RECONSTITUTION OF THE R^r COMPOUND ALLELE IN MAIZE[1]

H. K. DOONER AND J. L. KERMICLE

Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706

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ABSTRACT

The R^r:standard allele in maize, which conditions anthocyanin pigmentation in plant and seed tissues in the presence of appropriate complementary factors, is associated with a tandem duplication. The proximal member of the duplication carries *P*, the plant pigmenting determiner and the distal member member carries *S*, the seed pigmenting determiner. Derivatives from R^r that have lost *S* function are designated r^r. They represent either losses of the distal member of the duplication (*P* derivatives) or mutations of *S* to *s* (*P s*). Derivatives that have lost *P* function are designated R^g, and represent either losses of the proximal member of the duplication (*S* derivatives) or mutations of *P* to *p* (*p S*).——All four possible types of r^r/R^g heterozygotes were tested for their capacity to yield R^r reconstitution by crossing over. No R^r derivatives were obtained from *P/S* heterozygotes, a result consistent with the view that *P* and *S* occupy corresponding positions in homologous chromosome segments. R^r reconstitution was detected in both tandem duplication heterozygotes *P s/S* and *P/p S*, and was found to be about ten times more frequent in the latter. The ratio of R^r reconstitution in the two heterozygotes is a function of position of the anthocyanin marker within the duplicated segment. The data from these heterozygotes allow one to measure the distance between *P* and *S*, that is to say, the genetic length of the duplicated segment. This distance was found to be 0.16 map units. The highest frequency of R^r reconstitution was obtained from *P s/p S* heterozygotes, since direct pairing $\left(\frac{P}{p}\ \frac{s}{S}\right)$ as well as the *p//s* type of displaced pairing have the potential to produce R^r derivatives. One of the R^g derivatives used in this study, $R^g{}_6$, was found to back-mutate in some sublines to R^r. The basis for this instability remains unknown.

RECOMBINATIONAL analysis of complex loci in maize has revealed in several instances the presence at the locus of a small cytologically undetected, tandem duplication. (LAUGHNAN 1952; STADLER and NUFFER 1953; MANGELSDORF and GALINAT 1964). One such complex locus is *R*, which conditions anthocyanin pigmentation in various plant parts and in the aleurone layer of the mature seed. R^r symbolizes an allele that elicits pigment formation in both plant (seedling parts and anthers) and seed. *R* factors that lack the capacity to pigment either plant or seed tissues, or both, are designated as follows (EMERSON 1921): R^g (colored seed, green plant), r^r (colorless seed, red plant), r^g (colorless seed, green plant).

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Evidence obtained by STADLER and his associates (STADLER 1942; STADLER and NUFFER 1953; STADLER and EMMERLING 1956) led to the conclusion that R^r:Cornell was a compound allele comprising a plant pigmenting determiner P which was borne proximally and a seed pigmenting determiner S borne distally in association with a tandem duplication. DOONER and KERMICLE (1971) presented evidence in favor of the identity of R^r:Cornell and the allele utilized by them in their studies, R^r:standard. Data from R^r:standard homozygotes (tandem duplication homozygotes) and from heterozygotes of R^r:standard and certain of its R^g derivatives (tandem duplication homozygotes and heterozygotes) allowed them to establish the relative position of the genes P and S within the corresponding members of the duplication and to measure the genetic length of the duplication.

A consequence of the duplication model is that it should be possible to synthesize R^r, i.e., $P\ S$, from its component elements P and S. Yet, the scattered attempts at reconstituting R^r mentioned in the literature (EMERSON, in STADLER 1946; HAHN, in STADLER 1951) met with failure. A main purpose of the work reported here was to resynthesize R^r from its appropriately characterized R^g and r^r derivatives in R^g/r^r heterozygotes. The new data were used to place more precisely than heretofore the anthocyanin marker in each member of the duplication and to obtain an independent estimate of the genetic length of the duplication. The values obtained were found to agree closely with those reported earlier (DOONER and KERMICLE 1971).

