the term paramutation to cover also the enhanced pigmentation in the $R^r$ progeny of $R^r\,r$ ($r$ = colourless aleurone) heterozygotes. Thus paramutation is a directed change to either lower or higher level of activity of a gene. Although paramutation has been shown to take place in somatic cells (Sastry, Cooper and Brink, 1965), it has not been thoroughly studied in any tissue other than the aleurone. This was primarily due to comparatively low amount of anthocyanin in other parts of the plant in the maize strain used.

A relatively rare *R* allele, designated as $R^{ch}$, is known to condition a deeper and broader pigmentation pattern than $R^r$ (Sastry, 1969). Among other effects $R^{ch}$ controls the production of cherry coloured anthocyanin pigment in the pericarp. It appeared, therefore, that this allele might be suitable for a comparative study of paramutation in the triploid aleurone and diploid pericarp. The experimental results presented here, however, make it clear that while $R^{ch}$ is paramutable with respect to its action in the aleurone, its pigmenting ability in the pericarp is not affected by $R^{st}$ in an $R^{ch}\,R^{st}$ heterozygote. In attempting to interpret these results the possibility is raised that $R^{ch}$ is a compound locus in which the component that conditions pericarp pigmentation is different from that underlying aleurone colour and is insensitive to the action of $R^{st}$. The data presented here support this hypothesis.

## Material and Methods

All genotypes have been obtained in a standard Wisconsin inbred line W22 by repeated backcrossing. Since detailed descriptions are provided elsewhere (Brink, 1958; Sastry, 1969) only salient phenotypic features of the genetic materials will be presented here. *R* locus is situated on the short arm of chromosome 10. The following *R* alleles were used in the present study.

$R^r$: Purple aleurone, purple anthers, green silks and red seedlings; paramutable.

$R^{st}$: Stippled aleurone, green anthers, green silks and green seedlings; paramutagenic.

$r^r$: colourless aleurone, purple anthers, green silks and red seedlings.

$r^g$: Colourless aleurone, green anthers, green silks and green seedlings.

$R^{ch}$: Purple aleurone, purple anthers, pink silks and red seedlings. Unlike $R^r$ and other alleles mentioned above, $R^{ch}$ produces cherry pigment in the pericarp when an independent genetic factor, *Pl*, is also present. Three independent collections of $R^{ch}$ have been utilized in the present study; of these one was originally obtained from Dr. L. J. Stadler's collection (Stadler-$R^{ch}$) and two were from New Mexico (New Mexico-1 $R^{ch}$ and New Mexico-2 $R^{ch}$). From their preferential segregation for $R^{ch}$, both strains from New Mexico were inferred to carry a heterochromatic knob (designated as *K*) situated on chromosome 10, distal to the *R* locus. Rhoades (1942) estimated crossingover between *R* and *K* to be of the order of one percent. When the knob is present in homozygous condition in inbred W22, the plants become twisted and are usually shorter than the plants with the knob in heterozygous condition.

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*Pl*: Apart from reacting with $R^{ch}$ to produce cherry pericarp, *Pl* also produces anthocyanin in stems and glumes (Emerson, 1921). *Pl* is situated on chromosome 6. The *Pl Pl* $r^g$ $r^g$ stock employed here had green seedlings, red stem, green silks, purple glumes, green anthers, colourless aleurone and colourless pericarp. *Pl* has no effect on paramutation of $R^r$.

Details of other stocks used will be given at appropriate places in the text.

In all studies concerning paramutation, changes in aleurone phenotype were evaluated by testcrossing the given plants on $r^g$ $r^g$ ♀♀ and assessing the resulting ears by individually matching a sample of 50 kernels from each testcross ear against a standard set of kernels with graded pigmentation. The standard consisted of seven kernels defining classes ranging from 1 (colourless) to 7 (completely coloured). Mean values of pigmentation were calculated from the frequencies of seeds falling in each class. These values were used to denote the pigmenting ability of the *R* gene under study.

The plants in $R^{ch}$ families, in which the intensity of the pericarp was to be evaluated, were pollinated either by $r^g$ $r^g$ or $r^r$ $r^r$ ♂♂. The ears from *Pl* $R^{ch}$ segregant plants were scored on an ear basis against a standard set of ears defining four classes of pericarp pigment. (Since the pericarp is a maternal tissue it cannot be scored by matching the individual kernels.)

