GENETIC STRUCTURE OF BACTERIOPHAGE T4 AS DESCRIBED BY RECOMBINATION STUDIES OF FACTORS INFLUENCING PLAQUE MORPHOLOGY[1]

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IN the study of biological replication, the most promising methods of attack appear to involve the use of genetic specificities. For the investigation of this problem with bacteriophage, it is then important to make available a rather abundant supply of genetic markers and to learn their genetic properties. This paper deals with the hereditary characteristics of a class of previously undescribed genetic factors and their recombination frequencies with known *r* (rapid lysis) and *m* (minute) loci both of which influence plaque morphology (HERSHEY and ROTMAN 1948, 1949). The factors to be described are designated by the genetic symbol *tu* because they increase the turbidity of the plaque halo. Mention of some of the plaque types involved has been made previously in a brief report (DOERMANN and DISSOSWAY 1949).

HERSHEY and ROTMAN'S data (1948, 1949) for the recombination frequencies among *r*, *h* (host range), and *m* loci in phage T2 indicate some kind of linkage system but their data were sufficient to establish only that a linear arrangement is a possibility. The data presented in this paper indicate a linear order for at least five linked factors in one group, and three in another.

MATERIALS AND METHODS

Bacteriophage T4 was used throughout these experiments except where the purpose was the introduction of two of HERSHEY and ROTMAN'S (1948) *r* factors into T4 from T2. The single *m* factor used in T4 was obtained by isolating a mutant from a genetically complex stock. The mutant was crossed to wild type and T4*m* was obtained as a segregant. The *r* mutants originating in T4 were obtained by isolation of a single *r* from each of a number of mottled plaques. Each of these arose from an individual T4r^+ particle. Similarly all the *tu* strains were obtained from independent mutations in T4$r_{48}tu^+$. Large numbers of *tu*'s are readily collected since a 24-hour plaque of rtu^+ invariably contains a small proportion of *tu* mutants.

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The following system of nomenclature has been used in this paper. Names of HERSHEY and ROTMAN'S mutants are unchanged. Our isolates were all given a subscript number of 41 or higher. In cases where similar mutations occur in allelic or almost allelic positions, the letters a, b, c, etc. are attached to the subscript. Thus, r_{51a}, r_{51b}, and r_{51c} denote three independent *r* mutants which show little or no recombination with each other.

The culture medium used in all experiments was the usual 0.8 percent solution of nutrient broth (Difco) with 0.5 percent sodium chloride added. Phage stocks were cultured in the enriched nutrient broth medium used by HERSHEY and ROTMAN (1949). A T6-resistant mutant of HERSHEY and ROTMAN'S strain S was used for assay platings and as host bacterium in preparation of phage stocks. Strain B of *Escherichia coli* was used as host for all other purposes.

Assays were made by the customary agar layer plating method, using the same media as were used by HERSHEY and ROTMAN (1949). The plates were poured quite full, since the optimum differentiation of plaque types is produced with 50–60 ml per 100-mm Petri plate. Two drops of an aerated overnight culture of S/6 and a sample of the phage to be assayed were mixed in 2 ml of soft agar at 45°C and poured over the surface. The plates were incubated 20–24 hours at 33°C, which seemed to give the most satisfactory differentiation. In general, lowering the temperature of incubation increases the turbidity of the halos and decreases the size of the plaques. Another condition which influences the turbidity of the halos, and which may at times be used to advantage is the nutrient composition of the agar. Increasing the glucose and tryptose makes the halos more turbid. The dryness of the plate also influences differentiation. Plates were incubated at 37°C for 20 hours before they were used, and filter paper liners were placed in the lids for uniformity in dryness.

Preparation of stocks genetically homogeneous with respect to *tu* afforded some difficulties at first. This is because some of the strains used in the present experiments are genetically unstable in one respect, namely, that 24-hour plaques resulting from single phage particles contain a high proportion of phage which is genetically different from the original particle. It was discovered, however (DOERMANN and DISSOSWAY 1949), that young plaques (4–6 hours of incubation) contain a genetically homogeneous population, at least with respect to those factors that we are able to identify. By using young plaques to infect bacterial cultures it has been possible to obtain genetically homogeneous stocks even of those types which had previously appeared to be most unstable. It should be noted in this regard that permitting bacterial cultures to become excessively turbid before infecting them with the young plaque also resulted in genetic heterogeneity in the stock. Once a homogeneous stock was obtained, it was possible to subculture it in the usual way without resorting to the use of plaques, provided the bacterial culture was not too turbid.