MATERIALS AND METHODS

Description of R *alleles:* R^r:standard is an allele given to R. A. BRINK by the late R. A. EMERSON. It was then incorporated into the Wisconsin W22 inbred strain in which it has been maintained over many generations by self-pollination. R^r:standard exhibits all the properties of alleles belonging to group A (STADLER 1948) characterized by strong pigmentation in the roots, coleoptile, and leaf-tip of seedlings, the anthers of the mature plant, and the aleurone layer of the seed. In terms of the duplication model for the R locus, it can be represented as $P\ S$.

Numerous R^g and r^r derivatives have been isolated from the R^r:standard allele. Such derivatives may have retained both members of the duplication, in which case they are designated as $p\ S$ and $P\ s$, respectively, or they may have lost one member, the respective representations in this case being S and P. STADLER and EMMERLING (1956) devised a test to detect the presence or absence of p in R^g derivatives of R^r, which is based on whether $R^r/R^g{}_m$ heterozygotes yield a recombinant r^g class carrying the distal marker of R^r (see Figure 1). This test has been applied to $R^g{}_6$ and $R^g{}_1$, the two R^g derivatives used in the present study, by BRAY and BRINK (1966) and DOONER and KERMICLE (1971), respectively. (The subscript indicates the number of the R^g isolate from R^r:standard.) $R^g{}_6$ was found to have retained the duplication, and so is designated $p\ S$. $R^g{}_1$, on the other hand, has lost the duplication and is therefore of S constitution (see Table 1).

Only recently has a test been developed that establishes whether r^r derivatives from R^r:standard have retained the duplication, and so must be designated as $P\ s$, or have lost one member, and therefore, are P in constitution. This test (AXTELL and KERMICLE, unpublished) makes use of the presence in each member of the duplication of a factor that inhibits expression of the white leaf striping phenotype conditioned by sr_2 (striate-2, 10L, close to the distal end), and also causes a pronounced narrowing of the leaves. Chromosomes having 0, 1, or 2 doses of inhibitor (provisionally designated I^{sr}) can be readily distinguished, i.e., the inhibitor shows a marked dosage effect.*

* EMERSON, BEADLE and FRASER (1935) have called attention to an interaction between alleles of R and chlorophyll striping conditioned by *iojap* (chromosome 7) and by *japonica*-1 (chromosome 8).

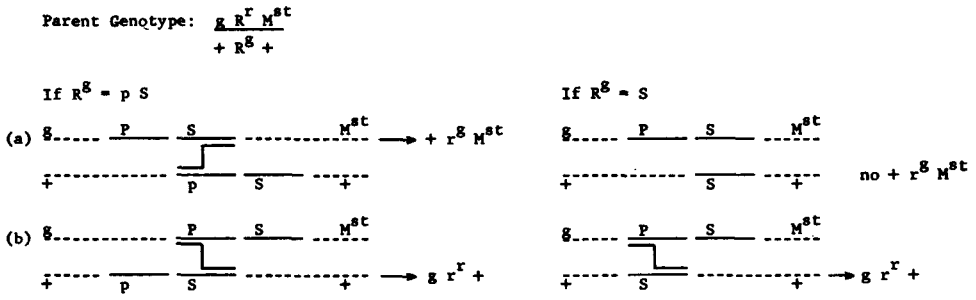


FIGURE 1.—Types of crossover colorless seed derivatives that can arise in R^r/R^g heterozygotes (adapted from STADLER and EMMERLING 1956).

The origin, composition according to the duplication model, and sr_2 interaction of the r^r derivatives used in the present study are given in Table 1. The abbreviations NCO and CO used in this table stand for noncrossover and crossover origin, respectively. The r^r derivatives n-35, n-101, n-19, and n-46 came from marked R^r/R^g_2 heterozygotes. The first two retained the R^r parental combination of outside markers, whereas the latter two had the proximal marker of R^r and the distal marker of R^g_2. The r^r (n-156) allele was obtained from a marked R^r homozygote and had both outside markers of one of the parental R^r strands. The r^r (N1–3–2) allele originated in a plant that was hemizygous for the entire marked region of chromosome 10. It carried, of course, the two markers borne by the R^r chromosome.

