PHENOTYPIC PROPERTIES OF HETEROZYGOTES IN THE BACTERIOPHAGE T4[1, 2]

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THE *r*II region of the genome of the bacteriophage T4 has been particularly useful for the investigation of mutation (BENZER 1955, 1957), recombination (CHASE and DOERMANN 1958), and gene function (KRIEG 1957). This is largely due to the differential response of phage mutant and nonmutant in this region to the bacterium *Escherichia coli* K12 (λ). While *r*II$^+$ phage may infect K12 (λ) with a subsequent liberation of progeny, *r*II phage absorb to and kill K12 (λ), but the complex does not produce any infectious progeny.

By using mixed infections with different combinations of phage genotypes, BENZER (1955) was able to show that the *r*II region of the T4 genome consists of two adjacent but autonomously functioning segments. These segments he designated the A and B cistrons (BENZER 1957). He found that K12 (λ) releases no phage when simultaneously infected with two different *r*A or with two different *r*B mutants, whereas infections of K12 (λ) with both an *r*A and an *r*B mutant do result in the production of phage progeny (BENZER 1955).

It therefore appears that both an unmutated A cistron and an unmutated B cistron must be present in an infected K12 (λ) bacterium for the production of mature phage progeny. Since the genetic material of phage heterozygotes might have a structural organization differing from the presumed "diploidy" of a mixed infection, it is of interest to know if both structural conditions display similar phenotypic properties.

From crosses of T2 involving a variety of markers, HERSHEY and CHASE (1951) found that two percent of the progeny particles recovered were heterozygous, i.e., showed subsequent segregation for a given pair of alleles. A heterozygote rarely segregates for more than one pair of alleles, except in cases of close linkage. Heterozygous *r*II markers in T4 also show this type of segregation pattern, and likewise are recognizably diploid only for short regions of the genome (DOERMANN and KATAJA 1957). LEVINTHAL (1954) has presented evidence that the heterozygotes are intermediate stages in recombinant formation and that they are

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usually recombinant for regions on either side of the heterozygous, or "overlap," region. The rest of the genome may be either hemizygous (Figure 1a), or diploid but homozygous (Figure 1b). These two alternative models are indistinguishable on the basis of the existing data. The most probable kinds of heterozygotes arising in crosses involving two closely linked *r*II markers are depicted in Figure 2. The functional properties, with respect to growth in K12 (λ), of two of these types, the single, or r^+ recombinant heterozygote (Figure 2a) and the nonrecombinant heterozygote (Figure 2c) are examined in this paper.

LEVINTHAL (1954) suggested that the phage genome might be little more than a DNA molecule and that in a heterozygous region, the two complementary chains (WATSON and CRICK 1953) may be derived from different parents. If this were true, it might be imagined that heterozygotes represent an association more intimate than that afforded by two homologous chromosomes within a nucleus, and might display phenotypic properties differing from those of corresponding mixed infections.

However evidence will be presented in this paper which indicates that heterozygotes display phenotypic properties similar to those of corresponding mixed infections. The bearing of these results on the analysis of recombination and on the structure and phenotypic action of the hereditary material of phage will be discussed.

MATERIALS AND METHODS

Two strains of bacteriophage T4 were used in these experiments, T4D (DOERMANN and HILL 1953) and T4B (BENZER 1955). It was found that progeny from

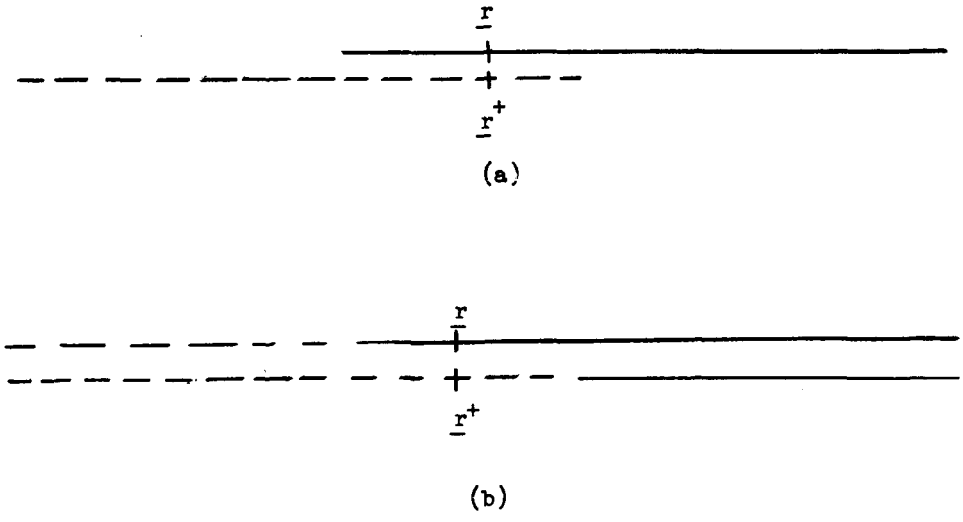

FIGURE 1.—The "partial replica" and "diploid" models for the structure of heterozygotes. Alternative models of the nature of heterozygotes, obtained from a cross of *r* (solid line) × r^+ (interrupted line) are depicted. Interrupted and solid lines are to indicate the source of the genetic material derived from one parental type or the other. Type (a) will be referred to as the "partial replica" model and type (b) as the "diploid" model.

crosses of the type T4D rII$^+$ × T4B rII exhibited the segregation of at least three unidentified genetic characters, and therefore subsequent crosses were made only within, and not between, strains.