Filtered lysates of *rm* stocks were usually not of sufficiently high titer for practical use and were therefore concentrated by centrifugation. Centrifuged stocks were resuspended in nutrient broth. Except for the *rm* stocks, it was not necessary to concentrate the filtrates.

The method of crossing is to perform a one-step growth experiment with mixed multiple infection (LURIA and DELBRÜCK 1941). Care was taken to make experiments as uniform as possible in all details. Bacteria at 2×10^7 cells per ml were concentrated in broth by centrifugation to *ca.* 4×10^8. An equal volume of the appropriate phage mixture was added to the culture. After 2½ minutes, adsorption was stopped by dilution. The unadsorbed phage was measured in one aliquot and inactivated by anti-T4 rabbit serum in another. Platings made prior to lysis from the diluted serum-treated culture gave the titers of infected bacteria. Genetic classification of the progeny was made on post-lysis platings from a 20-ml sample which contained the phage yield from about 200 bacteria. This sample was stored in the refrigerator, and, since later platings showed no loss of titer over a period of several months, replatings were made when it was desirable to obtain larger counts.

EXPERIMENTAL RESULTS

Classification of the mutant strains used. In establishing the group of *r* stocks used in these experiments, six *r* mutants were isolated from independent mutations in T4. These were first intercrossed with each other and with r_1 and r_7. The latter pair was obtained from DR. A. D. HERSHEY as T2H mutants (HERSHEY and ROTMAN 1948). They were introduced into T4 by making mixed infection with T2*r* and T4r^+, followed by isolation of *r* plaques from platings against B/2. The eight *r* loci fell into three groups on the basis of their recombination values. Intragroup crosses yielded recombination values (R_{ik}) of less than 1 percent. (These values are found by doubling the percentage of the r^+ recombinant since the complementary *rr* recombinant is not readily distinguishable from either of the parental types. In crosses where the two complementary recombinants are distinguishable, however, their sum has been used as a measure of R_{ik}.) Group A consisted of r_1, r_{48a}, and r_{48b}, group B of r_7 and r_{47}, and group C of r_{51a}, r_{51b}, and r_{51c}. Any member of group A shows 36–44 percent recombination with any member of either of the other groups. On the other hand, either member of group B shows 5–8 percent recombination with any member of group C. Thus similarity in the *r* linkage groups in T2 and T4 is indicated, since the r_1 and r_7 groups appear to be unlinked in both phages, and since in both, there are other *r*'s closely linked to r_7 (HERSHEY and ROTMAN 1948, 1949). In this connection, however, it should be mentioned that experiments to introduce the r_{13} locus from T2 into T4 were unsuccessful in several attempts. The reason for this is not known, but it may suggest some difference in the genetic structure of T2 and T4.

It is perhaps worth mentioning that, among the intercrosses of the eight *r* mutants, several cases were found where no wild type plaques were produced. Crossing r_{48a} by r_{48b} yielded no wild type in 1700 plaques examined, and r_{51b} by r_{51c} yielded none in 1054. This contrasts with the findings in T2 of HERSHEY and ROTMAN (1948) where no allelic pairs were found among 14 mutants tested, suggesting perhaps, that the number of factors controlling the r^+ phenotype is not as large as might have been predicted from their data.

Twenty-six *tu* mutants of independent origin were isolated and may be

grouped into five clusters as seen in table 1, which lists the *intra*cluster recombination value. These are based on the proportion of the tu^+ recombinant only. Since the number of tu^+ plaques per cross varied from zero to only 35, and since only a few of the possible intercrosses have been made, caution must be exercised in estimating the number of loci represented. Minimum estimates

TABLE 1

Recombination values within the clusters of near-allelic tu loci.