The two r^r derivatives that carried recombined outside markers (n-19 and n-46) would be dissociated from the duplication of R^r:standard if they originated by an exchange of the sort depicted in Figure 1 for *P S/S* heterozygotes. Their effect on sr_2 expression supports this mode of origin. Each of the two r^r cases modifies striping to the same extent as the (*S*) parent (one I^{sr} dose) rather than as the strongly inhibiting (*P S*) parent (two I^{sr} doses). The four r^r derivatives that bore a combination of flanking markers contributed by parental R^r:standard, on the other hand, inhibit striping to the same degree as R^r. At a gross level, therefore, the duplication was

TABLE 1

The origin, behavior in the sr_2 *interaction test, and composition of* R *and* r *alleles used in the* R^r *reconstitution study*

Allele	Origin	Number of I^{sr} units	Composition according to duplication model	
R^r:std	collected*	2	*P*	*S*
R^g_6	unmarked R^r/R^r	2	*p*	*S*
R^g_1	unmarked R^r/R^r	1		*S*
R^g_2	unmarked R^r/R^r	1		*S*
r^r(n–35)	marked R^r/R^g_2;NCO	2	*P*	*s*
r^r(n–101)	marked R^r/R^g_2;NCO	2	*P*	*s*
r^r(n–156)	marked R^r/R^r;NCO	2	*P*	*s*
r^r(N1–3–2)	$R^r/10^B$;NCO	2	*P*	*s*
r^r(n–19)	marked R^r/R^g_2;CO	1	*P*	
r^r(n–46)	marked R^r/R^g_2;CO	1	*P*	
r^r(W22)	inbred W22	0	*P*	

* Originally provided by R. A. EMERSON of Cornell University.

retained; the four represent instances of presumed *S* mutation. r^r(W22) is the *R*-locus allele carried in the commercial inbred line, Wisconsin 22. Although it possesses *P* function, it does not display I^{sr} activity. It could represent a partial deletion of that segment duplicated in the case of R^r:standard.

Markers: The markers used in this study were *g* (golden plant), which mapped 17.3 units proximal to *R*, and M^{st}, which mapped 6.2 units distal to *R*. M^{st} modifies the aleurone stippling pattern conditioned by R^{st}: in its presence R^{st} gives darkly stippled kernels, whereas in its absence, R^{st} seeds are lightly stippled. (Map distances were obtained from the cross $+ R^r + / g\ R^g{}_6\ M^{st}$ ♀♀ × $g\ r^g + ♂♂$. Interference = 0.7).

Selection and analysis of putative R^r *derivatives from* R^g/r^r *and* R^g/R^g: Appropriately marked R^g/r^r heterozygotes and R^g homozygotes were pollinated with $g\ r^g +$; *wx*. Since R^r conditions colored seed and colored plant and seedling, selection of putative R^r cases requires seedling screening of a selected kernel class. Accordingly, ears from the above cross were shelled individually and the colored kernels planted as separate families in sand flats in the greenhouse. During germination, temperature in the greehouse was kept at 27°, but since low temperature favors anthocyanin formation, the greenhouse temperature was lowered to 21° when the emerging coleoptile had reached a height of about two cm. From then on, a 21°–15° day-night temperature differential and continuous illumination were maintained until the time of screening. Seedlings with pigment in the coleoptile and in the tip of the first seedling leaf were selected as putative R^r derivatives. Selections yielding red-anthered plants were evaluated for the proximal marker (*g*) and either self-pollinated or backcrossed to $g\ r^r +$; *wx*. Authentic R^r derivatives were identified on the basis of their progeny segregation for $R^r : r^g$ and $Wx : wx$. Presence or absence of M^{st} was determined through crossing to an R^{st} stock not carrying M^{st}.

Selection and analysis of putative R *derivatives from* r^r *homozygotes:* Homozygous combinations of all six r^r derivatives used in the reconstitution studies were placed in a $g\ r^g +$; *wx* detasselling plot. The few colored kernels found among the colorless kernel progenies were field planted, scored for anther color and backcrossed to $g\ r^g +$; *wx* to test for $R^r : r^g$ and $Wx : wx$ segregation.

Calculation of frequencies: The effective gametic populations utilized in estimating frequencies were calculated by correcting the total number of kernels or seedlings screened by the proportion of selections successfully tested.