The r^+ and various rII mutants of T4B used were obtained from BENZER. These mutants are identified with numbers greater than 100, e.g., r_{145}. The relative map locations of their markers have been given by CHASE and DOERMANN (1958). The T4D r^+ and rII mutants r_{47}, r_{43}, and r_{51} were obtained from MISS M. CHASE. The mutant r_{73} was obtained from G. STREISINGER. The rest of the T4D rII mutants were all isolated from separate T4D r^+ plaques and all identified with numbers less than 100, e.g., r_{64}. With the exception of r_{51}, all the rII mutants used in this study have reversion indices of less than 10^{-5}. The reversion index of r_{51} is 10^{-4}. (The reversion index is the proportion of r^+ revertants in an rII stock, measured by plating on K12 (λ)). Less than 10^{-8} reversions were present in stocks of r_{168}, r_{145}, r_{47}, r_{61}, r_{64} and r_{73}. These mutants are apparently stable, but, with the exception of r_{47} (BENZER 1957), have shown no unusual behavior in crosses. The relative genetic locations of the markers of the T4D rII mutants, as determined by the present worker, are given in Figure 3 and more completely by

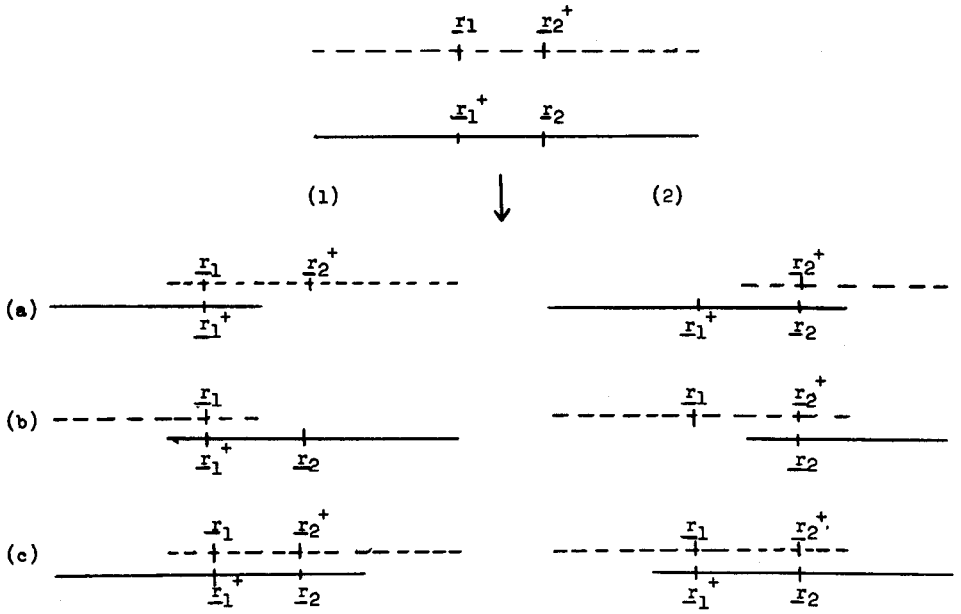

FIGURE 2.—Types of heterozygotes from $r \times r$ crosses. A two-factor cross, $r_1 \times r_2$, will give rise mainly to the three types of heterozygotes depicted in (a), (b), and (c). The $r+$ recombinant heterozygote (a) will give $r-+$ and either parental type, r_1 [as in (a)1] or r_2 [as in (a)2] as primary segregants. The r_1r_2 recombinant heterozygote (b) will give the r_1r_2 recombinant and and either parental type as the primary segregants. The nonrecombinants heterozygotes (c) produce the two parental types as primary segregants. The two types of recombinant heterozygotes (a and b) are formed as a consequence of the overlap including one but not both of the marked loci. The nonrecombinant heterozygotes only arise when the overlap region includes both marked loci.

EDGAR (1958). Some of these mutants carried the additional marker tu_{41} (DOERMANN and HILL 1953). This marker did not interfere with the scoring of mottled plaques (plaques containing both r and r^+ phage). No detectable differences between any of the rII mutants used in this study were noted with regard to the transmission coefficient, which under the conditions employed, was characteristically between 10^{-4} and 5×10^{-3}. (The transmission coefficient is the proportion of rII-infected K12 (λ) complexes which yielded progeny (BENZER 1955)).

Strain B of *Escherichia coli* was used as host for crosses. The plating bacteria were saturated overnight cultures of *E. coli* strain S/6, a derivative of B (DOERMANN and HILL 1953). The selective indicator used was *E. coli* strain 112–12 (λ *h*) No. 3, a derivative (WOLLMAN 1953) of K12, hereinafter called K. This strain was obtained from F. W. STAHL. It was used, in preference to K12 (λ *l*) used by CHASE and DOERMANN (1958), as it gives a lower transmission coefficient. This was advantageous in the experiments to be described.

