A GENETIC ANALYSIS OF BACTERIOPHAGE LAMBDA

Thesis by

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In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1955

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr. J. J. Weigle for his suggestions and encouragement in the course of the experiments and to Dr. Renato Dulbecco and Dr. Marguerite Vogt for their discussions and advice during the preparation of this manuscript. The author is indebted to Dr. Max Delbruck whose approach to biology was a constant inspiration.

This work was made possible by the financial assistance of the National Foundation for Infantile Paralysis. In addition the author was privileged to hold a McCallum Fellowship during the summer of 1951.

ABSTRACT

The genetic structure of the temperate bacteriophage lambda was analysed by means of crosses. Mutations in lambda which affected the size or type of plaque initiated by single particles were induced by ultraviolet irradiation. Each of four mutants so obtained was found to differ by one factor from the reference type. Two factor crosses demonstrated the existence of linkage between the 4 mutant factors and suggested a linear sequence which was later confirmed by three factor crosses. In addition 5 other factors were found to be linked to the first 4, thus all 9 factors studied seem to lie on the same linkage group.

A striking feature of three factor crosses was the high frequency of double crossover types. This "apparent negative interference" was traced to the yields of individual bacteria by means of single burst experiments.

Finally, the compatibility of the results of the lambda crosses with two different theories of phage recombination was examined.

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INTRODUCTION

It has been the object of this work to study the behavior of a temperate phage (phage λ) in genetic crosses.

The behavior of virulent phages, especially T2, in crosses has been extensively studied previously. Hershey (1) reviews this work. Operationally a cross consists in allowing two different but closely related phages to multiply in the same bacterium. When a bacterium is infected with two phage particles differing from each other by two heritable changes (mutations), the yield of new phage usually contains in addition to the parental types two new types. Each new type shows a characteristic of one parent combined with a characteristic of the other parent. Crosses with numerous markers in T2 have shown that the new types are recombinants, i.e. that they originate from the genetic interaction of the two parental types. The crosses also indicate that the markers are arranged in linkage groups, and that recombination between markers belonging to the parental phage occurs during a series of matings within an intracellular phage population.

A temperate phage differs from a virulent phage by its ability to establish a stable symbiosis with the bacterium it infects. A virulent phage is by definition one which can-

not do so. In the symbiotic state both the bacterium and the phage multiply, but the phage is non-infective. This non-infective state is called prophage. A bacterium carrying prophage transmits one or more copies of its prophage to each of its daughter cells, and they to theirs, etc. This property of carrying prophage is thus heritable. A bacterium which carries prophage is said to be lysogenic. Given the proper stimulus, a lysogenic bacterium will lyse producing phage identical with the original infecting phage. Infection by a temperate phage leads in most cases either to the lysogenic state or to lysis after a fixed latent period with the production of new phage. The latter behavior which is outwardly similar to infection by a virulent phage is called the "productive response." The physiological state of the bacterium before infection largely determines which pathway will be followed (2).

The capacity of temperate phages to give rise to a lysogenic response is also reflected in the appearance of their plaques which, in contrast to the clear plaques of virulent phages, contain a bacterial colony in their center. The colony consists of a mixture of lysogenic and sensitive bacteria. The degree of turbidity of the plaques of temperate phages can be used as an additional morphological character for the differentiation of plaque mutants.

A large body of work with lysogenic bacteria has led to

the interpretation of the lysogenic bacterium as containing an association of phage genetic material with bacterial genetic material. The evidence for this view will be only briefly sketched here; a more detailed review can be found in Lwoff (3). First, the superinfection experiments of Bertani (4,5,6) and of Jacob and Wollman (7) suggest that each lysogenic bacterium contains a number of prophages that is approximately equal to the number of its nuclei. Second, bacterial crosses show that the character "lysogeny" behaves as do other bacterial markers (8,9). An interpretation consistent with both sorts of experiments is that the establishment of lysogenesis consists in the combination of phage genetic material with bacterial genetic material at a specific locus.

There are at least two reasons for making a genetic analysis of a temperate phage. First, it may be possible to explore the relation between prophage and host with genetic experiments. An essential preliminary to these experiments would be the genetic study of a temperate phage itself. Second, it might be expected that temperate phages would interact with each other differently than would virulent phage in which a specific affinity for bacterial genetic material has not been found.

In the following the results of crosses between different mutants of the temperate phage lambda, a phage carried by

Bacterium coli K12, will be reported and their implications for the mechanism of recombination in a temperate phage discussed.

MATERIALS AND METHODS

Organisms

Strain D, a Bacterium coli K12 derivative, was used wherever λ sensitive bacteria were required for plating, as host for crosses, and for the preparation of phage stocks. This strain was preferred over K12 S (10) or coli C (11) because it yielded larger λ plaques. In order to avoid the effects of host controlled variations (11) in crosses, strain D was also used as the host bacterium.

Grown in tryptone broth supplemented with 10 micrograms of vitamin B1 per milliliter, D has a generation time of about 45 minutes at 37°C.

The steps in the derivation of D are given below. It should be noted that in this derivation several treatments with radiation were involved.

Strain	Genotype	Source	Agent
K12	lysogenic, wild type		
Y70	lys. $TLB_1^-Lac_1^-V_{1,5}^-$	K12	two X-ray treatments, one UV treatment--Lederberg
C60	defective lysogenic	Y70	superinfection with weak virulent--Appleyard
C600	sensitive	C60	UV treatment
D	sensitive	C600	single colony isolate

The treatments with radiation have not destroyed the ability

of D to become lysogenic. Several lysogenic strains have been prepared by infection of D with different λ mutants.

Lambda (λ) 26 was isolated from a λ-lysate prepared by ultra-violet induction of K12 (λ) and plated on D. Plaques similar to λ 26 represented about 0.1% of the total in the lysate. Lambda 26 forms large, turbid plaques on D. Lambda 26 was chosen for the reference type because it forms larger and more uniform plaques than the most common type found in lysates of K12 (λ). The plaques are also larger than those of λ_1, described by Lieb (2).

The latent period of λ26 on D is 45 minutes at 37° C and the average burst size is 50 under the conditions of a cross.

Media

a) liquid culture medium: Difco Bacto-tryptone 10g, plus 5g NaCl per liter

b) solid culture medium: Difco Bacto-tryptone 10g, plus 2.5g NaCl, plus 10g Difco Bacto-agar per liter

c) buffer: Na_2HPO_4 7g, KH_2PO_4 3g, $MgSO_4$ 0.2g NaCl 4g per liter

d) adsorption medium: M/100 $MgSO_4$ (in doubly distilled water)

Irradiations with ultra-violet light

A 15 watt GE "germicidal" lamp was used as the source of

ultra-violet radiation (UV). Samples were irradiated at a distance of 20 cm from the center of the lamp. The intensity at the sample was of the order of 1000 ergs per second per cm^2 at 2537 Å.

Preparation of phage stocks.

To obtain genetically pure stocks of different λ mutants, single plaque of the desired type was picked and replated. The picking and replating was continued until a plate containing only the desired type was obtained. From a uniform plate a single plaque was cut out and transferred to tryptone broth.

The suspended plaque was used to prepare a high titer stock by the agar layer method of Swanstrom and Adams (12). Enough phage was mixed with resting phase D and plated to obtain confluent lysis. The plates were incubated right side up 5 to 7 hours at 37° C--for shorter or longer times the phage yield was less. At the end of the incubation period the plates were inverted and about 1/2 ml of chloroform added to the lid of each petri plate, to kill the remaining bacteria by the chloroform vapor. The soft agar top layer was scraped off and eluted with buffer or M/100 magnesium sulfate. The agar and bacterial debris were centrifuged down, leaving most of the phage in the supernatant fluid. The stocks prepared in this way contained 10^{10} to 2×10^{11} infective λ per ml. Over a 4 months storage at 4° C there was no appreciable loss in titer. The stock fluids contained about 10% tryptone

broth eluted from the tryptone top agar.

This isolation technique insures that a stock contains the offspring of a single phage particle. Such stocks were found to be genetically quite homogeneous.

Plating of λ on D

For phage assay and determination of plaque type the agar layer method (13) was used with the modification that the plating bacteria were irradiated with UV (to 30% survival). The bacteria were irradiated to obtain uniformity of plaque size. On non-irradiated bacteria the plaques produced by a genetically homogeneous stock are quite variable in size.

Ordinarily the plating suspension was added to the tube containing melted soft agar, then the phage was added and the contents of the tube poured over the surface of an agar plate. In plating the yield from crosses for the classification of plaque types a variation on the agar layer technique, called preadsorption, was employed. The variation consisted in first mixing phage and plating bacteria in M/100 magnesium sulfate at 37° C for 10 minutes and finally adding the melted agar. The advantage of preadsorption is that adsorption of phage to bacteria is faster and therefore more uniform in time than on the plate under ordinary conditions. Preadsorption tends to increase the uniformity of the plaques of any one type.

In plating the yield of crosses the best differentiation of plaque types was obtained by plating on fresh plates (pre-

pared the same day as used) containing 40 ml of bottom agar in 100 mm petri dishes. The plates were incubated 17 hours at 37° C and then 5 hours at room temperature (ca 20° C). Examination of plates was made under 9x magnification with oblique illumination against a dark background.