RESULTS AND DISCUSSION

Combinations of various r^r and R^g alleles in heterozygotes were used in attempts to reconstitute R^r by crossing over. The r^r allele would presumably contribute *P*, the plant pigmenting determiner, whereas the R^g allele would contribute *S*, the seed pigmenting determiner. Tables 2 and 3 contain a summary of the R^r reconstitution results, indicating the pertinent genotype of the R^g/r^r heterozygotes used, the *R* locus constitution of the r^r and R^g alleles, the outside markers of the derived R^r strands, and the frequency of R^r reconstitution. Since R^g_6 was found unexpectedly to back-mutate to R^r Table 3 includes a column with the heading "R^r back mutation".

a) P/S *heterozygotes:* Neither of the r^r/R^g heterozygotes of *P/S* constitution tested yielded R^r derivatives. A total of 38,400 seedlings were screened and no true cases of R^r reconstitution were obtained (Table 2). Although the two genes necessary to reconstitute R^r are present in these heterozygotes, there is no opportunity for displaced pairing to occur. *P* and *S* therefore are not brought together into one chromosome by crossing over. In order to reconstitute a *P S* strand, at least one of the parental alleles must retain the duplication.

b) P s/S *and* P/p S *heterozygotes:* As can be seen in Tables 2 and 3, R^r deriva-

TABLE 2

R^r *derivatives obtained from* Rg_1/r^r *heterozygotes crossed to* g r^g + ; wx *males*

Genotype	R-locus constitution	Population	Outside marker composition				Total no. R^r	Freq. of R^r reconstitution ($\times 10^{-4}$)
g Rg_1 M^{st} / + r^r(n-46) +	S / P	11,400	0	0	0	0	0	
+ Rg_1 M^{st} / g r^r(n-19) +	S / P	27,000	0	0	0	0	0	
Subtotal	S / P	38,400	0	0	0	0	0	NIL
+ Rg_1 + / g r^r(n-35) M^{st}	S / P s	29,600	0	0	0	1	1	
+ Rg_1 + / g r^r(n-101) M^{st}	S / P s	26,000	0	0	0	3	3	
g Rg_1 M^{st} / + r^r(n-156) +	S / P s	13,400	1	0	0	0	1	
g Rg_1 M^{st} / + r^r(N1-3-2) +	S / P s	27,500	0	0	0	2	2	
Subtotal	S / P s	96,500	1	0	0	6	7	0.7

TABLE 3

R^r *derivatives obtained from* Rg_6/r^r *heterozygotes crossed to* g r^g+ ; wx *males*

Genotype	R-locus constitution	Population	Outside marker constitution a	b	c	d	R^r back mutation* No.	Freq. ($\times 10^{-4}$)	R^r reconstitution* No.	Freq. ($\times 10^{-4}$)
g Rg_6 M^{st} / + r^r(n-46) +	p S / P	22,900	25	1	4	21	34.7	15.1	16.3	7.1
g Rg_6 + / + r^r(W22) M^{st}	p S / P	21,300	17	1	2	11	22.3	10.5	8.7	4.1
+ Rg_6 M^{st} / g r^r(W22) +	p S / P	11,300	18	0	0	7	22.0	19.5	3.0	2.7
Subtotal (P-W22)	p S / P	32,600	35	1	2	18			11.7	3.6
+ Rg_6 + / g r^r(n-101) M^{st}	p S / P s	33,200	1	1	0	38	0	NIL	40	12.0
g Rg_6 M^{st} / + r^r(N1-3-2) +	p S / P s	38,200	46	2	2	35	56.9	14.9	28.1	7.3

* See text for the method utilized in the computation of these values.