The media used and the procedure for preparing phage stocks were those described by CHASE and DOERMANN (1958).

The crosses considered in this study were performed as described by CHASE and DOERMANN (1958). In addition, however, in order to obtain a high titer stock of progeny phage, the infected bacteria were diluted 100-fold into H-broth prior to serum treatment and allowed to lyse. The total progeny phage titer of such a lysate was usually about 2×10^8. As mentioned by CHASE and DOERMANN, the proportion of recombinants in such a lysate was often as much as a factor

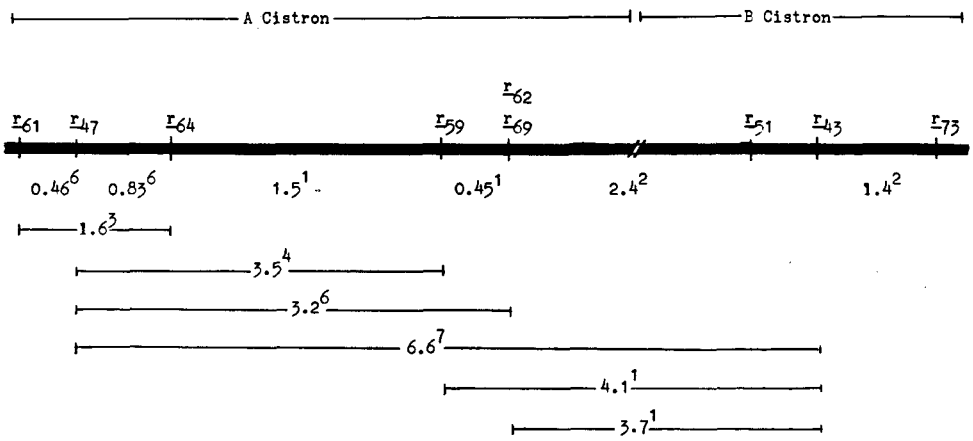

FIGURE 3.—A map of the rII region of T4D giving the markers and intervals referred to in this paper. The recombination values are calculated as twice the titer of progeny determined by plating on K divided by the titer of progeny obtained on S/6. The efficiency of plating on K relative to S/6 is usually about 0.8, and no correction is made for this factor. The superscript to the recombination values for a given interval indicates the number of crosses of which the given value is the mean. The standard deviation of these values was usually less than 20 percent of the mean. Other crosses with different rII markers (EDGAR 1958), not given in the figure, were also used to determine the best order of the markers.

of two greater than that in a more dilute lysate. The cause of this discrepancy is unknown.

The addition of chloroform to lysates as a precautionary measure to destroy unlysed complexes did not appear to influence the results to be discussed, and therefore its use was omitted.

Examination was made, by plating on S/6, of progeny and of progeny-K complexes (infective complexes formed by adsorption of the progeny to actively growing K). The procedure for preparing the progeny-K complexes was as follows. A growing culture of K was concentrated by centrifugation and resuspended in H-broth to a titer of between 2×10^8 and 8×10^8 cells/ml. Cyanide was added to give a final molarity of 0.005. Phage were added to this bacterial suspension such that the multiplicity of infection was less than 0.05. A ten minute adsorption period (in which over 95 percent of the phage were adsorbed) followed. At least 90 percent of the remaining unadsorbed phage were inactivated with anti-T4 rabbit serum. The infected bacteria were then diluted, and aliquots were plated on S/6.

Plaques on S/6 from direct plating of the progeny and from progeny-K complexes were scored as to whether they were phenotypically r, r^+, or mottled. The last type of plaque is made by infected bacteria which produce enough of both r and r^+ particles to give a plaque a mottled appearance. These may be produced either by heterozygotes or by bacteria mixedly infected with r and r^+ phage. Mottled plaques from the direct plating of the progeny are produced only by heterozygotes. The progeny-K complexes, on the other hand, depending upon the multiplicity of infection, produce a certain fraction of mottled plaques from cells mixedly infected with r and r^+ phage. Non-Poisson adsorption was found with K grown in H-broth. The extent of the deviation from random expectation was found to vary from day to day, but mixedly infected cells in control cultures, like the experimental tests in regard to multiplicity, usually occurred with a frequency twice the Poisson expectation. For this reason, the correction made for mixedly infected cells in Tables 1b, and 2 are, in all cases, two times the Poisson expectation. The multiplicities of infection in the experiments given in Table 1a were sufficiently low that no correction for mixedly infected cells was needed.

Since it was found that mottled plaques were misidentified if as many as 10^6 uninfected K bacteria were put on the plate together with the progeny-K complexes, very low multiplicities were not used in most experiments. The use of very low multiplicities necessitates fewer dilution steps from the adsorption tube, and thus a larger number of uninfected bacteria would be delivered to the plate.

It was found that mottling was most easily distinguished if the plates were incubated for 16 to 20 hours at 37° C. Therefore all plates were scored at this time.