To obtain a large number of ears by controlled pollinations for mutation experiments, the detasseling plot technique of Stadler (1946) was employed. In this technique, every fourth row in the field was planted with $r^r$ $r^r$ which functioned as the male parent, and the intervening rows were planted with appropriate female parents. The female parents were detasseled as soon as tassels appeared and well before anthesis. Putative mutant kernels produced on the resulting ears of female parents were studied further. The detasseling plots were isolated from other maize to minimize pollen contamination. The experiments were so designed that even if pollen contamination occurred, it could be detected by appropriate progeny testing.

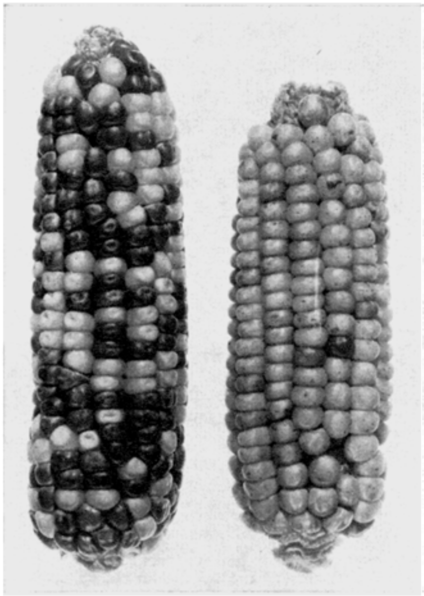

Fig. 1. Typical ears from $r^g$ $r^g$ ♀♀ × $R^{ch}$ $r^g$ ♂♂ (left) and $r^g$ $r^g$ ♀♀ × $R^{ch}$ $R^{st}$ ♂♂ (right) crosses, illustrating paramutation of Stadler $R^{ch}$

## Results

### (*i*) *Paramutation of* $R^{ch}$ *alleles*

The first series of tests was constituted to determine and to measure quantitatively the effect of $R^{st}$ on the aleurone and pericarp pigmenting capacities of the three $R^{ch}$ alleles. Plants from *Pl pl* $R^{ch}$ $r^g$ families were crossed with $R^{st}$ $R^{st}$ and $r^g$ $r^g$ plants to generate the required stocks. The $R^{ch}$ $R^{st}$ and $R^{ch}$ $r^g$ plants grown from the resulting ears were testcrossed on $r^g$ $r^g$ ♀♀. A record of the presence of *Pl* in $F_1$ plants was kept to ascertain if *Pl* in itself has any effect on paramutability of $R^{ch}$. The testcross data, summarized in Table 1, reveal that all three $R^{ch}$ alleles were affected by $R^{st}$. The degree of sensitivity to $R^{st}$, however, is different for the different alleles, the most highly affected one being Stadler $R^{ch}$. This is shown by comparing Fig. 1 with Figs. 2 and 3. In the cases of both the New Mexico alleles, there were many fully pigmented kernels in $R^{ch}$ $R^{st}$ testcrosses and their lowered pigmentation value scores were mainly due to the presence of a few kernels in classes 2 and 3. Since these two alleles showed only a small change (0.41 class in the case of New Mexico-1 $R^{ch}$ and 0.44 class in the case of New Mexico-2 $R^{ch}$) the paramutant plants of New Mexico-1 $R^{ch}$ (i. e. the plants of $R^{ch}$ $r^g$ kernels extracted from $r^g$ $r^g$ × $R^{ch}$ $R^{st}$ crosses) were again crossed with $R^{st}$ $R^{st}$ to obtain additional evidence. The testcrosses of these $R^{ch}$ $R^{st}$ plants made on $r^g$ $r^g$ ♀♀ exhibited a significant further change (compare entries 4 and 5 in Table 1). Thus, it is evident that the alleles from New Mexico are also paramutable. The low sensitivity of the New Mexico alleles can be attributed to the presence of the heterochromatic knob (*K*) introduced with the $R^{ch}$ alleles for Brink (1969) demonstrated that *K* exerts such an effect on the paramutation of $R^r$.