Cluster	Mutant	Cross indicating inclusion in cluster	Number of plaques examined	Percent recombination*
tu_{41}	tu_{41a}	....	....	....
	tu_{41b}	$tu_{41b} \times tu_{41a}$	557	0.0
	tu_{41c}	$tu_{41c} \times tu_{41a}$	819	0.0
	tu_{41d}	$tu_{41d} \times tu_{41a}$	487	0.0
	tu_{41e}	$tu_{41e} \times tu_{41c}$	531	0.0
tu_{42}	tu_{42a}	....	....	....
	tu_{42b}	$tu_{42b} \times tu_{42a}$	1137	0.4
	tu_{42c}	$tu_{42c} \times tu_{42a}$	509	0.0
	tu_{42d}	$tu_{42d} \times tu_{42b}$	3014	2.3
	tu_{42e}	$tu_{42e} \times tu_{42b}$	866	0.5
	tu_{42e}	$tu_{42e} \times tu_{42d}$	4445	1.3
	tu_{42f}	$tu_{42f} \times tu_{42b}$	686	0.0
	tu_{42g}	$tu_{42g} \times tu_{42f}$	788	0.2
	tu_{42h}	$tu_{42h} \times tu_{42b}$	795	2.3
tu_{43}	tu_{43a}	....	....	....
	tu_{43b}	$tu_{43b} \times tu_{43a}$	698	0.3
	tu_{43c}	$tu_{43c} \times tu_{43b}$	426	0.4
	tu_{43d}	$tu_{43d} \times tu_{43b}$	720	0.0
	tu_{43e}	$tu_{43e} \times tu_{43a}$	2946	0.1
	tu_{43f}	$tu_{43f} \times tu_{43e}$	1152	1.6
tu_{44}	tu_{44a}	....	....	....
	tu_{44b}	$tu_{44b} \times tu_{44a}$	1313	0.6
	tu_{44c}	$tu_{44c} \times tu_{44b}$	649	0.0
	tu_{44d}	$tu_{44d} \times tu_{44c}$	2395	0.7
tu_{45}	tu_{45a}	....	....	....
	tu_{45b}	$tu_{45b} \times tu_{45a}$	1835	0.0
	tu_{45c}	$tu_{45c} \times tu_{45a}$	1064	1.7

*Although the back-mutation rate from any *tu* to tu^+ has not been estimated accurately, it is evident that the occurrence of more than one tu^+ in 1000 plaques cannot be ascribed to back mutation. The stocks prepared for these experiments, which are the result of multiple infection with a single *tu* type, rarely contain one tu^+ per 1000 phage particles.

can be made, however. In the tu_{41} region only a single member has been found. In the tu_{42} cluster, at least three separate loci are evident. In the other three clusters, the minimum estimate would appear to be two in each.

Even though the experiments were not very satisfactory from a quantitative point of view, it may be said that a similar cluster of several *m* loci was discovered. HERSHEY and ROTMAN'S *m*, when introduced into T4 is quite close to m_{41}.