RESULTS

Since r^+ particles may grow successfully in K bacteria, whereas r do not, the relative frequencies of r^+ to mottled plaques on S/6, with and without prior adsorption to K, will reveal whether mottling particles from a given cross are lost

by passage through K. The ratio used is the fraction of plaques containing both r^+ and r (in other words, mottled plaques) to the sum of r^+ and mottled plaques. For the progeny-K complexes in some experiments, since multicomplexes are present, this ratio must be corrected by estimating, as indicated in the materials and methods section, the fraction of mottled plaques produced by mixedly infected bacteria. Such plaques may then be reclassified as r^+.

Single heterozygotes from rII × rII⁺ crosses

Table 1a presents data on the growth in K bacteria of single heterozygotes from *r*II × *r*II$^+$ crosses. From the χ^2 test for homogeneity, it may be seen that no loss of mottling particles from either the *r*IIA × *r*IIA$^+$ ($r_{205} \times r^+$) or the *r*IIB × *r*IIB$^+$ ($r_{43} \times r^+$) cross is detected after passage through K. The application of the methods of error analysis to the data in Table 1a (MARGENAU and MURPHY 1943) suggests that most, if not all, of the mottling particles function in K as *r*II$^+$ particles, i.e., produce progeny.

Heterozygotes, if not detected as mottled plaques, will be scored as either r or r^+ plaques. Variability is observed in the appearance of mottled plaques which might suggest such misclassification. An estimate of the magnitude of misclassification of single heterozygotes as r plaques can be made. Very few phenotypically r plaques were observed among the plaques obtained from the progeny-K complexes of the above mentioned crosses. The r plaques probably represent transmission complexes. They give, however, an upper limit estimate of the proportion of heterozygotes which can grow in K bacteria but which are scored as r. This limit is less than nine percent in the $r_{205} \times r^+$ cross and less than five percent in the $r_{43} \times r^+$ cross.

Since there is no apparent reason why heterozygotes would be more likely misclassified as r^+ than as r, it is reasonable to assume that virtually all single hetero-

TABLE 1a

Progeny and progeny-K complexes from rII × rII⁺ crosses

Cross type	Cross	Method of anal.	No. plaques examined: mottled	r^+	r	Obs. ratio*	p value of χ^2	Efficiency of growth of mottling particles in K
		P	149	5768	6262	0.025		
*r*A × r^+	$r_{205} \times r^+$	P-K	297	11882	26	0.024	0.8>p>0.7	0.97±0.09
		P	137	4975	4799	0.027		
*r*B × r^+	$r_{43} \times r^+$	P-K	113	4290	5	0.026	0.8>p>0.7	0.96±0.12

* Mottled/mottled plus r^+.

Tables 1a and 1b. For all crosses tabulated, the plaques counted are given for both the progeny (P) and the progeny-K complexes (P-K). The "cross type" indicates the cistrons in which the markers are situated. The ratio calculated from the raw data is the ratio of mottled plaques to the sum of mottled plaques and r^+ plaques. In Table 1a, the p value of a χ^2 test for the homogeneity of the data is given. The last column of Table 1a gives the value obtained by dividing the P-K ratio by the P ratio, together with its probable error, determined by the method suggested by MARGENAU and MURPHY (1943), page 498. This value is an estimate of the fraction of heterozygotes which grow in K (see text). In Table 1b, the corrected ratios are calculated from the observed ratios corrected as indicated in the text. The map distances (last column) are taken from Figure 3, with the exception of the cross $r_{145} \times r_{168}$, which is given by CHASE and DOERMANN (1958).

zygotes are scored as mottled plaques. With this assumption, these experiments permit the conclusion that most, if not all, single heterozygotes have an rII$^+$ phenotype in K.

Crosses involving two r*II markers*

As will be seen from Figure 2, two types (a) and (c) of heterozygotes neither homozygous nor hemizygous for either of the r markers, should be formed in crosses involving two different r markers. These are the r^+ recombinant heterozygote (a) and the nonrecombinant heterozygote (c). The relative frequencies of these two types among the progeny should depend upon the distance between the two markers employed. As will be seen, the r^+ recombinant heterozygotes are probably a constant fraction of the r^+ recombinants, independent of the interval between markers, and therefore a diminishing fraction of the population as the distance between the markers becomes shorter. The nonrecombinant heterozygotes, on the other hand, should be an increasing fraction of the population as the distance between the markers is reduced, since it will be increasingly likely that the overlap region will include both markers. Preliminary experimental evidence supports the correctness of this notion (DOERMANN and KATAJA 1957). This fraction should approach the frequency of single heterozygotes in T4, which, from the data in Table 1a, is a little over one percent.

The r^+ recombinant heterozygotes (a, in Figure 2) should give mottled plaques due to the segregation of the r and r^+ markers, and, since they are similar in structure to the heterozygous progeny from rII $\times$ rII$^+$ crosses, should give an rII$^+$ phenotype in K bacteria.

