RELATION OF UNEQUAL CROSSING OVER TO THE INTER-DEPENDENCE OF R^r ELEMENTS (P) AND (S)^{1, 2, 3}

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PREVIOUS studies have shown that the gene R^r behaves, with respect to spontaneous mutation, as if it were composed of two independently mutating elements: (P), affecting anthocyanin pigmentation of specific plant tissues, and (S), affecting anthocyanin pigmentation of the aleurone layer of the endosperm (STADLER 1948). When seed-color is lost by mutation, the level of plant-color expression remains unaltered. Similarly, the mutation of the plant-color determiner has no effect on the phenotypic expression of seed-color (STADLER 1951a).

Recent experiments have shown that (P) and (S) are separable by unequal crossing over (STADLER and NUFFER 1953). It should be noted that the symbols (P) and (S) are not intended to imply that there is only one separable element for plant-color and one for seed-color. Both could be multiple (PPSSSS) and give the same experimental results. These symbols indicate simply that all (P) determiners may be removed in a proximal segment that leave some (S), and that all (S) elements may be removed in a distal segment that will leave some (P).

Thus the gene R^r may be regarded in either of two ways. The R^r effect may be produced by the action of two separate genes of independent action, one affecting the aleurone-color and the other affecting the plant-color, or alternatively, it may be thought of as a single or compound structure with dual gene action.

These possibilities may be represented in two models:

The experimental distinction between these two models is in the structure of the gene following mutation. According to model A, the mutation of element (P) would have no effect on the subsequent mutation frequency of element (S), since (S)

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would be the same gene before and after the mutation of (P). With the model B, however, the mutation frequency of (S) cannot be predicted, since it would depend upon the mutability of the new gene designated R^{ps} .

When several mutant R^o alleles (designated pS) were compared with their R^r alleles (designated PS), it turned out that the frequency of aleurone-color mutation in the parent R^r alleles was approximately 10 times as high as that in the mutant R^o alleles (STADLER 1946 and 1951b). The results are those that would be expected with model B, a single or compound gene.

These facts taken together seem to indicate that while (P) and (S) are two distinct structural elements, they still have special inter-relationships as members of a functional complex.

The present paper is concerned with the behavior of one particular R^o Cornell allele, designated R^o -14, in subsequent mutational trials (STADLER and EMMERLING 1954). Briefly, the R^o -14 allele exhibits two peculiarities in its mutational behavior. (1) In the heterozygote R^o -14/ R^r the R^o gametes mutate at a lower rate than those of the R^r gametes. (2) In the backcross progeny the R^r gametes in R^o / R^r plants mutate at a higher rate than those of the R^r gametes in the homozygous sibs. The latter phenomenon will be referred to as the M_G effect.

Considering, then, these two characteristics of R^o -14, three alternative hypotheses can be formulated to account for the observations.

- (A). The reduction in the mutation frequency of R^o -14 is due wholly to a change in the stability of (S) following either mutation or loss of (P). The M_G effect is unexpected on this basis unless it can be attributed to a modifier of mutation rate. However, extensive trials have failed to separate the M_G effect from the R^o -14 gene. Thus the effect must be either closely linked or associated with the R^o -14 allele. In this connection it might be noted that an R -linked modifier, which depresses the frequency of seed-color mutation, has been identified previously in the R^o McBain stock (STADLER 1950).
- (B). The genic element (S) does not change in stability, but both effects are due entirely to unequal crossing over, yielding r^r crossovers in addition to r^r mutants from noncrossover mutation of R^r .
- (C). The stability of (S) is affected by mutation or loss of (P), and the occurrence of unequal crossing over increases the number of r^r mutants from R^o -14/ R^r , thus giving the M_G effect.

EXPERIMENTAL EVIDENCE

Preliminary data on the compound $G R^o$ -14 $k/g R^r$ Cornell K support the view that unequal crossing over is involved in the production of some of the seed-color mutations.

The aleurone-color mutations were secured from the cross $G R^o$ -14 $k/g R^r$ Cornell $K \times g r^o k/g r^o k$. The stock carried markers on either side of the R locus which permits the detection of unequal crossovers. The proximal marker used was golden plant (designated g), located 14 units to the left of R . Since no gene distal to R is known, it was necessary to use a cytological marker. A large heterochromatic knob located one unit to the right of R (RHOADES 1942) was used. This knob (designated

A If R^{a-14} is a noncrossover mutant (pS).B If R^{a-14} is a crossover case (-S).