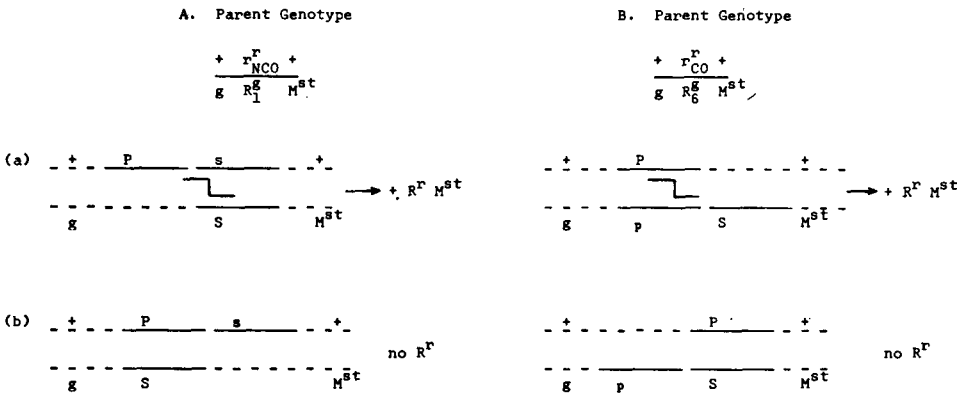


FIGURE 2.—Types of interhomologue pairing that are possible in the two kinds of duplication heterozygotes *P s/S* and *P/p S* (r^r_{NCO}/R^g_1 and r^r_{CO}/R^g_6). Note that given pairing type (a), an exchange within the homologous segment between *P* and *S* yields a CO R^r.

tives were obtained from both *P s/S* and *P/p S* heterozygotes. The preponderance of derivatives obtained from *P s/S* (r^r_{NCO}/R^g_1) heterozygotes had the proximal marker of the *P*-bearing strand and the distal marker of the *S*-bearing strand, as would be anticipated from the duplication model (Figure 2A). One of two major classes of R^r derivatives obtained from *P/p S* (r^r_{CO}/R^g_6) heterozygotes was similarly marked (Figure 2B). The second was nonrecombinant, bearing both of the markers flanking *p S*. Subsequent study of R^g_6 homozygotes showed that in the case of certain R^g_6 substrains, *p* back-mutates to *P* in the germ line (Table 5).

TABLE 4

Back mutation to R^r *in* r^r *and* R^g_1 *homozygotes*

Genotype	*R*-locus constitution	Population	R^r no.
g R^g_1 +	*S*	7,400	0
g r^r(n-19) +	*P*	42,800	0
+ r^r(n-46) +	*P*	47,000	0
g r^r(n-35) M^{st}	*P s*	27,100	0
g r^r(n-101) M^{st}	*P s*	44,000	0
+ r^r(n-156) +	*P s*	49,300	0
+ r^r(N1-3-2) +	*P s*	60,800	0

TABLE 5

Back mutation rates and their 95% limits of expectation in different lines of R^g_6

R^g_6	Effective population	No. R^r	Freq. ($\times 10^{-4}$)	Limits of expectation Lower No. R^r	Lower Freq.	Upper No. R^r	Upper Freq.
+ R^g_6 +	39,450	0	NIL	0	NIL	3.69	0.94
g R^g_6 +	50,500	54	10.7	40.56	8.03	70.47	13.95
+ R^g_6 M^{st}	19,550	49	25.1	36.27	18.70	64.76	32.90
g R^g_6 M^{st}	8,370	14	16.7	7.64	9.14	23.51	28.12

Therefore, R^r derivatives from R^g_6/r^r heterozygotes which carry the two outside markers of the R^g parental strand represent principally cases of back mutation of p of R^g_6 to P. Hence, a means of partitioning total R^r occurrences into those attributable to back mutation as compared with resynthesis by crossing over was developed, as follows.

If, coincident with R^r reconstitution or back mutation, an exchange may occur in either the proximal (g-R) or the distal (R-M^{st}) region, then six modes of R^r chromosome formation are defined. The six modes are allocated to the four flanking marker combination classes designated in the heading of Table 3 as follows: Column a comprises mostly back mutations, but should also include any case of R^r reconstitution having a second exchange in the proximal region. Column b represents R^r derivatives arising from reconstitution and a simultaneous exchange in the distal region. Column c represents instances of back mutation accompanied by an exchange in the distal region. Finally, column d includes the majority of R^r reconstitution cases and instances of back mutation having a proximal exchange.