The nonrecombinant heterozygotes (c, in Figure 2) could give mottled plaques due to the formation of r^+ recombinants as well as the regular r segregants. If even a small proportion of the nonrecombinant heterozygotes produce mottled plaques, it should be possible to determine whether they can grow in K bacteria, for, with closely linked r markers, the nonrecombinant heterozygotes should be more frequent than the r^+ recombinant heterozygotes, and their loss in K should be detected as a relative loss in mottling plaques from the progeny-K complexes compared to the direct plating of the progeny.

The results from experiments of this type are given in Table 1b and are graphically represented in Figure 4. It is seen that the crosses may be distinguished on the basis of whether or not a loss of mottling particles is observed in the progeny-K complexes.

Two-factor crosses involving r*II markers in the same cistron*

From crosses of two r's, both of which are within the A or both within the B cistron, it appears that two kinds of mottling particles are produced, which may be differentiated by their ability or inability to produce progeny upon infection of K hosts. Since the r^+ recombinant heterozygotes should grow in K, the type unable to grow in K must be the nonrecombinant heterozygote. It will be noted from Figure 4 that these particles represent an increasing fraction of the progeny rela-

tive to the r^+ recombinants, as the map distance becomes shorter. This is to be expected of the nonrecombinant heterozygotes on the basis of the observations of DOERMANN and KATAJA (1957) cited earlier.

Table 2 presents data for progeny-K complexes from repeated crosses involving various pairs of *r*IIA markers and a repeated cross of a distantly linked *r*IIA and *r*IIB pair. An analysis of variance was performed on the corrected ratios (mottled to mottled plus r^+) from these crosses. The corrections were made as indicated in the section on materials and methods. It was found that the variance among repeated crosses was sufficiently great to mask differences, if any, for this ratio between different pairs of markers. These data are thus not inconsistent with the notion that the r^+ recombinant heterozygotes represent a constant fraction of the r^+ recombinants, over the range of map distances employed.

The following observations show that the nonrecombinant heterozygotes probably have no greater chance of growing in K bacteria than do *r* particles. They therefore probably possess an *r*II phenotype in K. The progeny from the cross $r_{227}r_{320} \times r_{205}$ were adsorbed to K as described previously, except that the cells were not exposed to cyanide. The infected complexes were then superinfected with T6r_{47} (the r_{47} marker of T4, introduced into T6) at fifteen minutes and plated on S/6. The superinfection with T6r_{47} was performed to sterilize the uninfected K bacteria which in this experiment were in sufficient numbers, if allowed to grow on the plate, to obscure recognition of the phenotypes of the plaques. From

TABLE 1b

Progeny and progeny-K complexes from r*II* × r*II crosses*

Cross type	Cross	Method of analysis	No. plaques examined			Observed ratio mottled/ mot. plus r^+	Corrected ratio	Map distance
			mottled	r^+	r			
$rB \times rB$	$r_{43} \times r_{73}$	P	57	95	9522	0.38	...	1.6
		P-K	214	1042	11	0.17	0.12	
$rA \times rA$	$r_{145} \times r_{168}$	P	8	10	13705	0.44	...	0.14
		P-K	59	377	.. †	0.14	0.08	
	$r_{47}tu_{41} \times r_{61}$	P	22	25	9342	0.47	...	0.46
		P-K	154	896	.. †	0.15	0.12	
	$r_{61} \times r_{64}$	P	26	40	4975	0.39	...	1.6
		P-K	54	672	.. †	0.07	0.04	
	$r_{47}tu_{41} \times r_{59}$	P	36	194	9550	0.16	...	3.5
		P-K	144	1360	....	0.10	0.07	
$rA \times rB$	$r_{69} \times r_{51}$	P	46	61	8050	0.43	...	2.4
		P-K	184	236	.. †	0.44	0.39	
	$r_{69} \times r_{43}$	P	34	44	3178	0.44	...	3.7
		P-K	307	555	.. †	0.36	0.35	
	$r_{59} \times r_{43}$	P	90	202	11310	0.31	...	4.1
		P-K	420	1105	.. †	0.28	0.25	
	$r_{47}tu_{41} \times r_{43}$	P	62	258	9533	0.19	...	6.6
		P-K	126	653	.. †	0.16	0.13	

† *r* plaques were observed but are not tabulated.