Anti- λ serum

Immune serum was obtained from a rabbit which had received repeated subcutaneous injections of the *c* mutant (see the experimental section) of λ26. A 100 fold dilution of this serum at 37° C neutralized (prevented the growth of) more than 99% of the phage in a suspension containing 10^7 phage per ml in 10 minutes.

EXPERIMENTAL SECTION

I. Isolation of mutants

Recently Weigle developed a method for the induction of mutations in λ (14). The method consists in irradiating both the phage and the host bacterium. It was found that UV'd λ had a higher survival on UV'd sensitive bacteria than on non-irradiated bacteria. Among the plaques of UV'd phage plated on UV'd bacteria several percent contained mutants; of these, 98% were pure for the mutant type.

Weigle's method was used to obtain mutants of λ 26. Figure 1, in which the number of λ 26 survivors is plotted versus the UV dose, shows that the survival of UV'd λ 26 is greater on UV'd than on non-UV'd bacteria. A dose of 3 1/3 minutes (1.4×10^{-3} survival on UV'd D) gave more than 4% mutant plaques. Seven mutants to be used in the crosses, subsequently reported, were isolated from plaques on UV'd bacteria. All 7 mutants were obtained with doses which left from 0.1% to 5% survivors on UV'd D.

Two further mutants, c_1 and c_4, used in the present work were obtained when λ 26 and the m mutant of λ 26 were plated on UV'd D. It cannot be decided whether these two mutants were already present in their respective stocks or were produced by a mutagenic action of the UV'd D. The second possi-

bility has to be considered in view of the finding of Jacob (15) that UV treated K12S has a mutagenic effect on lambda. However, since the original mutant plaques were pure with respect to the mutant type, the first possibility appears more likely. In addition two other stocks, one stock of a recombinant with the phenotype of λ 26, and the other an s stock, produced the same proportion of plaques with the c phenotype on non-irradiated D as on UV'd D; thus, the irradiation of the bacteria appears not to be a factor in the origin of the c plaques.

II. Identification of Mutants

Nine mutants were used in the present work. A list of their denominations, plaque morphologies, and sources are given in Table 1. Figure 2 shows the more common mutant and recombinant types.

In general these mutants differ in one of two respects from their progenitor, λ 26; either they form smaller plaques (e.g. s and m), or they produce plaques with less bacterial growth within them (e.g. c, co).

When only plates with a technically good development of plaques are examined, the classification of plaque types is clear cut. With one exception none of the plaque size distributions of different plaque types overlap each other. The exception concerns + and s, the size distribution of + having a tail which overlaps the distribution of s. Difficulty in

distinguishing s from + arises when there are many + and a few s on the same plate. The situation existed in crosses where + was one of the parents and s one of the recombinants. In these cases all of the plaques suspected of being s were picked and retested. The test consisted of plating the unknown phage together with s co stock. The test makes use of the fact that + plaques are, on the average, larger than s co, while s plaques are smaller. Since many plaques arising from the one unknown plaque are involved in the comparison, the test becomes unambiguous. Control replatings of the same unknown phage or plating of stock mixtures has always given the expected result. In those cases where + is one of the parents and s a recombinant, some s plaques may be missed because the observer must judge whether or not a particular plaque should be picked and retested. Probably no more than 20% of the s plaques were missed, however. To minimize this difficulty, experiments were planned so that the s class would not seriously affect the results.

III. Genetic purity of different mutant stocks

Prepared by the method described earlier, the mutant λ stocks were found to contain only a small proportion of other genetic types, never exceeding 0.1%.

Table 2 shows the frequency of other mutant types present in the different λ stocks studied. To permit a comparison with the crosses to be described later the pro-

portions of the various mutant types in the stocks were measured on UV'd D.

IV. Crosses between different λ mutants and their recombinants

A. Cross procedure

A standard procedure was adopted for all crosses and is shown by the following schedule.

Host bacteria: A culture of K12D, grown in tryptone broth plus 10 μg/ml vitamin B1 to a concentration of 10^9 viable cells /ml, was centrifuged, resuspended in the same volume of M/100 $MgSO_4$, and aerated 60 minutes at 37°C to exhaust its food reserves.

0 minutes:	The adsorption mixture was prepared by adding 0.1 ml of phage 1, 0.1 ml of phage 2, and 2.2 ml of M/100 $MgSO_4$ to 0.6 ml of D. The adsorption mixture was incubated at 37° C.
5	A viable cell assay of the uninfected D culture was made.
15	Anti-lambda serum was added to an aliquot of the adsorption mixture at 37°C. The final serum dilution was 1/100.
16	The remainder of the adsorption mixture was centrifuged and the supernatant assayed for unadsorbed phage.
25	Phage growth was started by diluting the adsorption mixture $1/10^4$ into tryptone broth plus B1 at 37°C.
27	Assays of free phage, infected bacteria, and surviving bacteria were made from the serum-treated adsorption mixture. Anti-lambda serum was added to the plate on which the surviving bacteria were assayed to destroy the large

excess of phage released by the infected bacteria.

115 Chloroform was added to the growth tube to kill the surviving bacteria. Plates were made from the growth tube to examine the yield of the cross. The growth tube was stored at 4°C for further plating.

In crosses A, B, E, F, G, G1, H, L, L1, M, N, and AB, adsorption of phage to bacteria without phage growth was accomplished by adding phage to bacteria in tryptone broth containing M/100 KCN. (A technique described by Benzer and Jacob, (16).) In cyanide broth 45 minutes were allowed for adsorption.

B. General results of crosses

The amount of adsorbed phage was calculated by subtracting the unadsorbed phage from the input. Ninety percent or more of the input phage adsorbed in M/100 magnesium sulfate, but only 20 to 25% in cyanide broth. The average multiplicity of infection was found by dividing the number of adsorbed phage particles by the number of viable bacteria in the adsorption mixture.

It was generally found that 50% of the input bacteria gave the productive response, i.e. formed plaques when plated on sensitive bacteria, while 50% survived as colony formers. The concentration of free phage in the serum treated adsorption mixture was less than 0.25% of the concentration of infected bacteria.

C. Specific results of crosses

1) Introduction

In Table 3 all crosses are listed in alphabetical order. The multiplicities of infection and the numbers of the various types of progeny found in the different crosses are entered in the table.

In the experiments to be described, it will first be demonstrated that each of 4 of the mutants differs from the reference type, λ 26, by a single factor (one factor crosses). Next, two factor crosses will show the existence of a unique recombination frequency for each pair of factors and the possible existence of a particular serial order of these factors. Subsequently, three factor crosses will be described which confirm the serial order suggested by the two factor crosses. In addition, they reveal that the double crossover types occur much more frequently than expected. An hypothesis which would explain the excess of doubles in terms of an inhomogeneity in the population of infected bacteria is then excluded by means of single burst experiments. Finally, some aspects of the mechanism of recombination in λ will be examined. For this purpose an analysis of the distribution of recombinants among the yields of single cells and the results from a triparental cross will be discussed.

2) One factor crosses

The mutants s, c, co, and m, each obtained independently after a single UV treatment of λ 26 (see Table 1) were crossed to λ 26 and the results are given below.

Cross Name	Parents	Yield
A	s x +	parental types only in 4390 progeny
B	co x +	parental types only in 4868 progeny
C	m x +	parental types only in 1659 progeny
D	c x +	parental types only in 12, 500 progeny

As seen from the results, the absence of non-parental types in the progeny shows that each of the 4 mutants differs from λ 26 by a single factor.

3) Two factor crosses

All 6 possible two factor crosses between the 4 mutants s, c, co, and m were made with the following results.

Cross Name	Parents	Yield % of recombinant types		Total Number Examined
E	s + x + co	1.0 + +	1.0 s co	992
F	s + x + c	1.3 + +	1.4 s c	1413
G1	+ co x c +	0.06 + +		3600
G2	+ co x c +	0.1 + +		2700
H1	co + x + m	2.2 + +	2.1 co m	4239
H2	co + x + m	1.9 + +	2.8 co m	899
H3	co + x + m	3.0 + +	3.1 co m	1482
J	c + x + m	3.3 + +	2.9 c m	2577
K	s + x + m	5.1 + +	4.4 s m	1270

As may be seen from the table, in all crosses except G, the two expected recombinant types were observed at equal frequencies. Even the crosses G are probably no exception since it is very likely that the plaques of the recombinant c co cannot be distinguished from those of the c parent. It should be added that the recombinant types in all of the crosses occurred at frequencies much higher than those of spontaneous mutants (compare with Table 2).

The genetic constitution of some of the recombinants was verified by further crosses. (see the following table)

Cross Name	Parents	Yield % of recombinant types		Total Number Examined
L1	s co (E) x + + (E)	0.8 s +	0.6 + co	1397
L2	s co (E) x + + (E)	---	0.6	7230
M	s co (E) x + + (λ 26)	0.8 s +	0.6 + co	1900
N	+ + (λ26) x + + (E)	0.0 s +	0.0 + co	2310
O	co m (H) x + + (H)	2.4 co +	2.0 + m	1481

The symbol within parentheses indicates the origin of the particular stock used. E refers to cross E. H to cross H and λ 26 to the reference type λ 26.

The crosses L, M, and O yielded again the original parental types at equal frequencies, while cross N showed the genetic identity of a + + recombinant to the original reference type. It has also to be mentioned that the averaged frequency of recombinants in the crosses L (crosses in coupling) is, within sampling error, the same as that of cross E (cross in repulsion), i.e. 1.3 ± 0.1% and 2.0 ± 0.5% respectively.

