LINKAGE OF GENETIC MARKERS IN PHAGES T2 AND T4

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IN organisms that undergo meiosis, genetic linkage may be defined as the appearance (among the gametes produced by an F_1 of a dihybrid cross) of a frequency of genotypes different from that predicted on the basis of a free assortment of the alleles at meiosis. This criterion of linkage is not directly applicable in the case of bacteriophage because a cross cannot be performed in an analogous fashion. Indeed, the production of recombinant phage by a mixedly infected bacterial cell can be understood only if it is approached as a problem in population genetics (VISCONTI and DELBRÜCK 1953; HERSHEY 1958).

The measurements made in a phage "cross" are (1) the measurements of genotype frequencies prior to any opportunities for recombination, and (2) measurements of genotype frequencies in the population at later times, after the appearance of infectious phage within the cell. The initial constitution of the population of mating entities (vegetative phage) can be controlled by adjusting the frequencies of the genotypes which initiate the infection. The constitution at any time later, however, may depend on many factors. The number of opportunities for genetic recombination ("matings"), the way in which these matings are distributed in space (the degree of panmixia) and time, the number of vegetative phage particles participating in each mating, and the probability of recombination per mating, as well as the initial genotype frequencies, all may influence the subsequent genetic constitution of the population (HERSHEY 1958; BRESCH 1959).

It is clearly as inappropriate to apply the criterion of nonrandom association of genetic markers among the progeny of a phage "cross" as indicative of linkage as it is to assume that random or near-random association implies nonlinkage. A definition of linkage for two factors which relates to the probabilities of the possible genotypes of vegetative phage emerging from a single mating act would be more to the point. Such a definition would be awkward, however, unless one made specific assumptions about the mechanism of recombination (break-reunion or copy-choice), about the number of vegetative phage particles participating in a mating event, and about the degree to which the participating phages are able to share in the production of any recombinant. Such assumptions would at present be based on weak or nonexistent experimental observations. Furthermore, such

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a definition would have limited operational usefulness, since particles emerging from a single act cannot be directly examined.

In terms of the assortment of alleles at three loci, one can make a definition of linkage that is completely free of assumptions concerning either the kinetics or the mechanism of recombination.

In a phage cross involving linked loci, of the type $ab \times a^+b^+$, the recombinant classes, ab^+ and a^+b are examined for the frequency with which they include one of the two alleles at a third locus whose linkage to ab is in question. If the third locus, c, is not linked to ab, it stands, by definition, in a symmetrical relationship to a and b,

$$\begin{matrix} c \\ a\ b \end{matrix}$$

and the choice of recombinant class between the a and b loci will have no influence on the occurrence of c.

If locus c is linked to ab, however, then its relationship is no longer symmetrical, that is, the complete genotype may be written either as

$$\frac{a\ \ b \qquad\qquad c}{\qquad\qquad\qquad}$$

or as

$$\frac{c \qquad\qquad a\ \ b}{\qquad\qquad\qquad}$$

and the cross to test linkage is either

(1)
$$\frac{a\ \ b \qquad\qquad c}{\qquad\qquad\qquad} \times \frac{\qquad\qquad\qquad}{a^+\ b^+ \qquad\qquad c^+}$$

or

(2)
$$\frac{c \qquad\qquad a\ \ b}{\qquad\qquad\qquad} \times \frac{\qquad\qquad\qquad}{c^+ \qquad\qquad a^+\ b^+}$$

In cross (1) a single mating act should more often lead to the formation of ab^+c^+ than to the formation of ab^+c because only one event is required for the former, while two are necessary for the latter. This is true independently of whether the events are breaks and reunions, or switches from one parent to the other during the course of replication, and independently of whether mating is pairwise or groupwise. For cross (2) the frequencies are reversed.