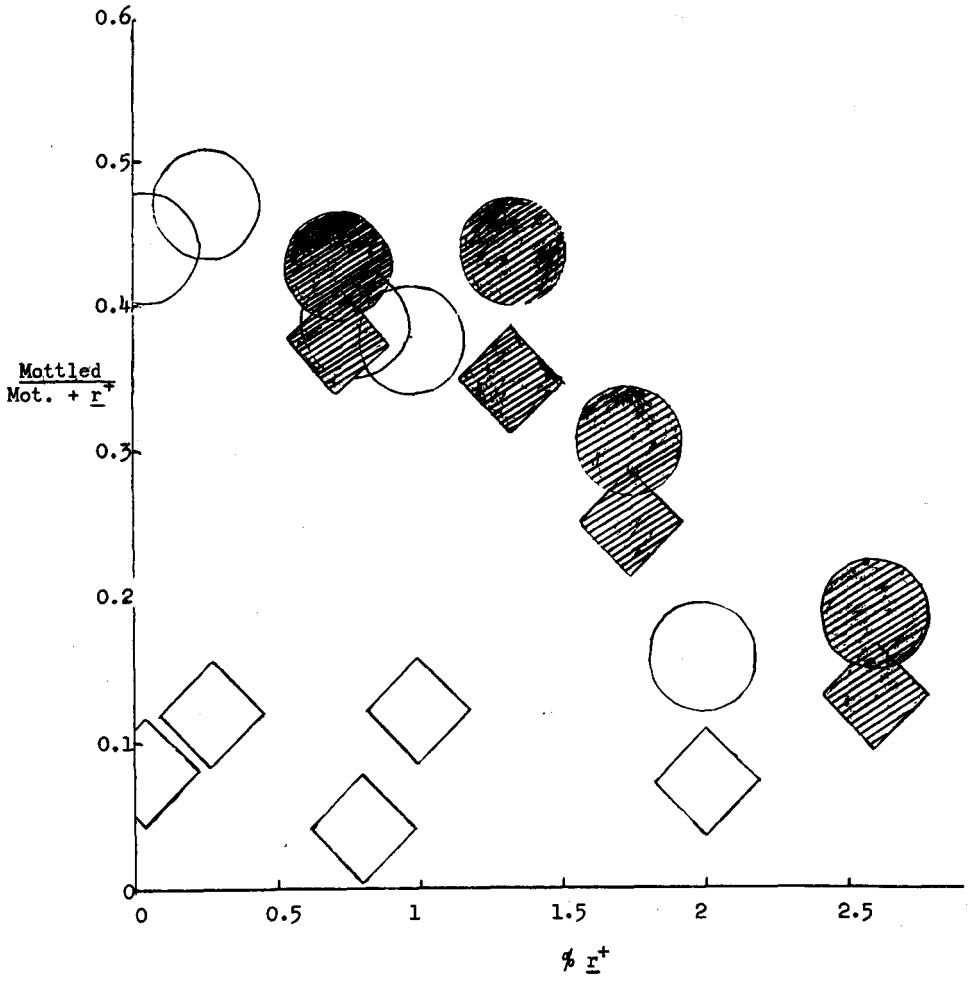

FIGURE 4.—A graphic representation of the results given in Table 1b. The ratio, mottled to mottled plus $r+$, of the progeny and progeny-K complexes, is plotted against the percent $r+$, calculated from the direct plating of the progeny, for each cross. For every circle, which represents progeny plated directly on S/6, there is a corresponding diamond, which represents the corrected ratio from the progeny-K complexes. The shaded symbols represent crosses involving two markers in different cistrons (rA $\times$ rB). The white symbols signify crosses of two markers within the same cistron (rA $\times$ rA, or rB $\times$ rB).

controls it appeared that after superinfection at this late time most of the T6r_{47} are excluded from the infected cells. The markers used are in the A cistron in the order r_{227}, r_{205}, r_{320} and are very closely linked, such that close to one percent nonrecombinant heterozygotes will be formed. Of the progeny-K complexes, about 0.01 percent gave r^+ and mottled plaques, and 0.01 percent produced r plaques. Of the former, one third were mottled. However, the relative frequencies of r^+ and heterozygotes cannot be determined reliably, since some of the mottled

TABLE 2

Repeated determinations of the ratio of mottled to mottled plus r+ plaques for various pairs of rII markers

Cross type	Cross	No. crosses	Corrected ratios (mottled/mottled and r^+)		Corrected mean ratio ± S.E. of mean
$rA \times rA$	$r_{47} \times r_{61}$	2	0.075	0.118	0.097±0.022
	$r_{47} \times r_{64}$	2	0.033	0.054	0.043±0.012
	$r_{47} \times r_{59}$	2	0.067	0.041	0.054±0.012
	$r_{47} \times r_{62}$	4	0.080, 0.076, 0.079, 0.044		0.07 ±0.009
$rA \times rB$	$r_{47} \times r_{43}$	4	0.053, 0.123, 0.091, 0.131		0.10 ±0.02

These crosses include the marker tv_{41} in one or other of the input parents. An analysis of variance on the corrected ratios gives an F ratio of 1.52, which is well below the F ratio of 3.63 for the 0.05 fiducial level.

plaques could possibly have resulted from superinfection of an r^+ infected bacterium by T6r_{47} which was able to enter the bacterium and recombine with the T4r^+ particles, thus giving a yield of T4r^+ and T4r_{47} on S/6.

As for the r plaques, they would be expected to be transmission complexes; however, they give an upper limit estimate of the efficiency of growth of the nonrecombinant heterozygotes, since, if these could grow in K bacteria, most of them should produce r plaques on S/6. Since one percent of the progeny-K complexes should consist of nonrecombinant heterozygote complexes, it can be concluded that less than one percent of the nonrecombinant heterozygotes produced in this cross are able to yield progeny upon infection of K.