Table 1. *Comparative paramutability of* $R^{ch}$ *alleles: mean aleurone scores of* $R^{ch}$ $r^g$ $r^g$ *kernels derived from* $R^{ch}$ $r^g$ *and* $R^{ch}$ $R^{st}$ *testcrosses on* $r^g$ $r^g$ ♀♀

| Entry | Staminate parent | No. plants tested | Mean score | Difference* | t or F[1] value |
|---|---|---|---|---|---|
| 1 | Stadler $R^{ch}/r^g$ | 18 | 6.23 | 2.20 | 15.71 |
| 2 | Stadler $R^{ch}/R^{st}$ | 28 | 4.03 | | |
| 3 | New Mexico-1 $R^{ch}/r^g$ | 17 | 6.35 | | |
| 4 | New Mexico-1 $R^{ch}/R^{st}$ | 12 | 5.94 | 0.41 | 114.50 |
| 5 | New Mexico-1 $R^{ch}/R^{st}$[2] | 18 | 4.64 | 1.71 | |
| 6 | New Mexico-2 $R^{ch}/r^g$ | 14 | 6.56 | 0.44 | 3.38 |
| 7 | New Mexico-2 $R^{ch}/R^{st}$ | 15 | 6.12 | | |

* Difference between normal and paramutant plants.

[1] All values are highly significant ($P < .01$)

[2] $R^{ch}$ was twice heterozygous with $R^{st}$ – see text.

To compare the pericarp pigmenting abilities of paramutant and normal forms of $R^{ch}$, plants were grown from the testcrosses of both the classes and were pollinated by $r\ r$ plants. The ears from $Pl$ plants were scored on an ear basis, as described earlier. It may be noted that each testcross ear used to evaluate aleurone pigment becomes a family at this stage. The mean scores for each genotype were calculated from family mean scores. These values point to the rather unexpected conclusion that $R^{st}$ did not reduce the pericarp pigmenting capacity of $R^{ch}$. In fact the non-paramutant families (controls) of Stadler $R^{ch}$ and New Mexico-2 $R^{ch}$ registered slightly but significantly lower scores than the families grown from kernels showing the paramutant aleurone phenotype. (The slight decrease of pigment in the control families was due to the presence of a few ears with very little pigment in the pericarp. A further study of the plants derived from these ears showed that the change was caused by a mutation of $Pl$ gene. When $R^{ch}$ from these ears was combined with a normal $Pl$, dark red phenotype of the pericarp was restored.)

While no quantitative data are available on the question whether the pericarp pigmenting ability is reduced if $R^{ch}$ is kept heterozygous with $R^{st}$ for more than one generation, we have stocks in which $R^{ch}$ was heterozygous with $R^{st}$ for three generations and plants from these families appear to have normal amounts of pericarp pigment.

Table 2. *Pericarp pigmenting ability of $R^{ch}$ alleles: mean pericarp colour scores of $Pl\ R^{ch}\ r^g$ plants grown from the crosses*
$r^g\ r^g$ ♀ × a) $R^{ch}\ r^g\ Pl$ ♂
× b) $R^{ch}\ R^{st}\ Pl$ ♂

| $R^{ch}$ allele | a) normal $R^{ch}\ r^g$ | | b) Paramutant $R^{ch}\ r^g$ | | Difference (a—b) | $t^{(c)}$ value |
|---|---|---|---|---|---|---|
| | No. families tested | Mean score | No. families tested | Mean score | | |
| Stadler | 9 | 2.80 | 14 | 3.66 | —0.86 | 3.58** |
| New Mexico-1 | 8 | 3.34 | 5 | 3.10 | 0.24 | 1.40 ns |
| New Mexico-2 | 18 | 2.97 | 24 | 3.02 | —0.05 | 2.24* |

(c) ns = $P > .05$. — * = $.05 > P > .01$. — ** = $P < .01$.

### (*ii*) *Mutation of $R^{ch}$ alleles*

Since there is evidence to suggest that changes at the $R$ locus result from intragenic recombination (see Gavazzi and Avila, 1969), only mutants isolated from $pl\ pl\ R^{ch}\ R^{st}$ plants were examined. However, the words „mutation" and „mutant" are used here since it could not be definitely established that intragenic recombination was the cause of changed phenotypes.

It may be recalled from the earlier description of the genetic stocks that $R^{st}$ is characterized by green anthers, green silks, stippled aleurone and colourless pericarp. All the features of $R^{ch}$ are dominant over those of $R^{st}$.