The same holds for the crosses involving the factors co and m (4.5 ± 0.5% and 4.8 ± 0.3%).

If we now compare the recombination frequencies for different pairs of factors, a rough additivity of these is suggested. Thus we find a recombination frequency of 1.4% for the pair s and co, of 4.8% for co and m, and of 9.5% for s and m. A similar situation holds for the frequencies of s and c 2.8%, of c and m 6.2%, and of s and m 9.5%. These results indicate that all 4 markers lie on the same linkage group in the sequence s - (c) co - m. Results from three factor crosses to be reported next confirm this conclusion.

4) Three factor crosses

a) Evidence for a single linkage group

In three factor crosses, the asymmetric distribution of one factor within the recombinant class for the other two factors indicates the presence of linkage. As can be seen in the following table, the factors m, co, and s show an asymmetric distribution in one of the two recombinant classes. (The data for the other recombinant class show a similar asymmetry and were therefore omitted from the table)

Cross Name	Parents	Number of m, m^+ Types among s-co recomb.			Number of co, co^+ Types among s-m recomb.			Number of s, s^+ Types among co-m recomb.		
V1	s co m x + + +	+ co	m	178	+ m	co	178	co +	s	477
			m^+	71		co^+	455		s^+	71
V2	s co m x + + +	+ co	m	32	+ m	co	32	co +	s	61
			m^+	13		co^+	51		s^+	13
W	s + m x + co +	+ +	m	121	+ m	co^+	121	+ +	s	273
			m^+	38		co	318		s^+	38

It can be concluded that m is linked to s or co (first column), co to s or m (second column), and s to co or m (third column), from which it follows that the three factors s, co, and m lie on the same linkage group.

b) Evidence for linearity

The additivity relation between the recombination frequencies found in the two factor crosses suggested that s, co or c and m are linearly arranged. Since the largest recombination frequency was obtained for the markers s and m, the particular order suggested was s - (c) co - m.

The three factor crosses given in the following table were made to confirm this sequence.

Cross Name	Cross Parents	Yield % of recombinant types		Total Number Examined
VI	s co m x + + +	3.4 s co +	3.2 + + m	14,171
		1.3 + co m	0.5 s + +	
		0.5 + co +		
V2	s co m x + + +	2.9 s co +	2.4 + + m	2,091
		1.5 + co m	1.4 s + +	
		0.6 + co +	0.2 s + m	
W	s + m x + co +	2.6 + co m	2.2 s + +	12,324
		0.9 s co +	1.0 + + m	
		0.2 s co m	0.3 + + +	

The genetic constitution of the triple mutant, s co m, derived in cross P and used in cross V had first been confirmed by crossing it to each of the three single mutants (see crosses R, T, and U).

Cross Name	Parents	Yield	Total Number Examined
R	s x s co m	all s	12,876
T	m x s co m	all m	10,766
U	co x s co m	all co	12,335

The absence of s^+, m^+, and co^+ respectively in the three crosses demonstrates that the genetic constitution of the mutant in s co m.

In crosses V and W the types which occurred with the lowest frequencies and which should, therefore, be due to double crossingover are those expected for the order s - co - m. They thus confirm the order suggested by the two factor crosses.

It should be mentioned that the recombination frequencies for the three factor crosses V and W are higher than those in the two factor crosses E, L, H, and O involving s and co, and co and m.

Cross	% crossing-over in the region s - co	co - m	s - m
V1	3.5*	7.6	9.1
V2	3.8	6.2	8.2
W	2.4	5.3	6.7
E and L	1.4		
H and O		5.0	
K			9.5

*value obtained by doubling the frequencies of the + co m and + co + classes.

The difference might be due to the fact that in crosses E, L, and H1 the infected bacteria were treated with cyanide.

This is supported by the fact that smaller differences were found in the case of two factor crosses involving s and m in which no cyanide was used.

5. Four factor cross

To determine the relative position of c and co the following cross was made.

Cross Name	Parents	Yield % of recombinant types		Total Number Examined
X	s + co m x + c + +	3.2 s + co +	2.9 + c + m	18,062
		0.7 s c + +	0.6 + + co m	
		0.25 + + co +		
		0.06 + + + +		
		0.07 s + + +	(13 plaques)	
		0.03 + + + m	(5 plaques)	

Although the numbers in the critical classes are small, the data suggest the following order s - c - co - m.*

6. Preliminary mapping of 5 other clear plaque mutants

In addition to c and co several other clear plaque mutants

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*Although the difference between the numbers of the class s + + + and the class + + + m is only on the borderline of significance (P = 0.06), it should be mentioned that in the case of the alternative sequence s - co - c - m, the + + + m class should not only be equal to but should be larger than the s + + + class.

The similarity between the numbers of the presumptive triple crossover type (+ + + m) and the presumptive single crossover type (s + + +) will be treated in the discussion section.

of λ 26 were approximately mapped c_1, c_2, c_3, c_4, and co_1. The following crosses are relevant to the mapping of these factors.

Cross Name	Parents	Yield % of recombinant types		Total Number Examined
AA	co x c_4 m	No + + m		4,932
AB	c x c_1	Parental types only		2,735
AC	c_3 x m	3.1 + +	3.4 c_3 m	1,724
AD	c_2 x m	3.2 + +	3.4 c_2 m	1,846
AE	co_1 x m	3.0 + +	3.6 co_1 m	3,666
AF	+ co_1 + + x s + co m	3.1 s co_1 +	3.2 + co_1 m	5,434
		0.9 s + + +		
		0.15 + + + +		
AG	+ c_2 + + x s + co m	2.5 s + co +	2.5 + c_2 + m	2,997
		0.7 s c_2 + +	0.5 + + co m	
		0.3 + + co +		
		0.1 s + + +		
		0.07 + + + +		
AH	+ c_3 + + x s + co m	3.2 s + co +	3.1 + c_3 + m	4,879
		0.9 s c_3 + +	1.0 + + co m	
		0.4 + + co +		
		0.1 s + + +		
		0.06 + + + +		

As may be seen from the table all 5 mutants are closely linked to c or co (0 - 1% recombination frequency), and less

closely to m (6.5%). The preliminary data suggest that co_1 is located between s and c, whereas c_4 appears to be allelic to co and c_1 allelic to c.

7. The "s effect"

In one of the crosses intended to verify the genotype of the triple mutant s co m, some unusual behavior was noted. The cross s x s co m showed a reduced recombination frequency between co and m. It may be seen from the following table that reduction of recombination frequency is only observed in those crosses in which the factor s is carried by both parents (crosses R and S).

Cross Name	Cross Parents	Average recombination frequency in co - m
H	co + x + m	5.5
O	co m x + +	4.5
P	s co + x + + m	more than 5.7 (double cross-over types not scored)
Q	s + + x + co m	more than 4.2 (double cross-over types not scored)
V	+ + + x s co m	7.4
W	+ co + x s + m	5.3
R	s + + x s co m	1.8
S	s co + x s + m	2.1

An explanation of this phenomenon cannot be given at present.

8. Apparent negative interference in multiple factor crosses

A feature common to all of the multiple factor crosses studied is the large frequency of double crossovers which exceeds significantly the product of the two regional cross-over frequencies. (see the following table)

Cross Name	Parents	% of crossover types between s and co	co and m	% of double cross-overs observed	expected
V1	s co m x + + +	3.5	7.6	1.0	0.27
V2	s co m x + + +	3.8	6.2	0.9	0.24
W	s + m x + co +	2.4	5.3	0.5	0.13

There are two main possibilities which may cause a high frequency of double crossovers:

1) An inhomogeneity among the bacteria such that a fraction of the total bacterial population gives rise to parental types only.

or

2) An unequal distribution of genetic exchanges in the phage population within every bacterium

a) A fraction of the phage population does not undergo genetic exchange.

or

b) Within every phage particle one genetic exchange increases the probability for a second. This is negative interference in the strict sense.

To test possibility 1) several single burst experiments were made. The results will be reported in the next paragraph.

9. Single burst experiments

Note on the technique of single burst experiments: Since a modification of the standard cross procedure had to be used, a short summary of the deviations will be given.

1) The host bacteria were irradiated with UV (to a survival of 50%) to eliminate as far as possible a selective advantage of the clear parent over the turbid one.
2) At time 25 minutes the suspension of infected bacteria was diluted and distributed in 0.5 ml samples such that on the average one sample in 3 or 4 contained an infected bacterium.
3) At time 115 minutes the whole content of each tube was plated.

In order to evaluate the single burst experiments, the theoretical fraction of bacteria yielding only parental types which would account for the observed excess of double cross-overs has to be calculated. Assume that a fraction "b" of the bacteria supports phage recombination and that the phage progeny from this fraction shows no interference. It follows that

$$\frac{R_d}{b} = \frac{R_{ab}}{b} \times \frac{R_{bc}}{b}$$ and therefore that

$$b = \frac{R_{ab} \times R_{bc}}{R_d}$$ where R_d is the observed frequency of double crossovers, R_{ab} is the observed frequency of single crossovers in s - co, and R_{bc} is the observed frequency of singles in co - m.

For crosses V and W, b is found to be 0.3. That is, 30% of the infected bacteria are expected to yield both parental types and recombinants, the remaining 70% parental types only.