A similar argument may be derived from the $r_{43} \times r_{73}$ cross (Table 1b). Here, because the transmission coefficient was low, the number of r plaques from the progeny-K complexes has been recorded. Their frequency is again 0.01 percent of the total progeny-K complexes and therefore could represent very few of the nonrecombinant heterozygotes.

In summary, these experiments indicate that two types of heterozygotes which arise in crosses involving two rII markers in the same cistron, may be distinguished by their ability or inability to grow in K bacteria. Those which do grow in K are r^+ recombinant heterozygotes, similar in structure to the heterozygous progeny obtained from an $r \times r^+$ cross. These appear to be a constant fraction of the r^+ recombinants within the range of map distances employed (0.1–4.0 percent). Of the other type, the nonrecombinant heterozygotes, at least 99 percent are unable to grow in K and produce progeny. They therefore have an rII phenotype.

Two-factor crosses involving rII markers in different cistrons

Crosses of rIIA × rIIB give no indication of yielding two classes of mottling particles differentiable on the basis of their ability or inability to infect K bacteria with the successful production of progeny. In these crosses the frequency of mottling particles relative to r^+ found among the progeny grown on S/6 is comparable to that found in crosses of the type rIIA × rIIA and rIIB × rIIB which give

a similar frequency of r^+ recombinants (see circles in Figure 4). In the latter crosses, especially for closely linked *r* markers, most of the mottling particles were demonstrated to be nonrecombinant heterozygotes, unable to grow in K. Since no such class is detected in the crosses of the type *r*IIA × *r*IIB, it would appear that the nonrecombinant heterozygotes formed in these crosses can grow in K bacteria and therefore exhibit an *r*II$^+$ phenotype.

Since K bacteria mixedly infected with *r*IIA and *r*IIB phage produce progeny, a lower multiplicity of infection for the progeny-K complexes was used than in the crosses involving *r* markers within the same cistron. Thus more infected K cells were put on the plates than in the latter experiments. This might account for the fact that, although the ratio for the progeny-K complexes is close to that of the direct plating of the progeny, it is consistently lower. In any event, the differences are quite small, inasmuch as the ratio of these values is in all cases greater than 0.8.

These experiments then, indicate that nonrecombinant heterozygotes from crosses involving *r*II markers in different cistrons may grow in K bacteria, exhibiting an *r*II$^+$ phenotype.

The detection of nonrecombinant heterozygotes

In contrast to the r^+ recombinant heterozygotes, it is probable that a considerable fraction of the nonrecombinant heterozygotes from $r \times r$ crosses are not detected as mottled plaques on S/6. From the experiments of DOERMANN and KATAJA (1957), it is to be expected that the absolute frequency of nonrecombinant heterozygotes among the progeny of an $r \times r$ cross should approach one percent as the distance between the markers is reduced. However, as can be derived from Figure 4, the absolute frequency of detected nonrecombinant heterozygotes remains less than this (less than 0.2 percent in the case of the $r_{47} \times r_{61}$ cross, and therefore about 20 percent of the expected number of nonrecombinant heterozygotes). It may be concluded that only a fraction of the actual nonrecombinant heterozygotes are detected as mottled plaques.

But in contrast to the estimated 20 percent of the heterozygotes which produce mottled plaques on S/6, less than one percent of B bacteria mixedly infected with these two *r* markers exhibit any detectable mottling. This is presumably due to the fact that, although many of these mixedly infected bacteria produce r^+ recombinants, too few are produced in each bacterium to give detectable mottling in the plaque. It therefore seems plausible that the formation of recombinants from heterozygotes is a more efficient process than from mixed infections. Additional experimental evidence to support this idea has been presented (EDGAR 1956), and a more detailed account will be published in a future paper.

These considerations do not influence the conclusion presented regarding the *r*II phenotype of nonrecombinant heterozygotes from crosses of two *r*'s in the same cistron, since, as was shown, it was possible to confirm this conclusion independently of scoring mottled plaques.

Such experiments were not performed to show that nonmottling nonrecom-

binant heterozygotes from crosses of two *r*'s in different cistrons have an *r*II$^+$ phenotype. However it would seem unwarranted, in this case only, and not the case above, to suggest that nonrecombinant heterozygotes are of two sorts with respect to phenotypic action.

DISCUSSION

The experiments described here indicate that phage heterozygous for a single pair of *r*II and *r*II$^+$ alleles are able to grow in K bacteria with very nearly the efficiency of *r*II$^+$ particles. This is also true of phage heterozygous for two *r*II markers in the *trans* position, provided the markers are in different cistrons. But if the markers are in the same cistron, the phenotype of the double heterozygotes is similar to that of *r*II mutants, in that they are unable to produce progeny upon infection of K. These findings parallel the observations of BENZER (1955) regarding K bacteria mixedly infected with phage of different genotypes corresponding to the heterozygote types above. It thus appears that no phenotypic properties have been found which are attributable to heterozygotes as distinct from the combination of parental types from which they arose.