Fig. 2. Representative testcross ears from matings of $R^{ch}\ r^g$, $R^{ch}\ R^{st}$ and $R^{ch}\ R^{st}$ plants (from left to right) on $r^g\ r^g$ ♀♀. The ear on the extreme right side came from a family in which $R^{ch}$ was heterozygous with $R^{st}$ for second time. The figure illustrates progressive paramutation of New Mexico-1 $R^{ch}$

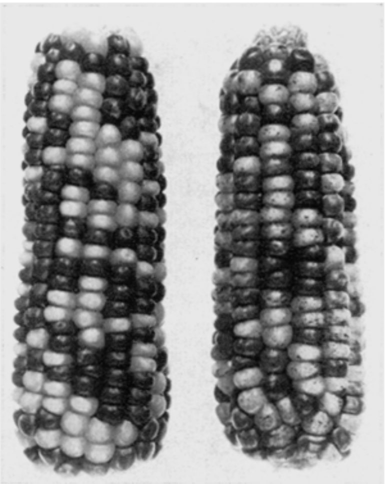

Fig. 3. Paramutation of New Mexico-2 $R^{ch}$. Representative ears from $r^g\ r^g$ ♀♀ × $R^{ch}\ R^{st}$ ♂♂ (right) crosses. On the average colour of the paramutant kernels is only slightly less than that of the normal kernels

The isolation and study of mutations from $R^{ch}\ R^{st}$ plants were carried out according to the following scheme:

$pl\ pl\ R^{ch}\ R^{st}$ ♀♀ × $pl\ pl\ r^r\ r^r$ ♂♂-Detasseling plots
$pl\ pl\ r^*\ r^r$ — (Putative mutants)

Cross on $Pl\ Pl\ r^g\ r^g$ ♀ ↓ Family-1 $\begin{cases} Pl\ pl\ r^g\ r^r \\ Pl\ pl\ r^g\ r^* \end{cases}$

Self ↓ $\left.\begin{matrix} pl\ pl\ r^r\ r^r \\ pl\ pl\ r^*\ r^* \\ pl\ pl\ r^r\ r^* \end{matrix}\right\}$ Family-2

(* mutant allele)

Table 3. *Mutants recovered from* $pl\ pl\ R^{ch}\ R^{st}$ *plants**

| Class | Phenotype | Stadler | | New Mexico-1 | | New Mexico-2 | |
|---|---|---|---|---|---|---|---|
| | | No. | Frequency ($\times 10^{-4}$) | No. | Frequency ($\times 10^{-4}$) | No. | Frequency ($\times 10^{-4}$) |
| | Colourless aleurone & pericarp | | | | | | |
| A | green anthers, green silks & *k* | 8** | 2.34 | 1*** | 0.41 | 4 | 0.57 |
| B | green anthers, green silks & *K* | — | — | 6 | 2.48 | 15 | 2.14 |
| C | red anthers, green silks & *k* | 3 | 0.88 | 1 | 0.41 | 2 | 0.28 |
| D | red anthers, pink silks & *k* | Nil | Nil | Nil | Nil | 1 | 0.14 |
| | Colourless aleurone & coloured pericarp | | | | | | |
| E | red anthers, pink silks & *k* | Nil | Nil | Nil | Nil | 1 | 0.14 |
| | Coloured (pale) aleurone & colourless pericarp | | | | | | |
| F | green anthers, green silks & *K* | Nil | Nil | Nil | Nil | 1 | 0.14 |
| | Total | 11 | 3.22 | 8 | 3.31 | 24 | 3.42 |

* Total numbers of gametes ($R^{ch}$ and $R^{st}$) in each case:

Stadler $R^{ch}\ R^{st}$ = 34,2000
New Mexico-1 $R^{ch}\ R^{st}$ = 24,200
New Mexico-2 $R^{ch}\ R^{st}$ = 70,200 .

** Since Stadler $R^{ch}$ did not carry *K*, this number represents total number of mutants with green anthers and silks. If classes A and B are added, the frequency of mutants with green anthers and green silks in New Mexico alleles will be equal to those from Stadler $R^{ch}$. One mutant in Class A (from Stadler $R^{ch}\ R^{st}$) was near colourless with occassional spots.

*** This was a near colourless mutant with occasional spots on the aleurone and was paramutagenic.

### Scoring of mutant types

The colourless and nearly colourless mutant kernels were studied along with some normal $R^{ch}$ kernels. (The expression of $R^{ch}$ was not affected by paramutation at this stage, since $R^{ch}$ was present in two doses in the triploid aleurone.) Segregation in family-1 and family-2 revealed the anther colour of the mutants. If both families segregate for red and green anthers, the appearance of green anthers must be due to the mutation. Observations on silk colour were also made at this stage. The pericarp pigmenting ability of the mutants was revealed by the plants in family-1. Presence of the heterochromatic knob (*K*) in the mutants derived from New Mexico alleles was assessed by checking the plant phenotypes in the families derived from selfed ears. (If *K* is present about 1/3 of the plants show twisted stalks as a consequence of the homozygous condition of *K*.) All mutants were studied for at least two more generations to confirm the original observations.