FIGURE 1.—The types of unequal crossovers expected by loss of seed-color.
 $G \ R^{a-14} \ k/g \ R^r \ K$: Cornell K

K) is known to segregate preferentially at megasporogenesis (RHOADES 1942). In the progeny of females heterozygous for knob 10, individuals carrying the knob are recovered about three times as frequently as those without the knob.

The $G \ R^{a-14} \ k/g \ R^r \ K$ plants were pollinated by $g \ r^r \ k$ pollen in a detasseled plot planting, and the aleurone mutations were identified by inspection of all seeds on the ears. The seed-color mutants were grown to maturity to determine the genotype and were pollinated by a recessive r stock to verify the mutation. Each mutant was sampled cytologically to determine whether unequal crossing over had occurred.

The classes of unequal crossovers expected by loss of seed-color are illustrated in figure 1. The classification of unequal crossovers indicated for the R^{a-14}/R^r compound is derived from the assumption that the R^{a-14} allele may be either (pS) or (-S) in constitution. Presumably the R^{a-14} allele of the (pS) type represents a noncrossover, plant-color mutation bearing a mutant null allele of (P) which has lost its phenotypic action but has retained its pairing affinity. Thus the compound $G \ R^{a-14} \ k/g \ R^r \ K$, including the (pS) allele, should yield the apparent mutants $r^r \ k$ and $r^r \ K$, since both distal and proximal displacement of the knob-bearing segment may occur (see fig. 1A). However, it is possible that R^{a-14} is deficient for the element (P) because of the result of a plant-color, unequal crossover in the parent R^r allele. In this case the compound $G \ R^{a-14} \ k/g \ R^r \ K$, including the (-S) allele, should produce $r^r \ k$ unequal crossovers, with none of the alternative class (see fig. 1B). This is based on the assumption that unequal crossing over occurs only when there are two or more synaptically homologous elements present in the gene string. Thus the critical crossover class, the $r^r \ K$ type, is expected from compounds of R^r with the R^{a-14} allele of (pS) constitution and not from the (-S) type.

But the absence of the critical crossover class would not be a valid criterion for distinguishing between (-S) and (pS) alleles if phenotypically inactive elements were present either distal to (S), or proximal to (P), or between (S) and (P). These null elements (designated N) could also be capable of pairing with (S) or (P) and thus produce unequal crossovers of the type expected only from the (pS) alleles.

Experimental detection of N elements either proximal to (P) or distal to (S) would come from the homozygote R^r Cornell (PS)/(PS), cytologically marked for the detection of unequal crossing over. If R^r Cornell consists of (NPS) or (PSN), unequal crossing over could yield r^r crossovers as well as $r^r \ k$ crossovers (see fig. 2).

FIGURE 2.—Unequal crossovers expected in the compound $g R^r K/G R^r k$ with either proximal or distal null elements. (Only crossovers yielding seed-color mutants are considered.)

FIGURE 3.—Unequal crossovers expected in the compound $g \ R' \ K/G \ R' \ k$ with a median null element.

The trials so far made with $g R^r K/G R^r k$ have given no r^o crossovers (STADLER and NUFFER 1953). The five colorless seed crossovers produced by this compound were in fact of type $G r^r K$, but the sample was too small to give any significant indication of the existence of such elements.

There is no evidence at present which excludes the possibility of null elements between (P) and (S). In experiments conducted with the homozygote, median N elements would be undetectable since the unequal crossovers would be all of the r^r crossover type (see fig. 3A). However, the subsequent behavior of plant-color crossovers should give evidence of median N elements. The homozygous (PNS) compound is expected to produce two classes of unequal crossovers among the plant-color mutants, the (-NS) and the (-S) types (see fig. 3B). In the case of the R^o crossover of (-NS) constitution, the (-NS)/(PS) compound should yield unequal crossovers among seed-color mutants showing both proximal and distal displacement. The (-S)/(PS) compound, which represents the other type of R^o crossover, should yield seed-color crossovers showing proximal but not distal displacement.

Thus the critical criteria by which to detect the presence and the specific location of these null elements would be as follows: (PS), absence of r^o crossovers in homozygous (PS) plants; (NPS) or (PSN), presence of r^o crossovers in homozygous (NPS) or (PSN) plants; and (PNS), absence of r^o crossovers in the homozygote (PNS) and the presence of two types of R^o crossovers.