The experimental data from the single bursts are given in table 4 and may be summarized as follows. Ninety-eight percent (52 out of 53) of the mixedly infected bacteria yielded both parental types and recombinants. The experimental value is, then, significantly higher than that required by an inhomogeneity of the bacterial population (possibility 1. page 25). More direct evidence for the homogeneity of the bacterial population can be obtained by analyzing the distribution of recombinants among the different single bursts. As may be seen from figure 3, this distribution is unimodal.

9. b) The distribution of recombinants among single bursts

i) The variance of the distribution of recombinants among single bursts.

It was of interest to know whether the available data provide any evidence for multiplication of recombinants after their formation within the original host bacterium. Such a multiplication would be reflected in a clonal distribution of recombinants. Since an important characteristic of a clonal distribution is its large variance (17), the variance of the numbers of recombinants among single bursts was calculated and compared with the theoretical variance of a random

distribution with the same mean.

Recombinant type	Experimental values of mean	Experimental values of variance	Theoretical variance of random distribution *
<u>+ +</u>	8.2	72.5	47.8
<u>c m</u>	6.6	89.5	32.4

Both the variance of + + and the variance of c m are significantly larger than the theoretical values for a random distribution. The observations, therefore, may be consistent with some multiplication of recombinants.

9 b) ii) Absence of correlation between complementary recombinants.

To test whether correlation between complementary recombinants exists in single bursts, the linear correlation coefficient was calculated and found to be 0.03. This indicates that there is no linear correlation between complementary recombinants in single bursts. The same conclusion can be drawn from an examination of the scatter diagram shown in figure 4.

10. Triparental Cross

A further examination of the mechanism of recombination in λ was made with a triparental cross. By means of a tri-

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*See appendix 2 for the calculation of the theoretical variance.

parental cross, it is possible to distinguish genetic exchanges involving two phages from exchanges involving three or more. The cross s co + x + co m x s + m was made with the following results.

Cross Name	Parents	Yield % of recombinant types		Total Number Examined
Y1	s co + x + co m x s + m	2.2	s co m	11,904
		2.0	+ co +	
		1.8	s + +	
		0.8	+ + m	
		0.05	+ + + (6 plaques)	
Y2	s co + x + co m x s + m	1.8	s co m	13,764
		1.7	+ co +	
		1.8	s + +	
		0.6	+ + m	
		0.02	+ + + (3 plaques)	

The existence of a + + + class shows that there are some genetic exchanges between 3 or more particles.

DISCUSSION SECTION

To evaluate the experimental results described above, several points will be discussed separately. The formation of lysogenic bacteria as a possible complication in the interpretation of crosses between temperate phages will be discussed first.

I. <u>The contribution of phage particles released by newly formed lysogenic bacteria to the phage yield of a cross</u>.

The possibility to be examined is whether a significant number of phage is released from lysogenic bacteria formed during a cross. The importance of this possibility is that the mechanism of recombination might be different in lysogenic bacteria than in bacteria giving the productive response. Since the phage yield was examined at 90 minutes, newly formed lysogenic bacteria would have had to be formed and to have started their spontaneous phage production before this time. In an exponentially growing culture of K12 (λ) about one bacterium in 2×10^4 spontaneously lyses per division time (10). If the probability of lysis in a newly formed lysogenic clone were the same, the contribution of the small fraction expected to lyse spontaneously would therefore be negligible. It might be mentioned that Lieb (2) has observed that in newly formed lysogenic clones of

K12 (λ), less than one clone in 100 contained a spontaneously lysing bacterium.

In the crosses reported previously at most 50% of the infected bacteria became lysogenic as judged from the fact that 50% of the infected bacteria survived infection. If we assume that 1/100 of the lysogenic bacteria lysed during the 90 minute growth period, no more than 0.5% of the phage in the yield could have come from lysogenic bacteria and therefore may be neglected.

II. The organization of hereditary determinants in λ

Several features of the results of the crosses described in the experimental part suggested a linear arrangement of the factors studied. These features were:

1. Recombinants with complementary genotypes appeared in pairs with equal frequency.
2. The frequency of recombination was characteristic for each pair of markers and the frequency was independent of whether the markers were introduced into the cross in coupling or in repulsion.
3. All of the three factor crosses studied led to the same linear sequence of the factors.

These features, which had been previously also found in T2 (1) and T4 (18), suggest that the hereditary factors studied in λ are arranged on a chromosome like structure.

III. The mechanism of recombination in λ

Any theory about the mechanism of recombination in λ has to account for the high degree of apparent negative interference, for the moderately high variance of the distribution of recombinants among single bursts, and for the lack of correlation between complementary types in single bursts.

A. Comparison of the results with the Visconti-Delbruck theory of phage recombination

In this section the compatibility of the experimental results with the Visconti-Delbruck theory (19) will be examined.

1) Assumptions of the Visconti-Delbruck theory

i) Non-infective, intracellular forms of the phage, which is said to be in the vegetative state, multiply up to the time of lysis.

ii) After the phage has multiplied to a certain point mating begins between pairs of vegetative particles. During a mating there is a possibility for reciprocal exchange.

iii) The acts of mating are random with respect to partner and random in time.

iv) A portion of the vegetative particles irreversibly mature into infective phage.

2) Calculation of the average number of rounds of mating for λ.

The results of a biparental three factor cross can be used to obtain a maximum estimate of the average number of rounds of mating if it is assumed that the interference between crossovers at each mating is either zero or positive.

Concomitant with the number of rounds of mating, crossover probabilities are obtained for the two regions involved in the three factor cross. The method of calculation is given in appendix 1, only the results will be given here.

Let M be the maximum estimate of the average number of rounds of mating, p_{12} the probability per mating of a crossover between s and co, and p_{23} the probability of a crossover between co and m. Then the values given in the following table are obtained.

Cross			Calculated values	
Name	Parents	M	p_{12} (s - co)	p_{23} (co - m)
V1	+ + + x s co m	0.66	0.11	0.25
V2	+ + + x s co m	0.65	0.12	0.21
W	+ co + x s + m	0.64	0.08	0.18

The most striking result is that the average number of rounds of mating for λ is no more than 0.66 compared with 5 rounds found for T2 (19). The lower value for λ would explain why there is more apparent negative interference for λ than for T2, since in λ about 50% of the vegetative particles do not mate at all ($p(0) = e^{-.65}$) while in T2 practically all mate ($p(0) = e^{-5}$). The low value of M, furthermore, would explain why the observed recombination frequencies are in general lower for λ than for T2.

6) Comparison of results of the triparental crosses with the expectations based on the Visconti-Delbruck theory

As a test of the validity of the Visconti-Delbruck theory for λ crosses, the results of the two triparental crosses can be compared with the expectation based on the values of M, p_{12}, and p_{23} calculated previously from the biparental, three factor crosses. (The calculation was made with equation 8 of Visconti and Delbruck (19)). The triparental cross was s co + x + co m x s + m.

Recombinant type	Observed frequencies Cross Y1	Y2	Calculated frequencies
s co m	0.022	0.018	0.037
+ co +	0.020	0.017	0.017
s + +	0.018	0.018	0.014
+ + m	0.008	0.006	0.007
+ + +	0.0005	0.0002	0.0003

For all classes except s co m the agreement between the observed and calculated frequencies is good. This test validates the assumption that mating is at random with respect to partner for λ. If mating were not at random, then the observed frequency of the triparental recombinant class, + + +, which depends on two subsequent matings should have been lower than the calculated value. This test for random mating is more critical in λ than in T2 due to the lower average number of rounds of mating in λ.

4) Apparent negative interference in the four factor cross

The compatibility of the Visconti-Delbruck theory with the results of the 4 factor cross $\underline{+\ c\ +\ +}$ x $\underline{s\ +\ co\ m}$ will be discussed next. A peculiar feature of this cross was the relatively high frequency of the triple crossover class $\underline{+\ +\ +\ m}$ which was found to be 1/3 as frequent as the single crossover class $\underline{s\ +\ +\ +}$ and 1/2 as frequent as the double crossover clas $\underline{+\ +\ +\ +}$. ("high apparent negative interference") It is interesting to see how well this result can be quantitatively explained by the Visconti-Delbruck theory. The ratio V_{cl} (19) is defined by Visconti and Delbruck as the proportion of phage within a class of recombinants for two closely linked markers which also have recombined for another pair of markers. If the class of triple crossovers $\underline{+\ +\ +\ m}$ is considered within the class of double crossovers for s - c and c - co, then their ratio $\frac{\underline{+\ +\ +\ m}}{\underline{+\ +\ +\ +}\text{ and }\underline{+\ +\ +\ m}}$ which is 5/15 is the observed value of V_{cl}. The calculated value of V_{cl}, using M and p_{23} derived from the three factor cross (M = 0.65, p_{23} = 0.21) is 0.25, assuming no interference. Since the standard error of the observed value is large, there is sufficient agreement between the observed (0.33) and the calculated (0.25).

5) Lack of correlation between reciprocal recombinants and the Visconti-Delbruck theory

Since the Visconti-Delbruck theory postulates that complementary recombinant types arise in the same act of

genetic exchange, a large positive correlation in the distribution of complementary recombinants in single bursts would be expected. However, it has been pointed out that random multiplication or sampling during maturation could obscure an originally perfect correlation (19,20).