In both crosses, the frequencies of $(ab^+)c^+$ and $(ab^+)c$ tend towards the same equilibrium value as the number of rounds of mating increases. Two variables can be manipulated to aid in maintaining the difference in frequencies. The opportunities for successive matings can be minimized by premature lysis, and the crosses can be performed with an unequal multiplicity of the two parents.

In practice, two crosses are performed: Cross I with $abc > a^+b^+c^+$ and Cross II with $abc < a^+b^+c^+$ (Figure 1), and bacteria are prematurely lysed in order to

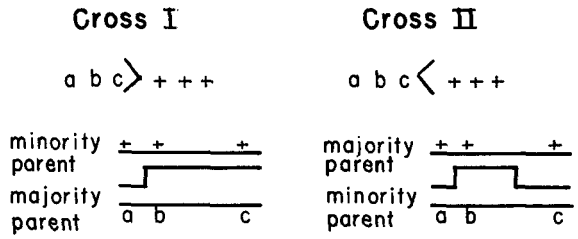

FIGURE 1.—The types of crosses used to detect linkage.

examine the phage population when the number of rounds of mating is small. The relative multiplicities of the majority and minority parents are the same for each cross. In each case ab^+ recombinants are selected and *among these* the frequency of the *c* allele contributed by the minority parent is measured. The frequency desired for Cross I is $\frac{ab^+c^+}{ab^+c + ab^+c^+}$; that for Cross II is $\frac{ab^+c}{ab^+c + ab^+c^+}$. If *c* is not linked to *ab*, the frequencies would depend solely on the relative multiplicities of the majority and minority parents, and should thus be identical for the two crosses. In the case of linkage, however, the frequency for Cross I (where the desired class may be the result of one event) should be higher than the frequency for Cross II (where two events are required). This criterion of linkage is entirely independent of the mechanism of mating or replication.

MATERIALS AND METHODS

Phage strains T2H *r*1, *r*2, and *r*7 were obtained from DR. A. D. HERSHEY; T4D *r*47, *r*48, and *tu*45 were obtained from DR. A. H. DOERMANN; and *r*73 was isolated in the course of this study.

Bacterial strains *Escherichia coli* H and S were obtained from A. D. HERSHEY, BB from G. STENT, K-12 (λ) from J. WEIGLE, and B from S. E. LURIA.

Experiments with T2

Media.—Broth: Bacto peptone, 10 gm; Bacto beef extract, 3 gm; sodium chloride, 5 gm; glucose, 1 gm; H_2O, 1 liter. Bottom agar: Bacto agar, 10 gm; Bacto tryptone, 10 gm; NaCl, 8 gm; sodium citrate, 2 gm; glucose, 1 gm; H_2O, 1 liter. Top agar: Has the same composition but contains only 0.7 percent agar. Buffer: Na_2HPO_4, 3 gm; KH_2PO_4, 1.5 gm; NaCl, 4 gm; K_2SO_4, 5 gm; gelatin, 0.01 gm; $MgSO_4$, 10^{-3} M; $CaCl_2$, 10^{-4} M; redistilled water, 1 liter. M-9: Na_2HPO_4, 7 gm; KH_2PO_4, 3 gm; NH_4Cl, 1 gm; H_2O, 1 liter.

Stocks were prepared on BB in M-9, and in the case of double *r* mutants were concentrated by centrifugation.

Crosses were performed on H grown in broth to a titer of about 1×10^8 per ml, washed and resuspended in buffer, and aerated for 45 to 60 minutes.

Premature lysis was accomplished by dilution into broth saturated with chloroform (SÉCHAUD and KELLENBERGER 1956).

Plating was on S or else on K-12 (λ). The bottom agar described below was used for the latter.

Experiments with T4

Media.—Broth: Bacto tryptone, 10 gm; NaCl, 5 gm; glucose, 10 gm; (added after autoclaving); H_2O, 1 liter. Bottom agar: Bacto agar, 10 gm; bacto tryptone, 10 gm; NaCl, 5 gm; H_2O, 1 liter. Top agar: Has the same composition but contains 0.7 percent agar.