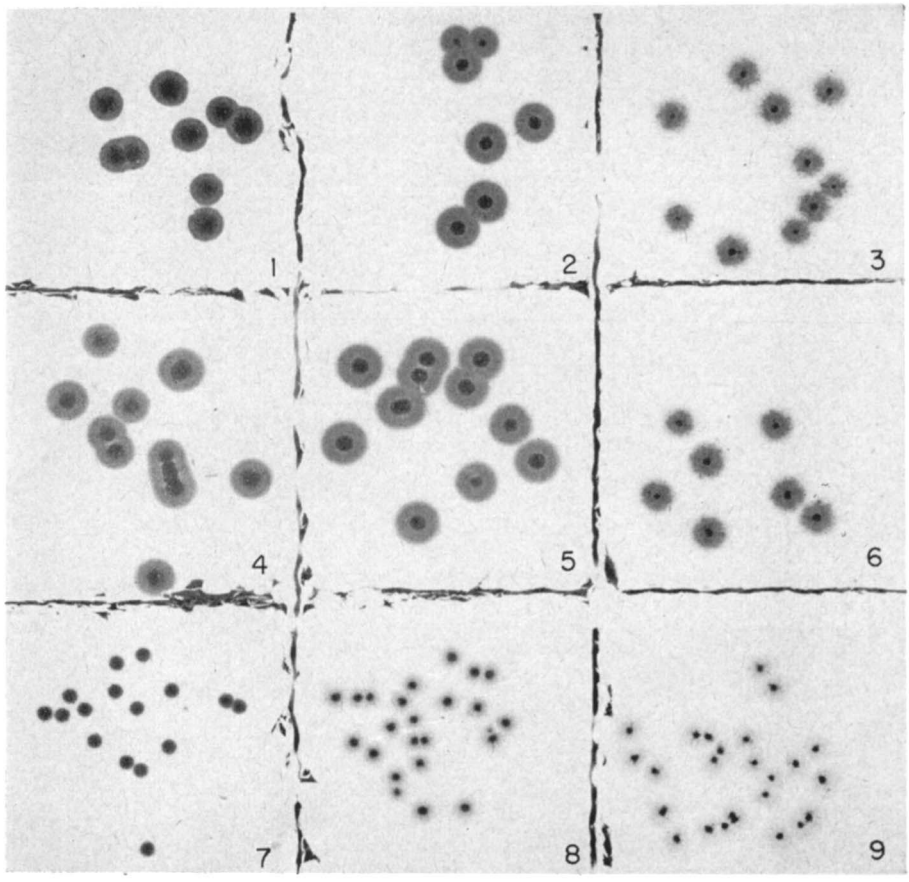

FIGURE 1.—Phenotypic expression of several *tu* factors in T4r_{48} and in T4r^+. In photographing the plaques it was necessary to give the r^+ plaques a slightly longer exposure than the r_{48} plaques in order to bring out the differences in their halos.

The genotypes of the plaques are as follows:

1. $r_{48}tu^+$	2. $r_{48}tu_{42a}$	3. $r_{48}tu_{41a}tu_{42a}$
4. $r_{48}tu_{44a}$	5. $r_{48}tu_{44d}$	6. $r_{48}tu_{44e}$
7. $r^+ tu^+$	8. $r^+ tu_{42b}$	9. $r^+tu_{41a}tu_{42b}$

Some of the morphological characteristics of *tu* plaques are shown in figure 1. Plaques in sections 1–6 show certain of the characteristics in a genetic background of r_{48a}. Plaques in sections 7–9 show some of them in the r^+ background. The top row illustrates the types that might be expected from a cross of two nonallelic *tu* factors which themselves are indistinguishable. The middle row (4–6) shows three phenotypically different *tu* mutants from the tu_{44} cluster, and the bottom row (7–9) shows r^+ with zero, one, and two *tu* factors. By using these types in appropriate combinations, three-factor crosses are possible where, under ideal conditions, all eight plaque types are differentiable.

The results to follow will deal with intercrosses among the described clusters of mutants as well as the *m* factor isolated in T4. The data for intercrosses of

all members of one cluster with those of another cluster have been pooled, unless the recombination value within the cluster was high enough to affect the intercluster recombination value significantly.

Statistical treatment of the data. Since it is known that the relative multiplicity (number of phage particles adsorbed per bacterium) of the two infecting parental types affects the recombination value, a criterion was set up for