It would be important to know whether the processes of random multiplication and maturation sampling can account quantitatively for the lack of correlation between complementary recombinants. However, it is not possible to give a complete quantitative treatment to this problem at the present time because a complete theory of phage recombination and maturation does not exist. The complete theory would have to take into account the spread in maturation time (19), the variation in the total number of vegetative particles, and the possibility of variation in the number of rounds of mating.

In the absence of the complete theory, an approximate treatment of the problem was made (the details of the calculation are given in appendix 2). The conclusion of the calculation is that the data are not inconsistent with the possibility that the randomizing effects are sufficient to destroy an initially perfect correlation. It is clear, however, that more extensive data and a more complete theory are needed before a satisfactory solution can be obtained.

B. Comparison of the results with the heterozygote theory (Levinthal theory) of phage recombination

1) Description of the heterozygote theory

Hershey (21) reported the discovery of single particles of T2 which give rise to two types of offspring. A particle heterozygous for r and r^+ produces both r and r^+ progeny. In addition Hershey found that most of the heterozygotes from a two factor cross segregated into one parental and one recombinant type.

The next step was taken by Levinthal (22) who showed that in a three factor cross, particles heterozygous for the central factor were almost always recombinants for the two distal factors. This finding led to the suggestion that heterozygotes were formed as the result of the replication of two genetically different phages in close association with each other such that only one new phage "chromosome" was formed from both. The new chromosome would have one of its ends from the first parent and the other end from the second parent with an overlap in the middle (heterozygous) region. Levinthal furthermore calculated that the frequency of heterozygotes was sufficient to account for all of the recombinants in the cross T2 h + x T2 + r_{13}. The heterozygote theory of phage recombination makes, therefore, two basic assumptions: 1 heterozygotes arise by a special kind of biparental multiplication and 2 all recombinants arise from heterozygotes.

2) Comparison of the heterozygote theory with the results of crosses

a) The lack of correlation between complementary recombinants

While the Visconti-Delbruck theory explains the absence of correlation between complementary recombinants in single bursts only by assuming an additional sampling process, the Levinthal theory predicts the absence of correlation.

b) The exclusion of one crossover by another (complete interference)

In the form of the heterozygote theory of phage recombination presented by Levinthal (22), replication was assumed to start at either one end of a chromosome or the other. This assumption leads to the expectation of one crossover excluding any other crossover in the same act of mating (or replication). It will be shown below that this expectation is not fulfilled by the λ crosses.

Let us consider the three factor cross <u>a b c</u> x <u>+ + +</u>. Let R_{ab} and R_{bc} be the observed frequencies of single crossovers in a - b and b - c, and R_d the observed frequency of double crossovers a - b - c. If there were complete interference, the exchanges in a - b and in b - c would be mutually exclusive in each act of mating, but independent in subsequent matings. It would follow that $R_d/2 = R_{ab}/2 \times R_{bc}/2 \times 2$.

$R_d/2$ is the frequency of one type of double recombinant, $R_{ab}/2$ and $R_{bc}/2$ are each the frequencies of one single crossover type. The final factor of 2 is necessary because the order of the two single crossovers is immaterial.

The pertinent results of the three factor crosses were reported earlier but will be repeated here.

Cross	Observed frequencies R_{ab}	R_{bc}	R_d	Expected frequency $R_{ab} \times R_{bc}$
V1	0.035	0.076	0.01	0.0027
V2	0.038	0.062	0.009	0.0025
W	0.024	0.053	0.005	0.0013

It may be seen from the last 2 columns that more double crossover types are observed than would be expected if there were complete interference.

A check on the expected relation for complete interference ($R_d/2 = R_{ab}/2 \times R_{bc}/2 \times 2$) can be obtained from the triparental cross which simulates complete interference in that the triparental recombinant requires two different matings for its formation. Considering both triparental crosses together it is found that:

	$R_{ab}/2$ (+ + m)	$R_{bc}/2$ (s + +)	$R_d/2$ (+ + +)
Observed in Y1 and Y2	0.0074	0.0178	0.0003 ± 0.0001
Expected			0.0003

The agreement between the observed value of 0.03% and the calculated value of 0.03% is good suggesting that the formula used is a valid test of complete interference.

The above objection to the applicability of the heterozygote theory could be removed if it were assumed that repli-

cation might start at several points along the phage chromosome.

C. General remarks

If one assumes that the Visconti-Delbruck or Levinthal theories give a fair representation of the intracellular events experienced by λ, then one is faced with the problem of explaining the low number of rounds of mating. Since all of the mutants were obtained by UV treatments, these may carry structural changes which inhibit pairing. So far none of the experimental data indicate the presence of structural changes.

Another possibility of explaining the low number of rounds of mating for λ may be connected with its temperateness. This is suggested by the fact that another temperate phage, one carried by <u>B. megatherium</u>, shows 0.25 rounds of mating. (Calculated from the data given by Murphy (23)). It may be speculated that in temperate phages a low number of rounds of mating is a reflection of a multiplication process confined to the bacterial nucleoids. Mating between opposite types may not occur until vegetative particles have entered the cytoplasm. This speculation is supported by the fact that in contrast to the infection of B by T2 in which the nucleoids are rapidly broken up (24,25), the nucleoids of K12S infected with λ retain their integrity until near the end of the latent period (26).

Table 1

Genotype	Stock Number	Origin	Stock Titer	Description of Plaque type on UV'd D
+	26	K12(λ)	1.7×10^{11}	large, turbid center
s	A3	2' UV treatment of λ 26	4.3×10^{10}	small, turbid center, differs from λ 26 only in size
cp	A9	3' UV treatment of λ 26	4.9×10^{10}	large, center clear except for small turbid ring
m	A10	3' UV treatment of λ 26	1.1×10^{11}	minute, turbid center, ring at outer edge
c	A12	3' UV treatment of λ 26	9.5×10^{10}	large, clear center with a few colonies scattered around
c_1	26-21	λ 26 plated on UV'd D	6.1×10^{10}	identical with c
c_2	A22	3 1/3' UV treatment of λ 26	1.3×10^{11}	identical with c
c_3	A26	3 1/3' UV treatment of λ 26	8.7×10^{10}	identical with c
+	K1	recombinant, cross E, <u>s</u> x <u>co</u>	1.0×10^{10}	identical with λ 26
+	L3	recombinant, cross H, <u>co</u> x <u>m</u>	6.5×10^{9}	identical with λ 26
co_1	A25	3 1/3' UV treatment of λ 26	9.5×10^{10}	large, center clear except for large turbid ring
sco	K2	recombinant, cross E, <u>s</u> x <u>co</u>	1.7×10^{10}	small, center clear except for small turbid ring
com	L67	recombinant, cross H, <u>co</u> x <u>m</u>	3.0×10^{10}	minute, clear except for turbid ring in center

Table 1 (continued)

Genotype	Stock Number	Origin	Stock Titer	Description of Plaque type on UV'd D
sm	Q89	recombinant, cross R, _s_ x _scom_	2.7×10^{10}	tiny, turbid, irregular edge
c4m	A10-1	A10 plated on UV'd D	1.1×10^{11}	minute, center clear with a few colonies scattered around
scom	M14	recombinant, cross P, _sco_ x _m_	9.0×10^{10}	tiny, semi-clear center

Table 2

FREQUENCY OF MUTANT TYPES PRESENT IN DIFFERENT PHAGE STOCKS

Stock genotype	c/c^+	co/co^+	c/co	c^+/c	co^+/co	m^+/m
+	$10/6\text{x}10^4$	$2/6\text{x}10^4$				
s	$1/10^4$	$6/10^4$				
co			$11/10^4$		$0/1.7\text{x}10^4$	
c				$0/2\text{x}10^4$		
s co			$10/2\text{x}10^4$		$1/2\text{x}10^4$	
co m					$0/1.4\text{x}10^4$	
sm						$15/2\text{x}10^4$
s co m						$2/5\text{x}10^4$

Each stock was plated on UV'd D and the number of mutant plaques of a particular phenotype counted. Each entry in the table consists of two numbers, the upper one giving the number of mutant plaques found, the lower one the number of plaques examined. Each column corresponds to a particular phonotypic change, for example $^c/c^+$ refers to the number of phenotypically c plaques in a c^+ stock.