The results of the tests of the seed-color mutants produced by the compound $G R^o-14 k/g R^r K$ are summarized in table 1. To calculate the most probable frequency of the noncrossover and crossover mutants the rate determined is stated in terms of the population in which analysis of the mutants was made. That is, 22 mutants were found, but only 13 of the 22 survived; thus the population of gametes tested is $13/22 \times 59,374 = 35,085$.

In a population of 35,085 gametes, this compound yielded 12 r^r mutants and one

TABLE 1

Frequency of noncrossovers and unequal crossovers among seed-color mutants in female gametes
 $G R^o-14 k/g R^r \text{ Cornell } K \times g r^o k/g r^o k$

Culture	Number of seeds examined	Number of colorless seeds	Fraction determined	Genotype of the mutant individuals					
				Noncrossovers			Unequal crossovers		
				$g r^r K$	$G r^r K$	$G r^o$	$g r^r k$	$G r^r k$	$G r^o K$
72:365	11,880	6	6/6	3	1*	0	2	0	0
366	8,052	4	4/4	2	0	0	1	1	0
73:1721	14,302†	2 + 1?	1/2 + 1?	0	0	0	1	0	0
1747	11,704†	5 + 1?	1/5 + 1?	0	0	1	0	0	0
1749	13,436†	3	1/3	0	0	0	1	0	0
Total	59,374	26 + 2?	13/20 + 2?	5	1	1	5	1‡	0

Adjusted mutation frequency 13/35,085.

* Intermediate mutant with faint aleurone color and a coincident crossover for g .

† Total population includes plants without K marker.

‡ Unequal crossover with a coincident crossover for g .

r^o mutant. Of the 12 r^r mutants found, five were $r^r K$ noncrossovers, one was a non-crossover $r^r K$ with faint aleurone color, and six were $r^r k$ crossovers. The only seed-color mutant lacking plant color was a noncrossover $G r^o k$ mutant.

The evidence that these mutants are unequal crossovers comes from the fact that only 1 percent of mutants analyzed should show a coincident crossover between R^r and the knob. The occurrence of six crossovers in this region among the 13 mutants investigated is highly significant evidence of a relation of crossing over to mutation ($P < 0.01$). The number of mutants expected by coincidence is 0.13.

The critical crossover class, the $r^o K$ type, was absent in this culture, although with equal frequency of occurrence of the two crossover types, 75 percent of the crossovers detected should be of this type, since the female transmission ratio for chromosomes with and without the knob 10 was approximately 75 percent K :25 percent k . This knob ratio was determined by seedling tests. The number of crossovers identified, in comparison with numbers expected on the basis of a 75 percent K :25 percent k ratio, is as follows:

	Total number of crossovers	$r^o K$ crossovers	$r^r k$ crossovers
Observed	6	0	6
Expected		4.5	1.5

Stevens' statistical tables (1942) can be used to determine whether the observed distribution of 0 $r^o K$: 6 $r^r k$ crossovers deviates significantly from the expected distribution of 4.5 $r^o K$: 1.5 $r^r k$ crossovers. According to Stevens' table, for $n = 0$, $1/N = 1/6 = 0.166$, the upper limit of expectation of zero mutants at the $P = 0.05$ level is 2.83. Thus the difference is clearly significant. The absence of the crossover class produced by proximal displacement of the K -bearing segment can be accounted for by assuming the R^o -14 is deficient for the element (P), that is, that this mutant is of the (-S) type.

The proportion of unequal crossovers among the r^r mutants in the $G R^o$ -14 $k/g R^r K$ compound, uncorrected for the knob ratio, is six out of 12, or 50 percent (table 2A). The relative frequency of mutant types adjusted for the transmission advantage of the chromosome bearing K is given in table 2B. Thus the frequency in megaspores, before preferential segregation shows that the number of $r^r k$ crossovers was 24, and the number of noncrossover mutants was eight r^r and four r^o . The corrected proportion of unequal crossovers among the r^r mutants is 24 out of 32, or 75 percent.

These results indicate that the excess of r^r over r^o mutants is due largely to unequal crossing over which increases the apparent rate of mutation of the opposite R^r Cornell allele.