Stocks were prepared on BB, by the plate method (SWANSTROM and ADAMS 1951), and were purified by centrifugation.

Crosses were performed on B grown in broth to a titer of about 1×10^8 per ml, washed and resuspended in buffer, and aerated for 45 to 60 minutes. Tryptophane (20 γ/ml) was added immediately before infection. Usually more than 90 percent of bacteria survived as infective centers.

Premature lysis is accomplished by dilution into broth saturated with chloroform.

Platings were performed on B or on K-12 (λ).

EXPERIMENTS

Linkage of r7 and r1 in T2: We chose two closely linked factors, *r*2 and *r*7, and tested for linkage to *r*1, a factor previously considered unlinked. Mutants *r*2 and *r*7 are members of the *r*II group (BENZER 1955) and do not form plaques on *E. coli* strain K-12 (λ) (=K). Only *r*2+ *r*7+ recombinants among the progeny of a cross of *r*2 × *r*7 phage will give rise to plaques when plated on K, and these plaques can readily be classified as *r*1 or *r*1+ by inspection.

Crosses of *r*2 *r*7+ *r*1 × *r*2+ *r*7 *r*1+ were performed with multiplicities of 1: ⩾10 or ⩾10:1 phage particles of each type per bacterium (Crosses Ia and IIa, Figure 2). In order to recognize any selective effects due to the presence of the *r*1 or the *r*1+ allele, similar crosses were performed with the positions of the two *r*1 alleles reversed (Crosses Ib and IIb, Figure 2).

The bacteria in which the crosses were performed were lysed as soon as they contained measurable numbers of intracellular phage particles. Differences in the frequencies of recombinant types are expected to be greatest at this time. Samples were also lysed at various later times, to observe the changes in recombinant frequencies to be expected because of repeated matings. The results are shown in Figure 2 where the frequency of the *r*1 allele of the minority parent, among *r*2+ *r*7+ recombinants, is plotted for each of the four crosses. The frequencies of the minority allele were different in Cross I and Cross II, indicating linkage among the three markers. The order of the markers is *r*2–*r*7–*r*1. Reversing the positions of the *r*1 alleles did not lead to important changes in frequencies, an indication that no strong selective effect can be ascribed to either of the *r*1 alleles. In experiments performed on any one day the difference in recombinant frequencies between the two types of crosses was more consistent than appears from the figure.

The efficiency of plating (e.o.p.) of T2 *r*II+ on K is 0.1–0.3 of that on B. The

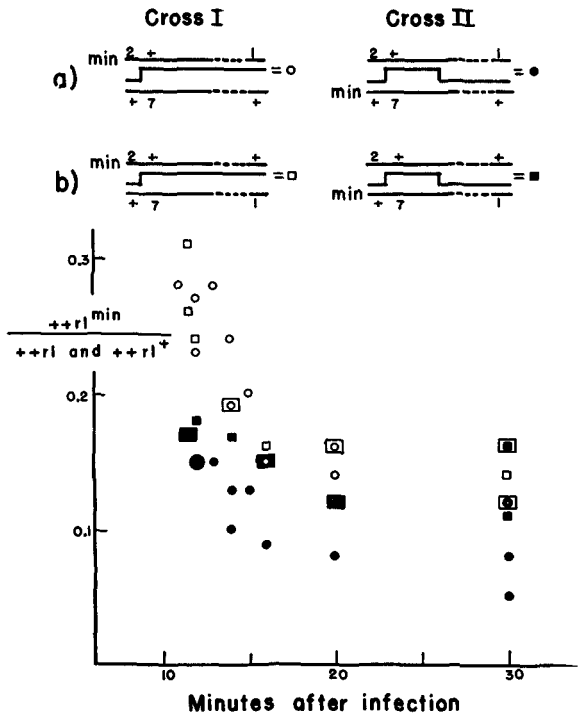

FIGURE 2.—Linkage between *r*1 and *r*7 in T2. Four crosses were performed as illustrated in the figure, where min indicates the minority parent. Results are plotted as frequencies of the *r*1 allele, contributed by the minority parent among recombinants (*r*2$^+$ *r*7$^+$) with respect to *r*2 and *r*7, for varying times of lysis (infection at time zero). The different symbols refer to the different crosses that are illustrated. Large symbols, or superimposed symbols, represent several points.