Table 3

Cross	Multiplicity and Genotype		Numbers of Various Types in the Progeny											
	Parent 1.	Parent 2.	+	s	co	m	s co	s m	co m	s co m	c	sc	cm	Total
A	2.0s	1.7+	524	466										4,390
B1	2.4co	1.0+	92		478									3,400
B2	9.0co	9.0+	707		761									1,468
C	5.0m	4.5+	736			923								1,659
D	9.5c	9.0+	925								840			12,500
E	s	co	10	439	533		10							992
F	s	c	19	808							566	20		1,413
G1	2.3co	2.7c	2											3,600
G2	8 co	2 c	3											2,700
H1	3.5co	3.5m	94		2980	1075			90					4,239
H2	8 co	8.7m	17		459	398			25					899
H3	24 co	23.5m	44		720	672			46					1,482
J	9.5c	11 m	84			1205					1213		75	2,577
K	3.8s	3.1m	65	647		502		56						1,270
L1	6.4sco	5.5+(E)	589	11	8		789							1,397

Table 3 (continued)

Cross	Multiplicity and Genotype		Number of Various Types in the Progeny											
	Parent 1.	Parent 2.	+	s	co	m	s co	s m	co m	s co m	c	sc	cm	Total
L2	3.5sco	5+(E)			45									7,230
M	11sco	22+(26)		16	12									1,900
N	8+(26)	8+(E)	2310	0	0									2,310
O	16+(H)	13com(H)	795		36	30			620					1,481
P	6.3sco	7.8m	88	+	54	1000	1950	+	+	+				3,092
Q	4.6com	9.3s	36	2774	96	+	+	+	1616	+				4,522
R1	8.0scom	9.0s		3950			49	--+		3950				7,949
R2	12scom	11s		1439			46	--+		1763				3,248
R3	11scom	11s		866			19	--+		794				1,679
S	10sco	11sm		70			3351	--*						
T	11m	11scom				5769		--+	128	4869				10,766
U1	8co	8.4scom			3691		441		407	2527				7,066
U2	5.2co	8.2scom			2798		255		248	1968				5,269
V1	4.6+	8.3scom	6936	71	71	455	477	--	178	5912				14,171
V2	11+	9.2scom	975	30	13	51	61	5	32	924				2,091
W	8.7co	10sm	38	273	5050	121	112	6389	318	23				12,324

Table 3 (continued)

Cross	Multiplicity and Genotype: Parent 1.	Parent 2.	Parent 3.	Number of Various Types in the Progeny: +	s	co	m	s co	sm	co m	s co m	c	sc	cm	Total
X	9.3c	7.7scom		10	13	46	5	299•		112	(c^+/c=.59)		122	271•	18,062
Y1	4.5sco	4.2com	5.7sm	6	210	234	98	4034	3293	3767	262				11,904
Y2	4.1sco	4.1com	6.4sm	3	241	231	89	3918	4350	4686	251				13,764
AA	8.4co	8.4c_4m				2640	0					142		2150	4,932
AB	c	c_1		0											2,735
AC	9.5c_3	11 m		54			757					855		58	1,724
AD	12.5c_2	14 m		60			863					860		63	1,846
AE	14co_1	14 m		109		1477	1949			131					3,666
AF	10co_1	10scom		8	51		1	171**		174**		26		50	5,434
AG	23c_2	14scom		2	4	9	0	76		16			21	74	2,997
AH	13c_3	11scom		3	5	18	1	157		49			45	149	4,879

*on plates with good sm plaques the number of sm equalled the number of sco
+not scored
•scored among first 9330 only
**sco and sco_1; com and co_1m.

Table 4a

Yields of single cells, cross c + m x + m
Input multiplicities 10 + m, 11 c +

Plate Number	c m	+ +	c +	+ m
	Numbers of various types			
1	13	12	88	71
2	1	0	31	13
3	1	0	9	18
4	5	9	142	30
5	3	4	39	93
6	6	15	30	72
7	4	7	44	38
8	6	4	127	154
9	0	1	4	5
10	3	18	151	192
11	1	0	24	0
12	2	0	279	9
13	0	2	7	22
14	0	1	6	13
15	6	2	33	130
16	8	1	47	129
17	0	4	2	675
18	0	0	0	111

In 100 plates 75 had no plaques, 6 had one plaque each and 1 had 2 plaques, the remaining 18 plates are given in the above tabulation. The average number of bursts per plate was 0.39. Two plates were expected to have more than one burst.

Table 4b

Yields of single cells, cross c + x + m
Input multiplicities 10 + m, 11 c +

Plate Number	Numbers of various types c m	+ +	c +	+ m
1	4	24	138	30
2	1	4	37	15
3	0	8	0	57
4	0	0	325	7
5	13	7	422	32
6	5	6	48	339
7	2	11	79	261
8	4	8	112	132
9	11	10	37	110
10	21	4	42	129
11	2	7	336	28
12	13	10	151	43
13	26	33	196	179
14	7	5	141	157
15	3	10	12	43
16	1	2	3	18
17	9	20	79	183
18	0	5	98	65
19	13	7	113	147
20	4	4	70	120
21	7	6	27	169

Table 4b (continued)

Plate Number	Numbers of various types c m	+ +	c +	+ m
22	13	7	123	71
23	4	1	32	38
24	6	5	36	100
25	0	0	22	5
26	58	25	123	118
27	1	2	11	20
28	1	0	28	13
29	23	39	142	135
30	1	0	3	2
31	19	13	148	104
32	4	22	99	85
33	10	19	134	35
34	5	16	100	276
35	0	5	14	10
36	0	7	21	119

Among 200 plates 157 had no plaques, 7 were too poor to be scored, the remaining 36 bursts are given in the above tabulation. The average number of bursts per plate was 0.24 and the expected number of plates with more than one burst, 5.

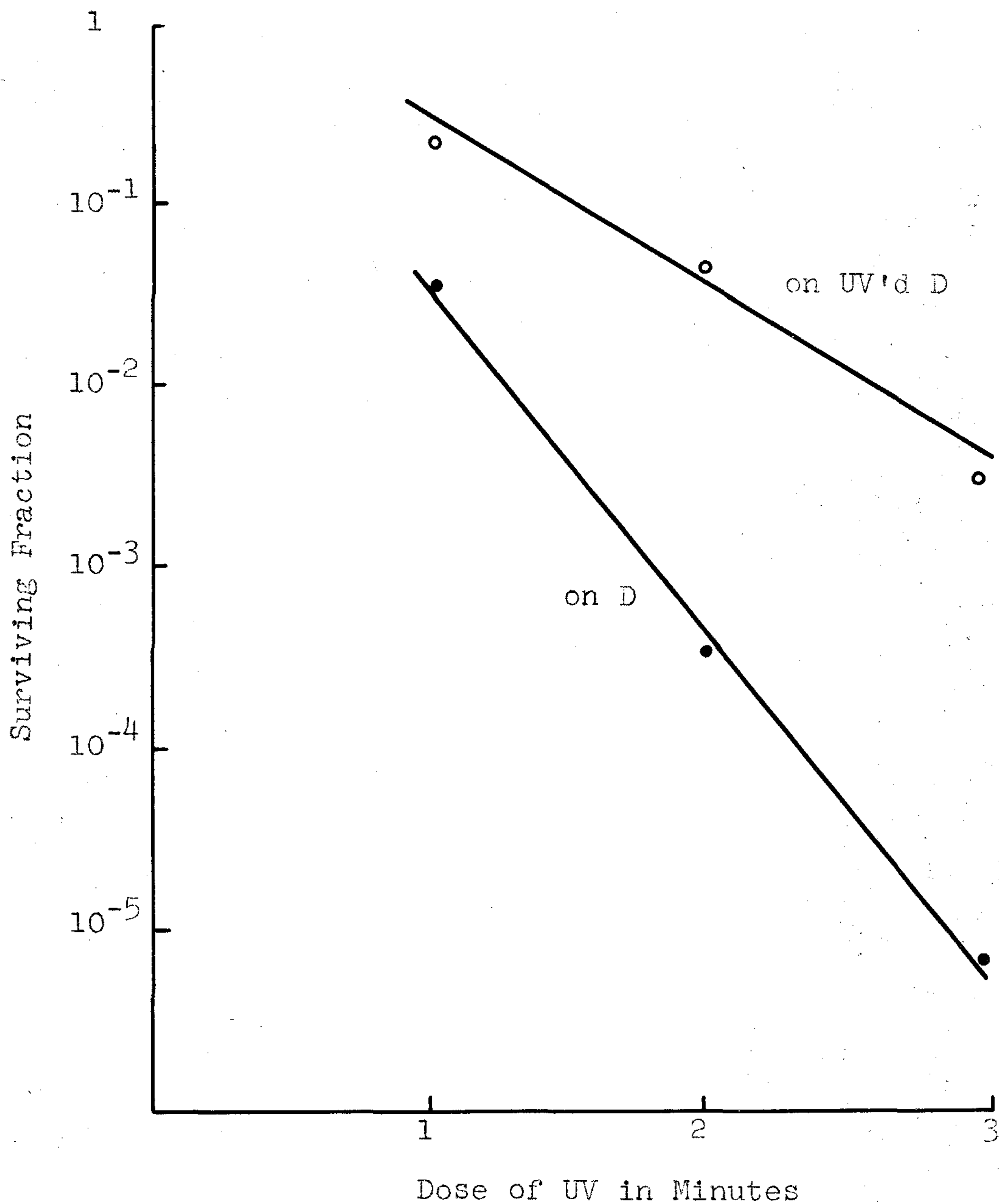

Figure 1. Survival of irradiated λ 26 plated on UV treated D (upper curve) and on non UV'd D (lower curve).