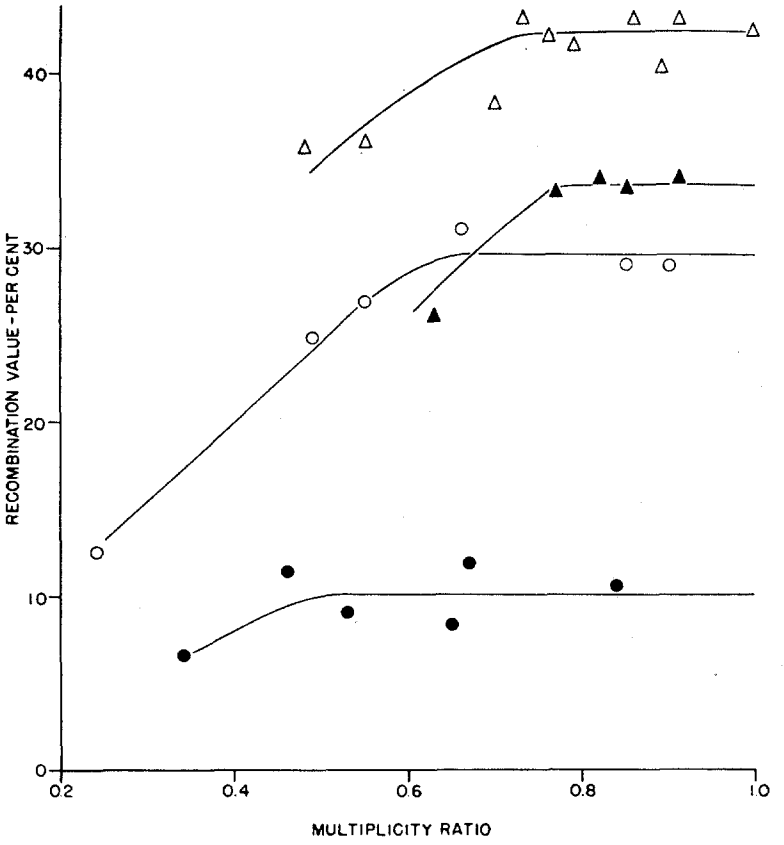

FIGURE 2.—Recombination value as a function of the inequality ratio of parental loci in the yield. The inequality ratio is the ratio of genetic markers from one parent to those from the other parent. The lower number has been divided by the higher in all cases.

△ Expts. with cross $r_{51} \times tu_{45}$
▲ Expts. with cross $r_{48} \times tu_{45}$
○ Expts. with cross $m_{41} \times tu_{42}$
● Expts. with cross $m_{41} \times tu_{43}$

rejecting any experiment where this bias would be expected to have an appreciable effect on the measured value. The most reliable measure of relative multiplicities appears to be the ratio in the yield of the genetic marker from one parental type to its allele from the other. In figure 2 the recombination values from several experiments are plotted against this ratio (always the lower to the higher number). It is seen that, if the data are taken from experi-

ments which have a ratio of 0.67 or greater, the recombination value generally falls on or near the flat part of the curves, giving maximum values. At lower ratios the recombination percentage is, in most cases, smaller. Although selection of a higher ratio might have given a little more precision, more data would have been eliminated. Therefore, the following criterion was established: When a ratio falls significantly below 0.67 (at the 5 percent level) then the experiment was rejected as not representative of the true recombination value. In cases where the ratio could not be determined from the yield, the multiplicity of infection was used as a criterion. All multiplicities are measured with approximately equal accuracy, but less accurately than the yield ratios. All experiments having multiplicity ratios of less than 0.67 were therefore rejected without testing the significance of the deviation.

Experiments were also selected on the basis of total multiplicity of either parent. When the multiplicity of infection of either parent was less than 3, the experiment was rejected, since in this case 5 percent or more of the bacteria could give no recombinants because they would not have been infected by one parental type. Of the total of 125 experiments, 16 were rejected for unsatisfactory ratios and 4 for low multiplicities.

The number of experiments per cross ranged from one to nine, and the number of plaques counted varied from about 400 to 4000 per experiment. In general, large numbers of plaques were counted for crosses yielding small recombination percentages. Since it was anticipated that extraneous factors would result in more total variation than could be expected on the basis of random binomial error, the method of COCHRAN (1943) was used to obtain appropriate weights for an analysis of variance. Over all experiments it was found that 55 percent of the total variation could be attributed to extraneous factors. Using this information, exact weights (as opposed to partial or binomial weights) were computed for each experiment. The weighted analysis of variance took the form of a randomized blocks experiment, and a modified form of the method described by TUKEY (1949) was used to separate the mean recombination percentages into statistically undifferentiable groups. In every case, the 5 percent level of significance was used.

The results of intercluster crosses. The data, selected as described are given in table 2. For the purpose of distinguishing linked and unlinked pairs the mean recombination values (R_{ik}) have been grouped into two families which are separated by the horizontal line in the table. The pairs in the lower group with recombination values ranging from 32.9 to 45.5 percent cannot, without more information, be considered as linked. Those pairs in the upper group are linked. The data further indicate that the nine loci fall into three groups which appear to be independent of one another. The only member of the group I is r_{48}. Group II contains three linked loci, r_{47}, r_{51}, and tu_{41}, and group III contains five, namely, tu_{42}, tu_{44}, tu_{43}, m_{41}, and tu_{45}.

DISCUSSION

Although the conventional three-factor crosses with the loci described in this paper are not yet available, it is possible to investigate whether, on the basis

TABLE 2

Recombination values observed in two-factor intercluster crosses.