The relative contributions of back mutation and recombination to each of the four outside marker classes can be calculated from the distribution of markers in combinations in which R^r back mutation or reconstitution is not observed. Where back mutation did not occur (R^g_1/r^r and $+\ R^g_6\ +/g\ r^r$(n-101)M^{st}), the following marker class distribution of R^r cases was found:

Marker class	a	b	c	d	Total
No. R^r cases	2	1	0	44	47

Based on three-point linkage information for the g-R-M^{st} region (cited in MATERIALS AND METHODS) the probability that any given case of back mutation (presumably recombination-independent) will fall under a particular marker class is:

Marker class	a	b	c	d
Probability	0.77	0.0	0.06	0.17

For any R^g_6/r^r combination in which both reconstitution and back mutation are occurring, the probability of an R^r falling in classes a, b, c or d can be expressed in terms of X, the fraction of R^r's from that combination which are due to reconstitution, namely,

$$\begin{aligned} a' &= 2/47X + 0.77(1-X) &&= 0.77-0.727X \\ b' &= 1/47X + 0(1-X) &&= 0.021X \\ c' &= 0/47X + 0.06(1-X) &&= 0.06(1-X) \\ d' &= 44/47X + 0.17(1-X) &&= 0.17 + 0.766X \end{aligned}$$

A combined estimate of X is obtained by the method of maximum likelihood. Application of this method to the data of Table 3 yields the following solutions:

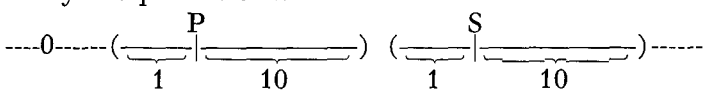
$g\ R^g_6\ M^{st}/+\ r^r$(n-46) $+$	$236.76X^3-311.50X^2+72.36+1.06=0$;	$X=0.32$
$g\ R^g_6\ +/+\ r^r$(W22)M^{st}	$144.40X^3-118.28X^2+35.16X+1.06=0$;	$X=0.28$
$+R^g_6\ M^{st}/g\ r^r$(W22) $+$	$117.61X^2-131.99X+14.38=0$;	$X=0.12$
$g\ R^g_6\ M^{st}/+\ r^r$(N1-3-2)$+$	$395.77X^3-520.41X^2+121.86X+2.12=0$;	$X=0.33$

The entries under the heading "R^r reconstitution" were obtained by multiplying these fractions by the total number of R^r cases from the respective combinations. The entries in the "R^r back mutation" column were computed by subtracting the adjusted number of reconstitutions from the total number of R^r cases.

The frequency of R^r reconstitution in $P\ s/S$ heterozygotes differs by an order of magnitude from that obtained in $P/p\ S$ heterozygotes (Table 2 and 3). This difference should reflect the position of the gene markers P and S in the duplicated segment. DOONER and KERMICLE (1971) used data obtained from R^r/R^r homozygotes to establish the relative position of the anthocyanin markers P and S toward the proximal end of the duplicated segment. Figure 2 depicts diagrammatically the types of interchromosomal pairing that are possible in $P\ s/S$ and $P/p\ S$ heterozygotes and the types of exchange that give rise to crossover R^r derivatives. Given pairing type (a) an exchange proximal to the genes in the duplicated segment yields a crossover (CO) R^r in $P\ s/S$ but not in $P/p\ S$ heterozygotes. Conversely, an exchange distal to the genes results in a CO R^r only in $P/p\ S$ heterozygotes. Therefore, since pairing type (a) should occur with equal frequency in both types of heterozygotes, the ratio of R^r reconstitution frequency from $P\ s/S$ heterozygotes to that from $P/p\ S$ heterozygotes gives the relative lengths of the duplicated segment that lie distally and proximally to the anthocyanin marker.

$$\frac{\text{CO } R^r \text{ from } P\ s/S}{\text{CO } R^r \text{ from } P/p\ S} = \frac{0.7 \times 10^{-4}}{7.1 \times 10^{-4}} = \frac{1}{10}$$

This ratio may be represented as:



A previous estimate of this ratio, obtained from the relative number of CO R^g to CO r^r derivatives from R^r/R^r homozygotes, was 1:16 (DOONER and KERMICLE 1971). The value of 1:10 given here should be more reliable since the relatively few CO R^g derivatives obtained in the previous study limited the precision of the estimate. Because there exists general agreement between the two estimates and because the estimate obtained from R^r/R^r homozygotes requires no assumptions about types and frequencies of pairing, the assumption that pairing type (a) occurs with equal frequency in $P/p\ S$ and $P\ s/S$ heterozygotes appears to be justified.