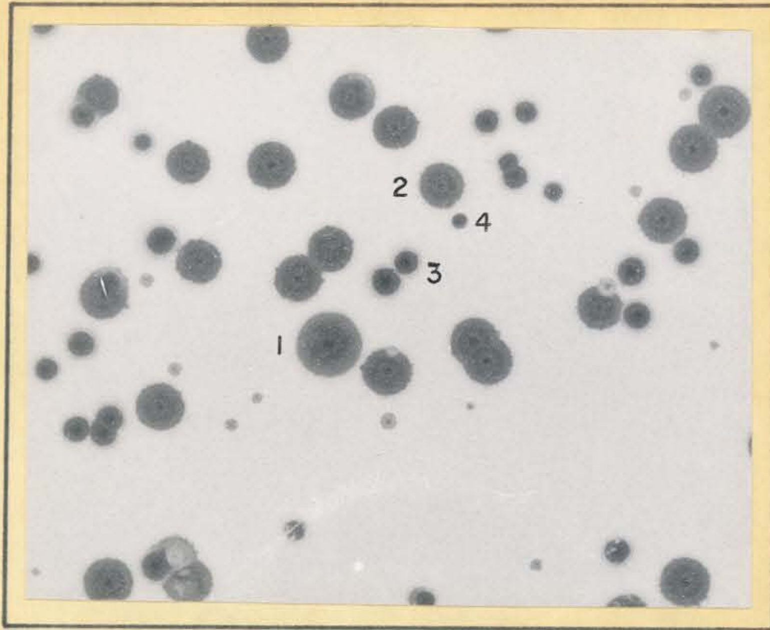

Figure 2a. Photograph of Mutants

1. <u>+ co +</u>
2. <u>s co +</u>
3. <u>+ co m</u>
4. <u>s co m</u>

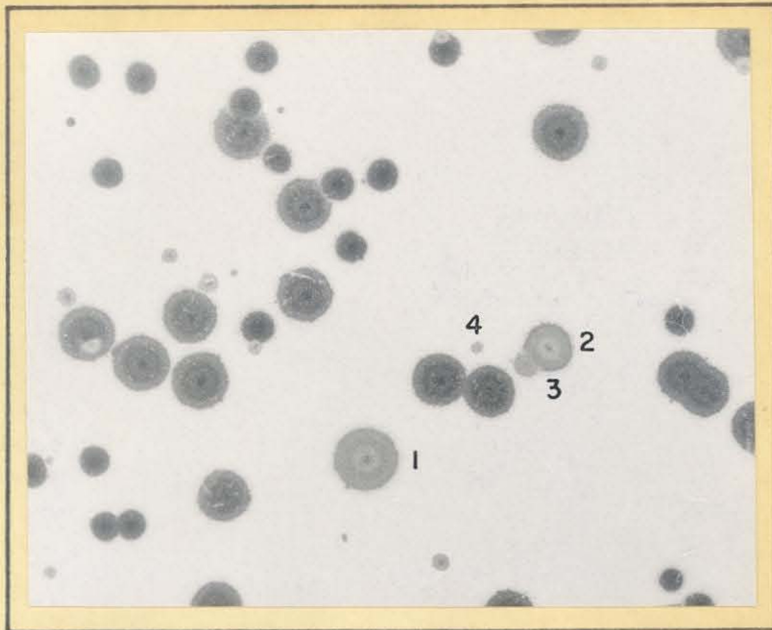

Figure 2b. Photograph of Mutants

1. <u>+ + +</u>
2. <u>s + +</u>
3. <u>+ + m</u>
4. <u>s + m</u>

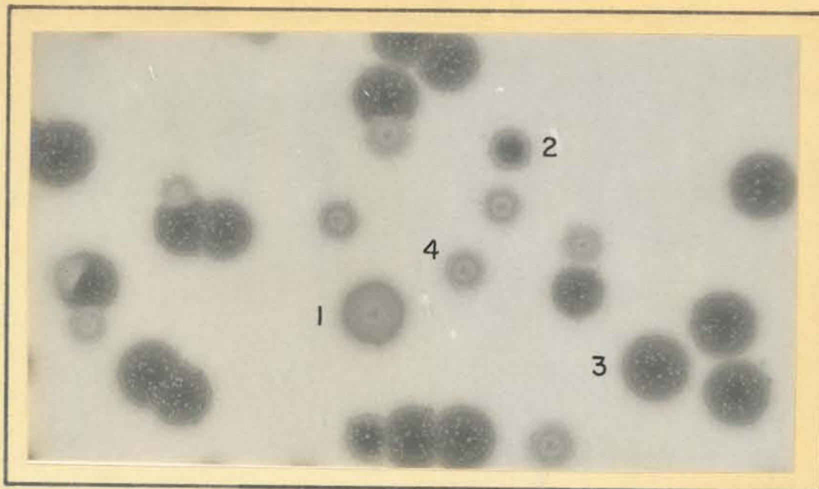

Figure 2c. Photograph of Mutants

1. + +
2. c m
3. c +
4. + m

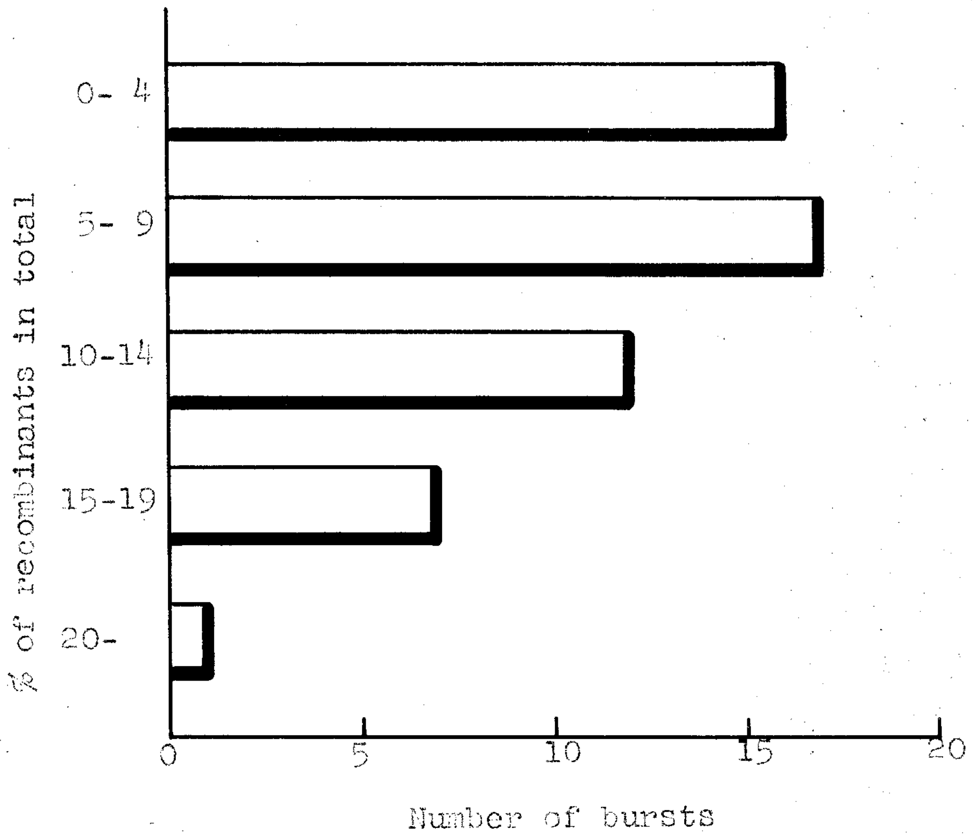

Figure 3. Distribution of recombinants among the yields of single cells for the experiments reported in table 4.