The experimental results presented above are incompatible with hypothesis A which ascribes the reduced mutation rate of (S) wholly to a change in stability of the genic element (S). The data, however, do not differentiate between hypotheses B and C. Such a differentiation requires data from backcross of $G R^o$ -14 $k/g R^r k \times g R^r K/g R^r K$, which permits direct comparisons of mutation frequency in sib plants of $G R^r k/g R^r K$ and $G R^o$ -14 $k/g R^r K$ constitution. At the present time there are no data available from backcross progeny marked for unequal crossover determinations, but there are data available from several cultures without crossover

TABLE 2
Proportion of unequal crossovers among r^r mutants in G R^a -14 k/g R^r K

A. Uncorrected for knob ratio	
Adjusted mutation frequency	13/35,085
r^r mutants obs.	12
r^a mutants obs.	1
r^r crossovers obs.	6
r^a crossovers obs.	0
r^r noncrossovers obs.	6
r^a noncrossovers obs.	1

Proportion of r^r crossovers among r^r mutants = $6/12 = 50\%$.

B. Corrected for knob ratio				
	r^r K noncrossovers	r^r k crossovers	r^a k noncrossovers	n
Number observed in megaspores	6	6	1	35,085
Number occurring in megaspores to yield above with 75 K:25 k ratio	8	24	4	140,340

Proportion of r^r crossovers among r^r mutants = $24/32 = 75\%$.

markers. Inefficient as these tests are, they do permit a tentative choice between hypotheses B and C.

The backcross progeny tested for the relative frequency of r^r and r^a mutations, which are shown in tables 3 and 4, were produced by crossing two different R^a -14 k/ R^r k F_1 plants (40:351 and 69:210) by homozygous R^r Cornell. These backcross cultures were grown in a detasseled plot with r^a g pollen. The genotype of each plant, as to R^a/R^r and R^r/R^r , was determined by seedling tests.

These results illustrate the two peculiarities of the R^a -14 allele. The data of tables 3 and 4 show the pronounced depression of the R^a mutation frequency and the M_G effect in the backcross progeny. In table 3 the total mutation frequency in R^a/R^r plants was 39/42,075, the mutations including 33 to r^r and 6 to r^a . The total muta-

TABLE 3
Seed-color mutation rate of R^a -14/ R^r Cornell versus parent R^r Cornell/ R^r Cornell in female gametes

F ₁ Culture	Genotypic class		R^a/R^r fraction determined	Mutants from R^a/R^r plants	
	R^a/R^r	R^r/R^r		r^a	r^r
40:351-4	14/10,719	4/7,925	12/14	2	10
351-6	6/11,424	8/8,239	5/6	1	4
351-7	23/24,247	12/12,645	22/23	3	19
Total	43/46,390	24/28,809	39/43	6	33

Adjusted mutation frequency 39/42,075.

TABLE 4

Seed-color mutation rate of R^a -14/ R^r Cornell versus parent allele R^r Cornell/ R^r Cornell in female gametes

F ₁ culture	Genotypic class		R^a/R^r fraction determined	Mutants from R^a/R^r plants	
	R^a/R^r	R^r/R^r		r^a	r^r
69:210-5	22/13,428	22/11,904	19/22	1	18
210-6	21/10,655	17/9,394	19/21	1	18
210-7	28/15,524	20/15,454	25/28	0	25
Total	71/39,607	59/36,752	63/71	2	61

Adjusted mutation frequency 63/35,144.

tion frequency, which is reported in table 4, was 63/35,144. Of the 63 mutants tested, 61 were r^r and only 2 were r^a . These data probably represent extremes of $r^r:r^a$ ratio in the heterozygotes examined.

As mentioned previously, the M_G effect applies to the increased mutation rate of the R^r allele in the R^a/R^r class over the rate in the R^r/R^r class. Of the 42,075 gametes tested in the R^a/R^r plants (table 3), about one half of the gametes were tests of the R^r allele and one half were tests of the R^a allele. Thus the mutation rate for R^r in plants of R^a/R^r constitution was 15.68×10^{-4} (33/21,037.5); in plants of R^r/R^r constitution was 8.33×10^{-4} (24/28,809). The latter rate was calculated from the total population, since each seed tested an R^r gamete. In table 4, the observed mutation rate in plants of R^a/R^r constitution was 34.72×10^{-4} (61/17,572), while in plants of R^r/R^r constitution the rate was 16.05×10^{-4} (59/36,752). Thus the R^r mutation frequency was approximately twice as frequent in R^a/R^r plants as in R^r/R^r plants. These data may be interpreted in terms of hypothesis B.