low e.o.p. does not influence the results described above since the e.o.p. of *r*II+ *r*1 and *r*II+ *r*1+ on K are identical (compare also the results of Crosses a and b, Figure 2). The low e.o.p. does, however, interfere with a precise determination of the frequency of *r*II+ phage among the progeny at various times of lysis. A determination of this frequency seemed necessary in order to compare the rates of replication and mating in crosses of types I and II. Crosses were therefore performed with T4, since the e.o.p. of T4 *r*+ on K is identical to that on B.

Linkage of r47 and r48 in T4: Experiments identical to the ones described above were performed with phage T4. The linkage of the *r*II mutants *r*47 and *r*73 to *r*48 (a mutant linked to the *r*1 of T2) was examined.

The types of crosses made and the results obtained are shown in Figure 3A. There was again a clear-cut distinction between crosses of types I and II, indicating linkage in the order *r*73–*r*47–*r*48. It seemed possible that the differences in frequencies of recombinant types measured in crosses of types I and II were due to different rates of replication and mating. To evaluate this possibility, the frequency of *r*73+ *r*47+ recombinants among the total progeny was measured in one cross of type I and one of type II (Figure 3B). The fact that the frequency of

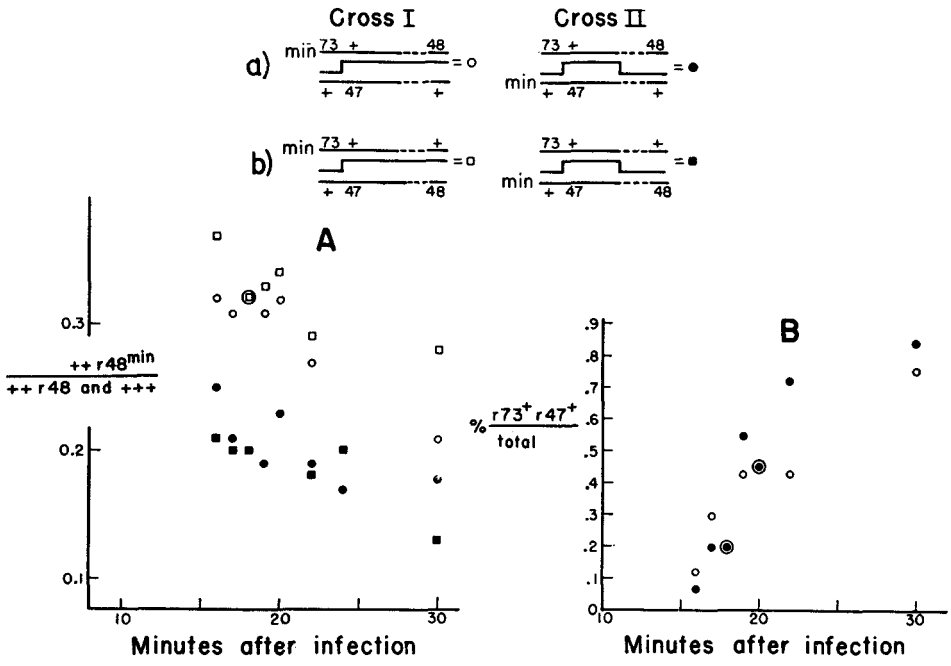

FIGURE 3.—Linkage between *r47* and *r48* in T4. Four crosses were performed, as illustrated in the figure, where min indicates the minority parent. The different symbols refer to the different crosses that are illustrated. Large symbols, or superimposed symbols, represent several points. Results in A are plotted as frequencies of the *r48* allele, contributed by the minority parent among recombinants ($r73^+$ $r47^+$) with respect to *r73* and *r47*, for varying times of lysis (infection at time zero). The percents of recombinants $r73^+$ $r47^+$ in the total population are plotted in B.

recombinants at various times of lysis was similar for the two crosses indicated that there were no large differences in rates of mating.