Without knowledge of the nature of the block to phage production in *r*II-K complexes, no decision can be reached at this time regarding which of the models for the structure of heterozygotes presented in Figure 1 is correct. However, that an unaltered and intact *r*IIA$^+$ and *r*IIB$^+$ cistron must be present in a phage-infected K bacterium for the successful production of progeny is suggested by the experiments of BENZER (1955) and of KRIEG (1957). This is most easily interpreted as indicating that a cistron functions as a unit, perhaps as a template, in the formation of the primary gene product. If the structure of the heterozygote is as depicted in Figure 1a, and must function as such in a K bacterium which it infects, it would be expected that often heterozygotes of this type would have an *r*II phenotype, since, in some cases, one strand will have an incomplete cistron while the other strand will be mutant (*r*).

If some multiplication of vegetative phage may proceed in K complexes before the expression of an *r*II$^+$ phenotype is required, both structural models of heterozygotes could predict the observed results, since the replication of the segregants of the heterozygote would presumably produce a complex similar to that formed from mixed infections with the corresponding genotypes. However, a model in which the "partial replicas" (Figure 1a) multiply as such would require the assumption that, if they are unable to supplement each other structurally during self replication, then these partial replicas may supplement each other in the formation of gene product. Otherwise the *r*II $\times$ *r*II$^+$ and the *r*IIA $\times$ *r*IIB crosses would yield a detectable number of heterozygotes which would be incapable of forming *r*II$^+$ gene product.

If, on the other hand, the *r*II$^+$ phenotype must be expressed before any multiplication of vegetative phage occurs, the observations reported in this paper would seem to require that *both* strands of the heterozygote independently initiate the production of gene product. But the observation that nonrecombinant heter-

ozygotes with two *r*II markers in one cistron are unable to grow in K would suggest that no "recombinant" products of the gene are formed.

The partial replica model for the structure of the heterozygote (Figure 1a) would then require the assumption that gene product may be formed by switching templates, but at the ends of an overlap region only. These assumptions, necessary for the partial-replica model under the conditions specified, would make the "diploid" model (Figure 1b) for the structure of the heterozygote the more appealing one.

Although some preliminary evidence (KRIEG 1957) may be interpreted to suggest that no phage multiplication occurs in *r*II-K complexes, it is suggestive only, and no final conclusions can be drawn from the above arguments until more decisive evidence is at hand.

The studies reported in this paper show that, from crosses of two *r*II markers, recombinant particles which are measured on K will include certain classes of heterozygotes as well as r^+ recombinants. In crosses involving markers within the same cistron, only r^+ recombinants and r^+ recombinant heterozygotes will produce plaques on K bacteria. Since the r^+ recombinant heterozygotes constitute a small, and probably constant, fraction of these particles, this method of determining map distances by two-factor crosses will yield results that differ only slightly from the direct measurement of recombinants, and will very likely be proportional to it. Although the recombinant heterozygotes presumably represent unsegregated recombinants, until their role as intermediates in recombinant formation is more fully understood, the interpretation of studies in which they are not distinguished from recombinants may possibly be subject to qualification.

It should be pointed out that the high negative interference phenomenon described for recombination in the *r*II region (CHASE and DOERMANN 1958) is not attributable to the scoring of heterozygotes. Less than one third of the progeny particles scored on K bacteria from the cross $r_{227}r_{320} \times r_{205}$ are r^+ recombinant heterozygotes. Since CHASE and DOERMANN found a negative interference factor greater than 15 for this cross, even the elimination of the heterozygotes from the data, if reasonable, would leave virtually all of the recombinants to be otherwise explained.

In crosses involving two *r*II markers in different cistrons, nonrecombinant as well as r^+ recombinant heterozygotes will produce plaques on K. The nonrecombinant heterozygotes from this type of cross may grow in K, and if r^+ recombinants are formed during growth, they can initiate the formation of a plaque on K. In the case of closely linked markers, the nonrecombinant heterozygotes will constitute nearly one percent of the total progeny. Since heterozygotes have a high efficiency of recombination (EDGAR 1956), these will constitute a large fraction of the plaque forming particles measured on K in such crosses. It is therefore apparent that the measurement of recombination by plating on K bacteria of progeny from crosses of two *r*II markers in different cistrons will, in many cases, give spuriously high recombination values. This phenomenon may well account for the apparent "gap" between the A and the B cistrons found by BENZER (1955).

SUMMARY

The experiments reported here indicate that all, or virtually all, phage particles heterozygous for a single pair of *r*II and *r*II$^+$ alleles have an *r*II$^+$ phenotype in K12 (λ) bacteria. On the other hand, particles heterozygous at two *r*II loci in the *trans* position have a mutant phenotype in K12 (λ), provided the two markers are in the same cistron. If the markers are in different cistrons, most, if not all double heterozygotes have an *r*II$^+$ phenotype.

Certain implications arise from these observations in respect to the analysis of recombination and the structure and phenotypic action of the phage genome. Recombination analyses which utilize the *r*II-K system may be subject to some qualification, for it has been shown that no reliable estimate of the map distance between two *r*II markers in different cistrons can be made from the measurement of plaque forming particles on K12 (λ) bacteria. Although the experiments reported here do not distinguish between the "partial replica" and "diploid" models of heterozygote structure, fewer assumptions need be applied to the latter model in order to account for the results obtained.

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