Hypothesis B involves the assumption that the stability of (S) is not changed, but the effects of R^a -14 are due entirely to unequal crossing over. We may assign identical noncrossover mutation rates to R^r and R^a -14 and then calculate how large a proportion of the r^r mutants from R^a -14/ R^r plants must be unequal crossovers. The frequency of mutation of the R^a -14 gene is taken as a measure of the gene mutation rate, assuming that all the r^a mutants observed are from mutations of the R^a allele and all are noncrossovers. If this speculation is wrong, the proportion r^r mutants due to unequal crossing over is still higher.

If the assumptions from hypothesis B were valid, the data of table 3 will show an expected number of noncrossover mutations for both R^r and R^a to be six, since the number of r^a mutants derived from the R^a allele is six (table 5A). Of the 33 mutants observed in heterozygous plants, the expected distribution of r^r crossovers and r^r noncrossovers will be 27 and six respectively. It is expected, therefore, that the 24 r^r mutants observed in the homozygous R^r plants will include 16 r^r crossovers and approximately eight r^r noncrossovers. Thus the percentage of r^r mutations ascribed to unequal crossing over will become 82 percent in the heterozygote and 67 percent in the homozygote.

The data of table 4 show two mutations of R^a -14 to r^a . Under hypothesis B, therefore, the 61 r^r mutants observed in the heterozygote will include 59 r^r crossovers

TABLE 5
Relative frequency or unequal crossovers among r^r mutants in R^g-14/R^r and R^r/R^r sibs
Hypothesis B

A. Data from table 3		
	R^g/R^r	R^r/R^r
Mutation frequency	43/46,390	24/28,809
Adjusted frequency	39/42,075	
r^r mutants obs.	33	
r^g mutants obs.	6	
r^r crossovers exp.	27	
r^r noncrossovers exp.	6	8 ⁺
r^g noncrossovers exp.	6	
Proportion of crossovers among r^r mutants in R^g/R^r plants = 27/33 = 82%.		
Proportion of crossovers among r^r mutants in R^r/R^r plants = 16/24 = 67%.		
B. Data from table 4		
	R^g/R^r	R^r/R^r
Mutation frequency	71/39,607	59/36,752
Adjusted frequency	63/35,144	
r^r mutants obs.	61	
r^g mutants obs.	2	
r^r crossovers exp.	59	
r^r noncrossovers exp.	2	4 ⁺
r^g noncrossovers exp.	2	
Proportion of crossovers among r^r mutants in R^g/R^r plants = 59/61 = 97%.		
Proportion of crossovers among r^r mutants in R^r/R^r plants = 55/59 = 94%.		

and two r^r noncrossovers; in the homozygote the expected number of r^r crossovers and noncrossovers is 55 and 4 respectively. Thus 97 percent of the r^r mutants in the heterozygote and 94 percent of the r^r mutants in the homozygote may be attributed to the occurrence of unequal crossing over.

The same data may be interpreted in terms of hypothesis C. This hypothesis involves the assumption that the stability of (S) is affected by mutation or loss of (P), and the occurrence of unequal crossing over increases the number of r^r mutants in the heterozygote. The percentage of r^r mutants that must be ascribed to unequal crossing over in the R^g/R^r class may be derived from the mutation rate of r^r mutants in the R^r/R^r plants. In this case, the gene mutation frequency of R^r will be identical in the heterozygote and the homozygote. This implies that the r^r mutants from the R^r/R^r are all noncrossovers and that there is a similar occurrence of r^r noncrossovers from R^g-14/R^r .