That pairing type (a) occurs with equal frequency in $P/p\ S$ as in $P\ s/S$ would follow as a corollary if alternative pairing types (a) and (b) in these heterozygotes occurred with equal frequency. Such an assumption is not without observational precedents. LAUGHNAN (1952) proposed that the low recovery of $\alpha\ a$ strands, relative to α strands, in $A^b/a\ (\alpha\ \beta/a)$ heterozygotes in maize could be simply explained in terms of the position of α, β, and a in their respective segments, if one assumed that $\alpha//a$ and $\beta//a$ pairing occurred equally frequently. GREEN (1966) found that differential recovery of markers carried in the right and left halves of a direct tandem duplication in duplication/standard females of *Drosophila melanogaster* could be explained in terms of the position of the genetic

marker in the duplication, provided the two types of interchromosomal pairing possible in these heterozygotes occurred equally frequently. This assumption makes it unnecessary to postulate preferential or polarized pairing as a cause of the differential frequency of recovery.

Whether only pairing types (a) and (b) are possible in tandem duplication heterozygotes depends on the possibility of intrachromosomal pairing between the proximal and distal members of the duplication, as has been envisioned by LAUGHNAN (1961). DOONER and KERMICLE (1971) found no evidence for the occurrence of this type of pairing in *P S/S* heterozygotes, and concluded that the frequency of intrachromosomal *P//S* pairing in such heterozygotes could be taken to be negligible relative to the frequency of interhomolog *P//S* pairing. If this conclusion is accepted, the frequencies of pairing types (a) and (b) in tandem duplication heterozygotes become 0.5. This value is used in estimating the genetic length of the *R* duplicated segment from the R^r reconstitution data.

Cases of R^r reconstitution from *P s/S* and *P/p S* heterozygotes arise following (1) a type of pairing occurring with a frequency of 0.5, and (2) an exchange in the duplicated segment. The frequency of R^r reconstitution in *P s/S* heterozygotes, representing derivatives arising from an exchange proximal to the anthocyanin marker in the duplicated segment, is 0.7 per 10^4 colored kernels tested. Similarly, the frequency of R^r reconstitution in *P/p S* heterozygotes, representing derivatives arising from an exchange distal to the gene in the duplicated segment, is 7.1×10^{-4}.

An expression for estimating genetic length of that portion of the duplicated segment lying proximal to the anthocyanin gene then becomes:

$$0.7 \times 10^{-4} \times 1/0.5 \times 100 = 0.014$$

and of that portion lying distal to the anthocyanin gene:

$$7.1 \times 10^{-4} \times 1/0.5 \times 100 = 0.142 .$$

The total genetic length of one element of the duplication is: $0.142 + 0.014 = 0.156$. This value compares with 0.16, an estimate obtained by DOONER and KERMICLE (1971) utilizing fractionation, rather than resynthesis, of the R^r complex.

Table 3 also gives the frequency of R^r reconstitution obtained from R^g_6 heterozygotes with r^r(W22), the *R*-allele carried by the commercial inbred line W22. This r^r is peculiar in that while having retained *P* function it has an effect on sr_2 expression equivalent to *R*-locus deficiencies. It may, therefore, represent a deletion of part of the duplicated segment. The frequencies of R^r reconstitution in two different R^g_6/r^r (W22) heterozygotes were found to be 4.1 and 2.7 per 10,000 gametes. The weighted average of 3.6×10^{-4} compares with an R^r reconstitution frequency of 7.1×10^{-4} in R^g_6/r^r(n-46) heterozygotes. The probability that this difference is due to chance alone is less than 0.05. Thus, it appears that the absence in r^r(W22) of a portion of the R^r duplicated segment that includes I^{sr} causes a significant reduction in the frequency of recombination within the *R*-locus. Similarly, a reduction in separation of components of the *R*-stippled complex in r^r(W22) heterozygotes has been reported (KERMICLE 1970).