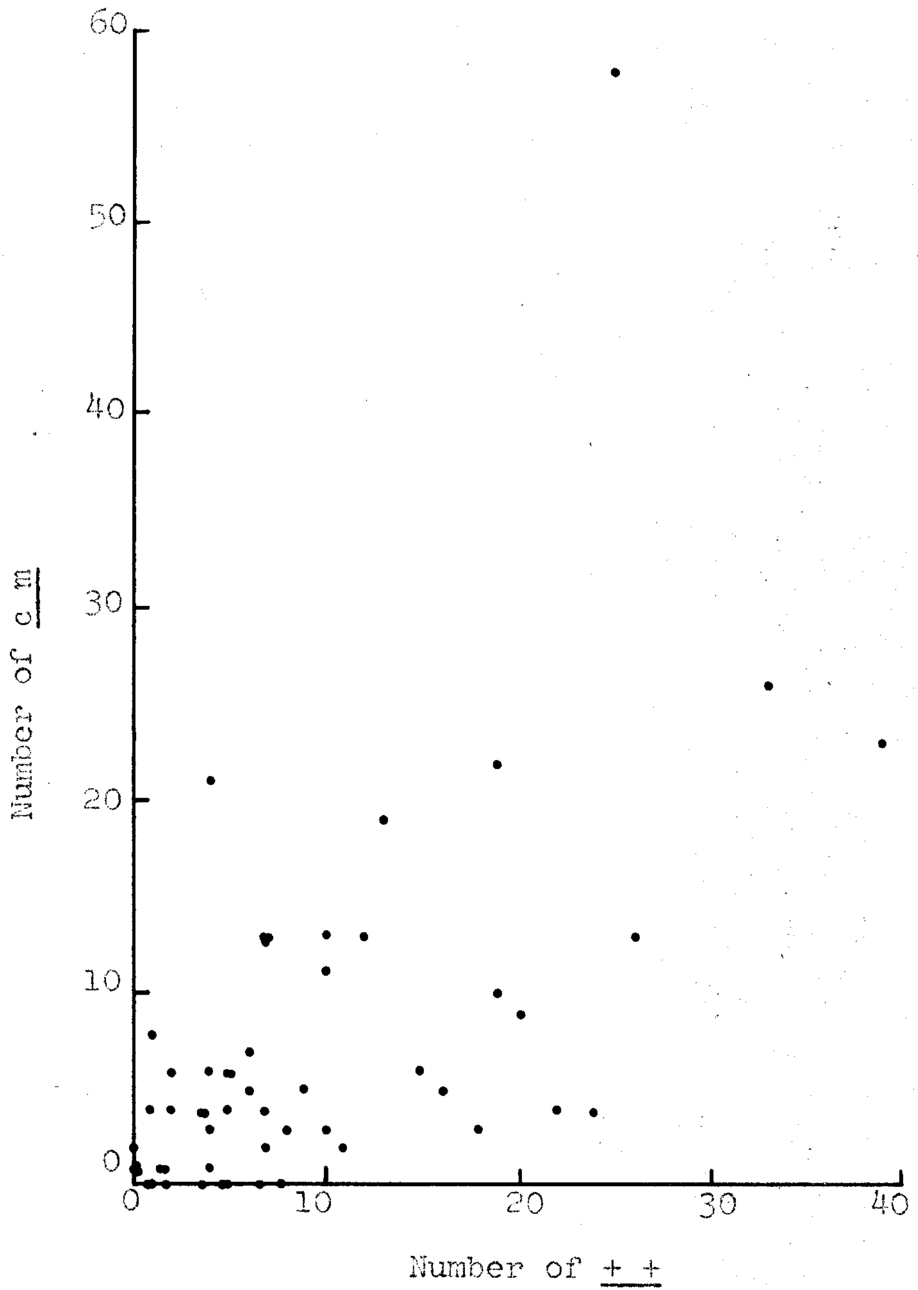

Figure 4. Scatter diagram for single burst cross c + x + m

APPENDIX 1

Calculation of a maximum estimate of the number of rounds of mating and the crossover probabilities by the Visconti-Delbruck theory

I. Theory of the two factor cross with equal input

Consider a pool of vegetative particles which are mating with each other. Assume that mating is random in time and that the probability of a particular mating is proportional to the product of the frequencies of the two types involved. Let a be the frequency of each of the two recombinant types in the pool, let m be the average number of rounds of mating per particle, and let p be the probability of crossingover between the two factors considered.

During a short time interval in which m increases by dm, a will increase due to matings between parental types which result in crossingover by an amount

$$\frac{2}{2}\left(\frac{1-2a}{2}\right)\left(\frac{1-2a}{2}\right)p\ dm.$$

Also during dm a will decrease due to matings with crossingover between two recombinants by an amount $\frac{2}{2}\ a^2\ p\ dm$. Therefore the net change in a will be

$$da = \frac{2}{2}\left(\frac{1-2a}{2}\right)^2 p\ dm - \frac{2}{2}\ a^2\ p\ dm$$

The solution to this equation is $a = \frac{1}{4}(1 - e^{-mp})$ if a = o when m = o. This equation was first derived by Visconti and Delbruck (19) from the same assumptions but by a different method.

II. Theory of the biparental, three factor cross with equal input.

The recombination frequencies for the cross b c d x + + + must also satisfy the equations for the two factor crosses bc x + +, c d x + +, and b d x + +. If the sequence is b - c - d and the probability of crossingover in b - c is p_{12}, in c - d p_{23}, and in b - d p_{13} then the following relations will hold.

$$\text{(A)}\quad \begin{aligned} a_{+cd} + a_{+c+} &= \frac{1}{4}(1 - e^{-mp_{12}}) \\ a_{bc+} + a_{+c+} &= \frac{1}{4}(1 - e^{-mp_{23}}) \\ a_{bc+} + a_{+cd} &= \frac{1}{4}(1 - e^{-mp_{13}}) \end{aligned}$$

In addition $p_{13} = p_{12} + p_{23} - 2\alpha p_{12} p_{23}$ where α is a parameter measuring the amount of interference between crossingover in b - c and crossingover in c - d ($\alpha = 1$ for no interference; $\alpha = o$ for complete interference). Equations (A) and the relation between the p's can be solved simultaneously for the ratio m/α ($\equiv M$).

$$M = \frac{2\,A\,B}{A + B - C} \quad \text{where} \quad \begin{aligned} A &= -\ln_e\,(1 - 4a_{+cd} - 4a_{+c+}) \\ B &= -\ln_e\,(1 - 4a_{bc+} - 4a_{+c+}) \\ C &= -\ln_e\,(1 - 4a_{bc+} - 4a_{+cd}) \end{aligned}$$

Since α is always greater than zero but less than one, $M \geq m$. If no interference is assumed, $M = m$, then equations (A) can be solved for p_{12}, p_{23}, and p_{13}.

APPENDIX 2

I. Calculation of the theoretical variance for a random distribution of recombinants

Let $g(n)$ be the probability that a bacterium will yield a total of n mature phage particles. Let $f(x,n)$ be the probability that a bacterium yields x recombinants of a particular type if it yields a total of n particles. Assume that if recombinants arise at random that $f(x,n)$ is a binomial probability function, i.e.

$$f(x,n) = \binom{n}{x} p^x (1-p)^{n-x}$$

where p is the theoretical proportion of recombinants among the vegetative particles. The probability that a bacterium yields x recombinants is therefore $f(x,n)\ g(n)$.

The mean and variance of x can now be found.

(A) mean $x = E(x) = \sum_{n=1}^{\infty} \sum_{x=o}^{n} x\ f(x,n)\ g(n) = Np$

where N is the mean burst size $= \sum_{n=1}^{\infty} n\ g(n)$

$$\text{var}(x) = E(x^2) - [E(x)]^2$$

$$\text{var}(x) = \sum_{n=1}^{\infty} \sum_{x=o}^{m} x^2\ f(x,n)\ g(n) - N^2p^2$$

(B) $var(x) = p(1-p)\ N + p^2\ var(n)$

Estimates of N and var(n) may be obtained from the single burst experiments, they were found to be 197 and 62,000 respectively. The theoretical proportions of recombinants p_{cm} and p_{++} may be estimated from the observed average number of the recombinant and the estimate of N, using relation (A), the result is $p_{cm} = 0.034$ and $p_{++} = 0.04$. The theoretical variance of x is found by substituting these values into equation (B), giving var(cm) = 32.4 and var (++) = 47.8. It should be noted that the expected variance is much larger than the mean. This is due to the inclusion of the effect of the burst size distribution.

II. Effect of random sampling on the correlation between complementary recombinants.

The model to be considered is a pool containing recombinants and parental types from which samples are drawn. If X is the number of recombinants of one type which are withdrawn from the pool which originally contained R of this type of recombinant, then X = R - T where T is the number of this type left in the pool. Similarly, for the other recombinant, Y = S - U. Now X, Y, R, S, T, U are all random variables by our assumption of random sampling.

Applying the expectation operator E,

$E(X) = E(R) - E(T)$

$E(X^2) = E(R^2) - 2E(RT) + E(T^2)$

$E(Y) = E(S) - E(U)$

$E(Y^2) = E(S^2) - 2E(SU) + E(U^2)$

$E(XY) = E(RS) - E(TS) - E(RU) + E(TU)$

$\mathrm{var}(X) = E(X^2) - [E(X)]^2 = \mathrm{var}(R) + \mathrm{var}(T) - 2\ \mathrm{cov}(RT)$

$\mathrm{var}(Y) = \mathrm{var}(S) + \mathrm{var}(U) - 2\ \mathrm{cov}\ (SU)$

$\mathrm{cov}(XY) = E(XY) - E(X)\ E(Y)$

$\mathrm{cov}(XY) = \mathrm{cov}(RS) - \mathrm{cov}(TS) - \mathrm{cov}(RU) + \mathrm{cov}(TU)$

It may be assumed that cov(TS), cov (RU), and cov(TU) are each small compared with cov(RS) and that cov(RT) is small compared with var(R) since the sampling process is random.

Thus

$$\mathrm{var}(X) = \mathrm{var}(R) + \mathrm{var}(T)$$

$$\mathrm{var}(Y) = \mathrm{var}(S) + \mathrm{var}(U)$$

$$\mathrm{cov}(XY) = \mathrm{cov}(RS)$$

In addition, since the recombinants represented by R and S are present in approximately equal numbers and are subject to the same fluctuations var(R) = var(S) and var(T) = var(U), the correlation between X and Y is

$$r_{XY} = \frac{\mathrm{cov}(XY)}{[\mathrm{var}(X)\ \mathrm{var}(Y)]^{\frac{1}{2}}}$$

$$= \frac{\mathrm{cov}(RS)}{\mathrm{var}(X)} = r_{RS}\ \frac{\mathrm{var}(R)}{\mathrm{var}(X)}$$

Therefore, the reduction in correlation due to the sampling process is approximately

$$\frac{\text{var }(R)}{\text{var }(X)}$$

The next step is the calculation of the reduction factor to be applied in the case of the single bursts reported here. The estimate of var(X) is the observed variance of one of the recombinants, while var(R) which is the variance before sampling will be assumed to be the theoretical variance of a random distribution calculated in section I of this appendix. For the recombinant <u>cm</u> the values are var(X) = 89.5 and sampling variance = 32.4 (see experimental section 9 b)ii). The reduction factor is therefore $\frac{32.4}{89.5}$ = 0.36. Thus, if the correlation before sampling were 1 (perfect correlation), the observed would be expected to be 0.36. Actually a correlation of 0.03 was observed. However, the observed correlation 0.03 is not significantly different (at the 1% level) from the expected correlation 0.36. The above calculation shows that the amount of sampling may be adequate to destroy an originally perfect correlation. Since several unjustified assumptions were made and since the compatibility is weak, the problem needs to be analysed further.

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