But the assumption of no crossing over in R^r/R^r plants is not entirely plausible, since results already at hand indicate that crossover mutants occur in the homozygote (STADLER and NUFFER 1953). These were at a rate of 7×10^{-5} and constituted about one third of the r^r mutants. Their relative frequency among r^r mutants, however, must be calculated on a different basis for comparison with those

TABLE 6

*Relative frequency of unequal crossovers among r^r mutants in R^g-14/R^r and R^r/R^r sibs
Hypothesis C*

A. Data from table 3	
Adjusted mutation frequency for r^r mutants in R^g/R^r	33/42,075
Mutation frequency in R^r/R^r	24/28,809 = 8.33×10^{-4}
r^r noncrossovers exp. in R^g/R^r	17.5
$\frac{8.33}{10,000} = \frac{X}{21,037.5}$	
r^r crossovers exp. in R^g/R^r	15.5
Proportion of crossovers among r^r mutants in R^g/R^r plants = $15.5/33 = 47\%$.	
B. Data from table 4	
Adjusted mutation frequency for r^r mutants in R^g/R^r	61/35,144
Mutation frequency in R^r/R^r	59/36,752 = 16.05×10^{-4}
r^r noncrossovers exp. in R^g/R^r	28.2
$\frac{16.05}{10,000} = \frac{X}{17,572}$	
r^r crossovers exp. in R^g/R^r	32.8
Proportion of crossovers among r^r mutants in R^g/R^r plants = $32.8/61 = 54\%$.	

in the heterozygote, since in the $G R^r k/g R^r K$ compound there are two chromosomes with R^r instead of only one. The fact that crossovers and noncrossovers are distinguishable here only by g classification badly weakens the data, since one seventh of the crossovers may be misclassified due to coincident crossing over between G and R . Thus the percentage of crossovers among r^r mutants, very inaccurately estimated, is 38 percent. This is low, but the determination has practically no significance.

The data of table 3 show that the mutation rate of R^r Cornell in R^r/R^r plants is 8.33×10^{-4} (24/28,809). If the assumptions from hypothesis C were correct, the expected number of r^r noncrossovers in plants of R^g/R^r constitution will be 17.5 (table 6A). This hypothesis implies that there is an equal mutation rate of r^r noncrossovers in R^r/R^r and R^g/R^r . Of the 33 r^r mutants observed in the heterozygote, 17.5 will be noncrossovers and here, 15.5 will be crossovers. Thus the proportion of r^r crossovers to the total number of r^r mutants in the heterozygote becomes 15.5/33 (or 47 percent). The reduction in gene mutation rate of the element (S) is from 8.33×10^{-4} prior to mutation of (P) to 2.84×10^{-4} following mutation of (P).

Applying the assumptions from hypothesis C to the data in table 4, the number of noncrossover mutants of R^r in R^g/R^r plants becomes 28.2, since the mutation rate of R^r in R^r/R^r plants is 16.05×10^{-4} . Thus the observed number of 61 r^r mutants in R^g/R^r plants will include 32.8 r^r crossovers and 28.2 r^r noncrossovers. The proportion of r^r mutations ascribed to unequal crossing over becomes 32.8/61 (or 54 percent). In this case, the reduction in gene mutation rate of the element (S) is from 16.05×10^{-4} to 1.13×10^{-4} . Thus the gene mutation rate of R^r may be much

higher than that of R^a -14, even though the apparent increased rate of R^r mutations in R^a -14/ R^r is due to crossing over.

DISCUSSION

Three hypotheses have been proposed to account for the lowered mutation rate of R^a -14 in the heterozygote R^a -14/ R^r and the pronounced increase in r^r mutation frequency of R^a -14/ R^r over R^r / R^r .

The data reported on the compound $G R^a$ -14 $k/g R^r K$ (table 1) give little support to hypothesis A, which ascribes the effects of R^a -14 to a change in mutability of (S). Of the 12 r^r seed-color mutants found, six were r^r crossovers. This experiment indicates that the increased frequency of r^r mutations in the heterozygote is due largely to the occurrence of unequal crossing over.

The results, however, do not differentiate between hypotheses B and C. In order to distinguish between these two hypotheses, data are required on the relative mutation frequency of r^r mutations in knob-marked comparisons of R^a -14/ R^r and R^r / R^r sibs. These results are not yet available, but some indication of the percentage of r^r mutations due to unequal crossing over may be obtained from back-cross progeny unmarked for the determination of unequal crossing over.

Under the hypothesis B, which assumes no change in the stability of (S) following loss of (P), but rather implies that the effects are due entirely to unequal crossing over, the gene mutation frequency of R^r would be as low as that found in R^a -14. This is illustrated in table 5 with data from tables 3 and 4. In table 3 the expected percentage of crossovers among r^r mutations would be 82 percent in the heterozygote and 67 percent in the homozygote. The proportion of unequal crossovers becomes still higher when data from table 4 are considered. In this case the percentage of unequal crossovers would include 97 percent of the r^r mutations in the R^a -14/ R^r plants and 94 percent in the homozygous R^r sibs. Therefore, this hypothesis would seem to be questionable for the reason that it requires an unreasonably high frequency of unequal crossing over.