The salient features and frequencies of various mutant types are assembled in Table 3. As can be seen from this table, a majority of the mutants had green anthers, green silks, colourless aleurone, colourless pericarp and, in the case of New Mexico alleles, the heterochromatic knob (classes A and B). The data also reveal that the pericarp and the aleurone pigmenting abilities are closely associated but can be separated; in fact only one mutant with colourless aleurone and coloured pericarp (class E) could be isolated. However, the mutant in class F also supports this contention. Anther colour and silk colour also are closely associated but could be separated.

### (*iii*) *Interaction of* $R^{ch}$ *with other genetic factors*

Many genetic factors are known to affect the expression of the *R* locus. Some of them effect only pigment in the aleurone, and some of them affect both aleurone and plant pigment. The following experiments were carried out to see how far the conclusions drawn from mutation experiments agree with differential interaction of $R^{ch}$ with other genetic factors.

*C* is one of the complementary genes required to produce anthocyanin in the aleurone. None of the alleles of *C* affect anther colour. $C^I$, a dominant allele of the *C* inhibits aleurone pigment. Plants with $Pl\ pl\ C\ C\ R^{ch}\ r^g$ constitution were crossed on $pl\ pl\ C^I\ C^I\ R^r\ R^r$ ♀♀. The resulting ears were colourless, showing that $C^I$ inhibits aleurone pigment of $R^{ch}$ also. A number of *Pl* plants grown from these colourless kernels ($Pl\ pl\ C^I\ C\ R^{ch}\ R^r$ and $Pl\ pl\ C^I\ C\ R^r\ r^g$) were selfed. All the selfs of the plants with pink silks had dark cherry pericarp showing that $C^I$ has no inhibitory effect on pericarp and silk colour.

Similarly studies with *pr* (chromosome 5) suggested the functional independence of different components of $R^{ch}$. The presence of *pr* gene in *R* plants changes the aleurone colour from purple to red and, if *Pl* is also present the anther colour changes from red to deep scarlet. Plants with $Pl\ pl\ R^{ch}\ R^{ch}\ pr\ pr$ constitution were found to have red aleurone and scarlet anthers but the cherry pericarp and pink silk colour were not affected.

In a different series of experiments, *bz* (chromosome 9; bronze coloured aleurone, anthers and stem) was found to inhibit completely the silk pigment whereas it had no effect on the pericarp pigment of *Pl* $R^{ch}$ plants.

## Discussion

Stadler and his associates envisaged the *R* locus as consisting of two structurally and functionally discrete elements designated as (*P*) for plant colour and (*S*) for aleurone colour (cf. Emmerling, 1958, for details). Immediately after the discovery of paramutation at the *R* locus an interesting question was raised: whether the whole *R* locus was affected by paramutation or only the (*S*) component was changed. This question became more important when it was realized that paramutation itself takes place in somatic cells of $R^r R^{st}$ plants (Sastry, Cooper and Brink, 1965).

In 1958, Brink and Mikula reported that the coleoptile and leaf sheath colour of paramutant plants was significantly less than in the plants of normal $R^r$, suggesting that the effect of the paramutagenic allele was parallel on both (*P*) and (*S*) components of the locus. However, as these authors point out the empirical methods employed to rear the seedlings along with other technical difficulties placed severe restrictions on further experimentation. Comparatively low amounts of anthocyanin in the coleoptiles and leaf sheaths of even normal plants of the particular inbred strain used contributed another problem in measuring the effect of paramutation.

The first series of the present experiments were designed to overcome the above mentioned difficulties. The capacity of $R^{ch}$ to promote pigment formation in the aleurone and pericarp (which is a diploid tissue like coleoptile and leaf sheath) was utilized to study further effects of the paramutagenic allele, $R^{st}$. Two principal facts emerged from these experiments:

(*i*) All three $R^{ch}$ alleles tested showed a decreased amount of aleurone pigment following heterozygous association with $R^{st}$ and hence $R^{ch}$ can be designated as paramutable.

(*ii*) Pericarp pigment of the paramutant plants, however, was not lowered.

These facts, in contrast to the observations of Brink and Mikula (1958), demonstrate that all components of the *R* locus are not equally sensitive to the action of $R^{st}$. This raises many questions. For instance, is there a distinct component of the *R* locus that is concerned with pericarp pigmentation and that is insensitive to the action of $R^{st}$? If it is present, is it the same or different from the plant pigmenting component (*P*)? Answers for these questions are essential for a valid interpretation of the paramutation data on the basis of differential sensitivity.