Cross	Mean recombination value (R_{1k})*	95 percent confidence limit		Recombination per mating (P_{1k})**
		Lower	Upper	
	percent	*percent*	*percent*	*percent*
$r_{47} \times r_{51}$	5.4	4.1	6.8	2.4
$r_{51} \times tu_{41}$	23.7	20.8	26.7	12.8
$r_{47} \times tu_{41}$	25.0	21.3	28.9	13.8
$tu_{42} \times tu_{44}$	9.5	8.1	11.1	4.2
$tu_{42} \times tu_{43}$	25.5	22.0	29.0	14.1
$tu_{42} \times m_{41}$	30.1	25.8	34.7	18.3
$tu_{42} \times tu_{45}$	32.6 †	27.9	37.4	21.0
$tu_{44} \times tu_{43}$	20.5	18.1	22.9	10.6
$tu_{44} \times m_{41}$	24.9	14.9	36.4	13.7
$tu_{44} \times tu_{45}$	30.3	26.7	34.1	18.5
$tu_{43} \times m_{41}$	10.2	8.4	12.0	4.5
$tu_{43} \times tu_{45}$	21.8	19.2	24.4	11.5
$m_{41} \times tu_{45}$	15.5	13.1	18.1	7.4
$r_{48} \times tu_{45}$	32.9	29.2	36.7	
$tu_{41} \times tu_{43}$	34.9	29.2	40.8	
$r_{48} \times m_{41}$	35.9	27.5	44.8	
$r_{47} \times tu_{44}$	36.1	31.0	41.3	
$r_{48} \times tu_{43}$	36.4	31.8	41.2	
$r_{48} \times tu_{42}$	36.4	28.9	44.3	
$tu_{41} \times tu_{42}$	36.9	34.6	39.3	
$tu_{41} \times tu_{44}$	37.0	27.9	46.7	
$tu_{41} \times m_{41}$	37.1	31.3	43.0	
$r_{48} \times r_{51}$	37.4	30.3	44.8	
$r_{47} \times r_{48}$	37.7	29.8	46.0	
$r_{51} \times tu_{44}$	38.8	27.3	50.9	
$r_{51} \times tu_{42}$	39.1	35.1	43.2	
$tu_{41} \times tu_{45}$	39.2	34.7	43.9	
$r_{51} \times m_{41}$	39.3	33.4	45.3	
$r_{47} \times tu_{42}$	40.3	27.8	53.5	
$r_{48} \times tu_{44}$	40.8	31.3	50.7	
$r_{47} \times tu_{45}$	41.3	32.9	50.9	
$r_{48} \times tu_{41}$	41.6	25.3	58.7	
$r_{51} \times tu_{45}$	41.7	39.1	44.3	
$r_{47} \times m_{41}$	43.2	37.2	49.3	
$r_{47} \times tu_{43}$	43.6	35.2	52.1	
$r_{51} \times tu_{43}$	45.5	35.9	55.2	

*The weighted mean.

**Calculated from the mean recombination value as described in the Discussion, using equation 2 and m = 5.

† The variance analysis would place this value below the line separating linked from unlinked loci. The linkage of these loci is obvious, however, from their linkages with intermediate markers.

of the two-factor crosses, a linear arrangement of the loci is indicated. When they are placed in the order given in figure 3, a linear arrangement is compatible with the data, since we see that the recombination value for any pair of nonadjacent loci is always larger than the recombination value for any of the intermediate pairs. To be more explicit, let the loci be indicated by 1, 2, 3,

4, and 5 for the larger linkage group in the figure, and let R_{12} be the recombination value for 1 (tu_{42}) and 2 (tu_{44}). R_{23} is the recombination value for 2 and 3 (tu_{43}) and R_{13} for 1 and 3. Then it is seen that R_{13} is greater than R_{12} or R_{23}, and, in fact, this is true for any set of three loci in either linkage group.