Hypothesis C assumes that the stability of (S) is affected both by mutation or loss of (P) and by unequal crossing over. This hypothesis implies that the frequency of unequal crossing over among mutants in the R^a / R^r class will be lower, to an extent corresponding to the increased frequency of noncrossover mutation of R^r / R^r . Its minimal value may be calculated by using the mutation rate of R^r / R^r determined in accounting for the r^r noncrossover mutations from the R^a -14/ R^r class. In table 4 with a high rate of R^r / R^r mutation (16.05×10^{-4}), the proportion of r^r mutants due to unequal crossing over need not be high (i.e. 32.8/61 or 54 percent), while the assumption of full effect would require an extremely high percentage (97 percent). Thus it appears more likely that many of the r^r mutants in the R^a -14/ R^r plants represent gene mutations rather than unequal crossovers. At the present time it cannot be concluded that hypothesis C is more valid than hypothesis B, since hypothesis C is based on the assumption that unequal crossing over is absent in the homozygote R^r / R^r . Data already at hand indicate that some unequal crossing over occurs in the homozygote.

A more definite basis for estimating the reduction in gene mutation rate for the

(S) element may be obtained from a direct comparison of noncrossover r^r mutation frequency in $G R^r K/g R^r k$ and r^o noncrossover mutation frequency in $G R^o-14 K/g R^o-14 k$ sibs. The M_G effect may also be measured directly in $G R^o-14 k/g R^r K$ and $G R^r k/g R^r K$ sib comparisons. Both of these tests are now in progress.

If hypothesis C were valid, the question might be asked, why should the compound $(-S)/(PS)$ give more frequent crossovers than $(PS)/(PS)$? The high frequency of unequal crossing over could be due to the mode of pairing in $(-S)/(PS)$ compound. Pairing initiated proximally would, in the R^o/R^r compound, result in PS with opportunity for crossing over, while pairing initiated distally would result in $\overline{S}$ in PS without this opportunity. Both types, in the R^r/R^r compound, would result in $\overline{S}$ in PS . In the latter compound, therefore, unequal pairing would be expected only when pairing is initiated at the (S) or (P) locus.

An additional feature of considerable interest in the analysis of R^o-14 is the indication that the gene R^o-14 is deficient for the element (P). This type would be expected to result from a crossover mutation in the parent R^r Cornell allele. It does not necessarily follow that noncrossover mutations would be all (pS), since the loss of (P) could be due to some mechanism which is independent of crossing over. If, however, R^o crossovers differ from R^o noncrossovers by the physical loss rather than by recessive mutation of element (P), it may be possible to demonstrate by the unequal crossover mutants resulting from pairing differences the presence of an element (p) in the R^o noncrossovers and its absence in R^o crossover alleles. This would also provide a criterion for distinguishing between gene mutation and physical loss in other R^o mutations, specifically those induced by irradiation. Several R^o mutants of known crossover origin are being tested at the present time.

SUMMARY

The occurrence of unequal crossing over in the $G R^o-14 k/g R^r K$ compound indicates that the R^o-14 gene is deficient for the element (P). Presumably the plant-color element was lost by the occurrence of unequal crossing over in the parent allele.

Three alternative hypotheses were proposed to account for the peculiarities of R^o-14 behavior which indicated both the lowered mutation rate of the element (S) and the M_G effect. Hypothesis A implied that the peculiarities of R^o-14 were due to a change in mutability of the element (S) while in B they were due to unequal crossing over and in C to unequal crossing over as well as a change in mutability of (S).

Hypothesis A was excluded since 50 percent of the r^r mutants from the $G R^o-14 k/g R^r K$ compound were unequal crossovers. Application of hypothesis B to backcross data showed an unreasonably high frequency of unequal crossovers in both the R^o/R^r and R^r/R^r plants while hypothesis C demonstrated a moderate frequency of unequal crossovers in R^o/R^r and R^r/R^r sibs. These data, however, do

not permit a definite choice between hypotheses B and C since the stocks were unmarked for the detection of unequal crossovers.

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