From the results of the mutation experiments (Table 3) one can deduce that there is a separate entity in $R^{ch}$ which promotes cherry colour in the pericarp. In fact these data demonstrate that there is more structural discreteness within the $R^{ch}$ locus than was originally suspected. Since there were mutants with red anthers, green silks and colourless pericarp (class C in Table 3), it may be inferred that the pigmentation of silks is conditioned by a different element or elements than that of anthers. Hence the function of (*P*) may be confined to anther pigment and possibly seedling colour since these two functions were not separated in the present experiments. The mutant with pink silks and colourless pericarp (class D) suggests that the colour in these two tissues is conditioned by two different elements which are tentatively designated as (*Si*) for silk colour and (*Ch*) for pericarp colour. Isolation of the mutant with colourless aleurone and coloured pericarp in the presence of *Pl* (class E) indicates that (*Ch*) and (*S*) (aleurone colour component) are separate entities. The mutant with coloured aleurone and colourless pericarp (class F) also supports this contention. Anderson and Emerson (1923) described naturally occurring alleles ($r^{ch}$) with the properties of the class E mutant.

The general consensus of opinion among students of the *R* locus is that a majority of the changes at that locus arise as a result of intragenic recombination (Emmerling, 1958; Ashman, 1965; Gavazzi and Avila 1969). In the present experiments, $R^{ch}$ was not marked by flanking genetic markers, since the main intention was not a detailed structural analysis of the locus. However, if one considers the heterochromatic knob (*K*) as a distal marker, then its distribution among the mutants suggests the sequence of different components as: Centromere — (*P*) (*Si*) (*Ch*) (*S*) — *K*. Confirmation of this sequence will be the subject of future experimentation.

At this stage the possibility that the mutants described above might have resulted from $R^{st}$ alone should be considered. This cannot be ruled out for class A mutants but they did not enter into the present discussion. However, mutants in the rest of the classes, have at least one property from $R^{ch}$ or carry the heterochromatic knob. These features clearly point out that either the mutants have arisen from $R^{ch}$ alone or as a result of intragenic recombination between $R^{ch}$ and $R^{st}$.

Different interactions of $R^{ch}$ with other genes support the conclusions drawn from mutation experiments. It appears that while $C^I$ can only affect (*S*), *pr* can modify the action of (*P*) and (*S*) but not (*Si*) and (*Ch*). The specific inhibition of silk colour by *bz* suggests that it acts only on (*Si*). These different genetic factors should have affected pigmentation uniformally in all tissues, if $R^{ch}$ were an entity. Thus it appears that structural discreteness within the $R^{ch}$ region is associated with a certain amount of functional independence.

Even if the compound nature of $R^{ch}$ is accepted, the results from paramutation experiments are hard to explain on any simple hypothesis. Brink (1964), Sastry et al. (1965) and Brink et al. (1968) postulated that paramutation involves a heterochromatic segment at the *R* locus composed of varying numbers of a common repeating unit called a metamere. The effect of the metameres is to repress *R* gene action. The degree of repression is assumed to be proportional to the number of metameres. According to this model, the essential feature of paramutation is the change in the number of metameres making up the repressor segment.

If the weakly pigmenting action of the paramutants is a consequence of repressing metameres, then such elements may not have the same effect on all components of the *R* locus, and components like the one that conditions pericarp colour may be insensitive. The insensitivity of (*Ch*) is not caused by the the apposition of (*Ch*) and (*S*). This is shown by the fact that the pericarp colour of the class E mutant (Table 3) and naturally occurring $r^{ch}$ is not affected by $R^{st}$ (Sastry, unpublished).

Then, is it justified to assume a component of *R* locus for the pigmentation of each principal tissue affected? From the studies reported here, the answer appears to be yes. A comparative study of various *R* alleles should throw more light on the structure and evolution of complex genetic regions in higher organisms.

## Acknowledgements

The author wishes to thank Dr. R. A. Brink, under whose supervision this work was carried out. It is a pleasure to acknowledge many suggestions offered by Drs. R. A. Brink, J. R. S. Fincham, D. B. Roberts and J. D. Axtell. Many discussions with Dr. E. G. Barry have been most beneficial. This study was aided by grants to Dr. R. A. Brink from the Wisconsin Alumni Research Foundation and the National Science Foundation. The manuscript was prepared under the tenure of Beit Memorial postdoctoral fellowship.

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Received March 16, 1970

Communicated by G. Melchers

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