One can then proceed to test the question, which naturally follows, whether the recombination values are statistically independent of each other or are

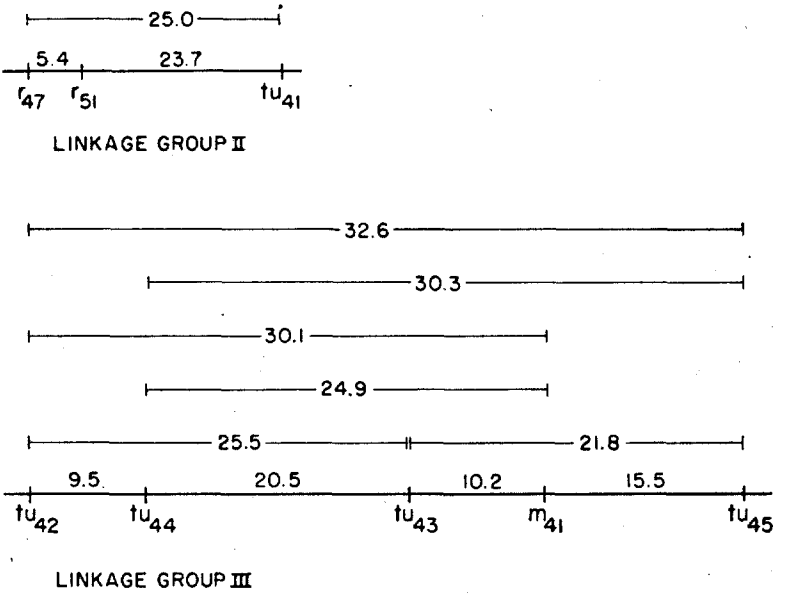

FIGURE 3.—The linear order of loci in two linkage groups of T4. The numbers indicate the observed recombination percentages (R_{ik}). Linkage group II is probably linked with linkage group B discovered in T2 by HERSHEY and ROTMAN (1949) and group III with their group C.

correlated in some way. If they are statistically independent, then the chance of observing a recombination between 1 and 3 could be written as follows (HALDANE 1918):

$$R_{13} = R_{12}(1 - R_{23}) + R_{23}(1 - R_{12}) = R_{12} + R_{23} - 2R_{12}R_{23}. \qquad (1)$$

This calculation has been made for all groups of three loci in both linkage groups and the comparison of the calculated values with those observed is made in table 3. The observed value is, in all cases except one, lower than the calculated value. This suggests that R_{12} and R_{23} are positively correlated, that is, when a recombination occurs between 1 and 2 a recombination in the adjacent region between 2 and 3 has a greater than average chance of occurring. Thus the region between 1 and 3 would be estimated low since the doubles are unobservable in the two-factor cross. We will refer to this apparent excess of double recombination as a positive correlation in the occurrence of adjacent recombinations. It is noteworthy that HERSHEY and ROTMAN (1948) observed a similar positive correlation in three-factor crosses.

Several possible interpretations could be advanced to explain this positive correlation: (1) The loci could be in some order other than a strictly linear one. (2) The mechanics involved in recombination may be such that multiple breaks occur excessively frequently due to difference in fragility of the genetic structure in different individuals. (3) The individual particles which participate in the process of reproduction may vary in the number of opportunities they have for genetic recombination. (A model based on similar considerations has recently been proposed by ROTHFELS (1952) to account for an excess of double recombinants in *Escherichia coli.*)

The last interpretation appears to present the most plausible explanation. To take an extreme example, suppose that only 50 percent of the phage population within one bacterium participated in genetic recombination. Then, considering only this half of the population, the proportion of double recombinants

TABLE 3

Examination of the observed recombination data for statistical independence of individual recombination values.

Series of loci involved			Recombination value between terminal loci (R_{13})		Calculated minus observed
1	2	3	Calculated from equation 1	Observed R_{13}	
			percent	*percent*	*percent*
r_{47}	r_{51}	tu_{41}	26.5	25.0	+1.5
tu_{42}	tu_{44}	tu_{43}	26.2	25.5	+0.7
tu_{42}	tu_{44}	m_{41}	29.7	30.1	−0.4
tu_{42}	tu_{44}	tu_{45}	34.1	32.6	+1.5
tu_{42}	tu_{43}	m_{41}	30.5	30.1	+0.4
tu_{42}	tu_{43}	tu_{45}	36.2	32.6	+3.6
tu_{42}	m_{41}	tu_{45}	36.3	32.6	+3.7
tu_{44}	tu_{43}	m_{41}	26.5	24.9	+1.6
tu_{44}	tu_{43}	tu_{45}	33.0	30.3	+3.0
tu_{44}	m_{41}	tu_{45}	32.7	30.3	+2.4
tu_{43}	m_{41}	tu_{45}	22.5	21.8	+0.7

might be precisely that predicted from the frequencies of the single recombinants. If, however, the whole population is used for estimating the frequencies of single recombinants (as is done in the phage experiments), the predicted number of doubles would be only one-half the number observed.