Since the deletion of I^{sr} amounts in a functional sense to a recessive mutation,

it should be possible to place I^{sr} relative to P and S within the duplicated segment by applying the sr_2 test to the reconstituted R^r's derived from R^g_6/r^r (W22). Such a test is now in progress.

c) P s/p S *heterozygotes:* The frequency of R^r reconstitution in one of the two P s/p S heterozygotes studied (penultimate line of Table 3) was found to be higher than in either P s/S or P/p S heterozygotes. At least half of the possible pairing configurations in P s/S or P/p S heterozygotes cannot yield CO R^r derivatives. In P s/p S combinations, where both the R^g and r^r parental strands carry the duplication, R^r reconstitution by crossing over can result from the direct pairing configuration $\frac{P}{p}\ \frac{s}{S}$ and from displaced pairing of the type $\frac{P}{\ }\ \frac{s}{p}\ \frac{\ }{S}$. However, neither the second sort of displaced pairing, namely $\frac{\ }{p}\ \frac{P}{S}\ \frac{s}{\ }$, nor intrachromosomal association (pairing of P with s and of p with S) can produce CO R^r derivatives. Since the two types of displaced pairing should occur equally frequently, and since the frequency of intrachromosomal, relative to interchromosomal, association is low in R^r duplication homozygotes (DOONER 1971), considerably more than half the possible pairing events in P s/p S heterozygotes have the potential to yield CO R^r derivatives. These relations account for the higher frequency of R^r reconstitution in P s/p S combinations than in P s/S or P/p S heterozygotes.

It should be noted that of the two P s/p S combinations studied, R^g_6/n^r(n-101) and R^g_6/r^r(N1–3–2), the latter yielded a lower frequency of R^r reconstitution $X^2 : 1\ df = 4.16;\ P<0.05$). Conceivably, r^r (N1–2–3)—which arose in R^r hemizygotes—may represent an instance of (S) "mutation" by chromosome segment loss. This r^r derivative retains the effect of the parental R^r on sr_2 expression, indicating retention of the duplication. However, r^r(N1–3–2) could have arisen by loss of a chromosome segment containing S and a portion of the duplication, but neither of the two I^{sr} loci. Also retained necessarily was the R^r duplication junction, since S/P s combinations involving r^r(N1–3–2) yielded instances of R^r resynthesis.

d) *Back-mutation to* R^r *in* r^r *and* R^g_1 *homozygotes:* Table 4 gives the frequencies of back mutation to R^r of R^g_1 and the six r^r derivatives utilized in this study. All these derivatives were found to be stable in homozygotes, i.e., no cases of back mutation were detected in populations as large as those screened from R^g/r^r heterozygotes. This finding is in agreement with the observation that R^r derivatives obtained from R^g/r^r heterozygotes seldom have a parental combination of outside markers. They have, instead, the proximal marker of r^r and the distal marker of R^g, the flanking marker combination expected on the basis of a recombinational, rather than mutational, mode of origin.

e) *Back mutation to* R^r *in* R^g_6 *homozygotes:* As was mentioned earlier, R^g_6 was found quite unexpectedly to back-mutate to R^r. Although the precise nature of R^g_6 instability is not known, a peculiarity of its instability which is pertinent to the present discussion should be mentioned. Several R^g_6 sublines have been derived and maintained by selfing since the original isolation of R^g from R^r. Back-muta-

tion tests were made on four of these sublines which carry different outside marker combinations. As can be seen in Table 5, R^g_6 behaves as a stable derivative and does not back-mutate to R^r in one of the four sublines ($+ R^g_6 +$). This subline was used to synthesize the R^g_6/r^r(n-101) heterozygotes reported in Table 3 as producing no apparent cases of R^g_6 back-mutation. In the three other sublines tested, R^g_6 was found to be highly unstable, yielding from 1 to 2.5 R^r back mutations per 1000 gametes. This is the type of high instability that has come to be associated in maize with "mutable" genes, but the presence at R^g_6 of a controlling element has yet to be established.

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