A comparison of the order r*II B cistron,* r*II A cistron and* r*1 (r48) in T2 and T4:* The mutants *r47* and *r73* of T4 belong to cistrons A and B, respectively (BENZER 1955; EDGAR 1958). It could be shown, by spot-test crosses against a set of standard T4 deletions as described by BENZER and FREESE (1958), that *r7* of T2 belongs to the A cistron, and *r2* of T2 to the B cistron. The order B cistron—A cistron—*r*1 (*r48*) is thus the same in T4 and T2.

Linkage of r*47,* r*48, and* tu*45 in T4:* We examined the linkage of *r47* and *r48*, already shown to be linked, to *tu45* previously considered unlinked to the others.

The design of the experiments was similar to those previously discussed. Crosses of *r47 r48 tu45* × *r47+ r48+ tu45+* were performed at a multiplicity of 1:10 and 10:1, and, to control possible selection, the cross was repeated with the position of the *tu* alleles reversed. The progeny were plated on K, and the *r* plaques appearing were classified as *tu* 45 or *tu45+* (Figure 4). The *r* plaques on K represented *r47+ r48* recombinants; classifying these as *tu* or *tu+* distinguishes the single-recombinant from the double-recombinant types. It is clear from the results

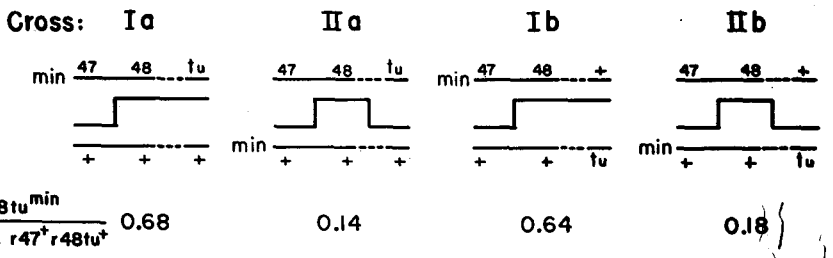

FIGURE 4.—Linkage between *r*48 and *tu*45 in T4. Four crosses were performed, as illustrated. The frequencies of the *tu*45 allele, contributed by the minority parent, among recombinants (*r*47$^+$ *r*48) with respect to *r*47 and *r*48, in cultures lysed 11½ minutes after infection, are presented.

presented in Figure 4 that there is strong linkage between *r*48 and *tu*45 and that the order is *r*47–*r*48–*tu*45.

DISCUSSION

Our data indicate that the known genetic markers of phages T2 and T4 are linked as had already been found for the smaller phages. Thus all the known bacteriophages have a continuous genetic map, and therefore, presumably, a continuous genetic structure. This continuity simplifies the problem of the assembly of all of the genetic material of a bacteriophage into one particle during the course of maturation.

Our results confirm the association of two groups previously considered unlinked that was independently described by BAYLOR, HURST, ALLEN, and BERTANI (1957) and are compatible with the findings of MCFALL and STENT (1958) that an anomaly of the genetic structure of a certain strain of T2H involves part of the *r*II region and at the same time is linked to *r*1. SEKELY (personal communication) has recently shown linkage between all known markers of T2L.

SUMMARY

This report describes sensitive tests of linkage, which have indicated that all the known markers of the T-even phages can be arranged in one linkage group. The results presented here agree with the independent findings of BAYLOR *et al.* (1957), who found a marker linked to both of previously unlinked groups in T2, and with those of MCFALL and STENT (1958), who described a genetic anomaly affecting markers on each of the remaining two groups, thus linking them.

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