The plausibility of such an interpretation of the positive correlation is indicated by the results of a number of experiments by several investigators. These results have been brought together into a theory, developed by VISCONTI and DELBRÜCK (1953), which treats the phage cross as a population genetics problem. The basic assumptions of the theory are: (1) that a pool of so-called prophage particles is formed, and that within this pool the particles mate pairwise and at random with respect to partners; and (2) that the prophages engage in several rounds of mating, the number being distributed randomly (Poisson) among the particles. This theory leads to the following relationship between R_{ik} (recombination value for i and k determined in the final yield

from a cross) and P_{ik} (recombination value for i and k in an individual mating of the two particles):

$$R_{ik} = \frac{1 - e^{-mP_{ik}}}{2} \tag{2}$$

where m is the average number of matings per phage particle. From several types of experiments they have furthermore estimated the value of the parameter m to be approximately 5. Thus we may calculate the values of P_{ik} for all pairs of linked loci, and can then proceed to test the assumption that the values of P_{ik} are independent of each other, i.e.,

$$P_{13} = P_{12}(1 - P_{23}) + P_{23}(1 - P_{12}) = P_{12} + P_{23} - 2P_{12}P_{23}. \tag{3}$$

P_{13}, obtained by substituting R_{13} in equation 2, is compared in table 4 with P_{13} calculated on the assumption of independence between P_{12} and P_{23} (equation 3). A good fit to the assumption of independence is obtained. It is also seen in table 4 that the data are quite incompatible with any hypothesis which requires complete interference where

$$P_{13} = P_{12} + P_{23}. \tag{4}$$

TABLE 4

The recombination values (P_{ik}) for individual matings.

Series of loci involved			Recombination values calculated for terminal loci (P_{13})		
			From P_{12} and P_{23}		From R_{13} †
1	2	3	Assuming no interference*	Assuming complete interference**	
			percent	*percent*	*percent*
r_{47}	r_{51}	tu_{41}	14.6	15.3	13.8
tu_{42}	tu_{44}	tu_{43}	14.0	14.8	14.2
tu_{42}	tu_{44}	m_{41}	16.8	17.9	18.2
tu_{42}	tu_{44}	tu_{45}	21.1	22.7	20.7
tu_{42}	tu_{43}	m_{41}	17.4	19.1	18.2
tu_{42}	tu_{43}	tu_{45}	22.4	26.0	20.7
tu_{42}	m_{41}	tu_{45}	22.9	25.6	20.7
tu_{44}	tu_{43}	m_{41}	14.1	15.1	13.7
tu_{44}	tu_{43}	tu_{45}	19.6	22.0	18.5
tu_{44}	m_{41}	tu_{45}	19.1	21.1	18.5
tu_{43}	m_{41}	tu_{45}	11.2	11.9	11.4

* From equation 3.
** From equation 4.
† From equation 2.

It should be pointed out that the condition of statistical independence of the P_{ik} values is not very sensitive to changes in the value of m. If m is reduced to 3, a slight negative correlation (interference) is observed, and when m is increased to 7, a slight positive correlation is obtained. The crucial point is, however, that if one makes the assumption that the opportunities for genetic mixing are randomly distributed and not precisely equal in the individual members of the phage population, then the observed linkages appear to fit a

linear organization very well, and the recombination values are approximately independent of each other.

SUMMARY

A new group of mutants which affect plaque type in bacteriophage T4 has been described. Recombination has been measured among members of this group and also between these and other genetic factors. Clusters of mutants showing less than 1 percent recombination within a cluster were found as well as mutants showing 1 to 45 percent recombination with each other.

No linkage groups in addition to those previously found by HERSHEY and ROTMAN (1949) in phage T2 were discovered. The mutants were found to fall into two of the original linkage groups in such a way that a linkage group of five loci and one of three loci are evident. The data indicate that, in each of the two linkages, the loci are distributed in a linear sequence with a small positive correlation in occurrence of neighboring recombinations (negative interference). It seems likely that the correlation arises from the possibility that the individual particles may mate different numbers of times. If one calculates the recombination values per mating by application of the theory of VISCONTI and DELBRÜCK (1953), the correlation disappears entirely.

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