THE SIZE OF THE MATING GROUP

IN BACTERIOPHAGE T4

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

1962

ACKNOWLEDGEMENTS

I am very grateful to:

Max Delbrück, who teaches by example and points the way by searching for it.

Robert Edgar, to whose counterpoint of encouragement and criticism I have danced and from whose advice I have learned much.

Charles Steinberg, on whose knowledge, insight, and patience I have made incessant demands.

Don Ridgeway and Richard Epstein, who have listened patiently and commented instructively.

Barbara, my wife, who has typed my thesis, endured my tantrums, brightened my world.

The U.S. Public Health Service, The National Foundation, and The Arthur McCallum Fund, who have provided financial support.

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ABSTRACT

In unequal-input, three-factor phage crosses, the kinetics of recombination should depend on the number of vegetative phages participating in each mating. Among a selected class of progeny recombinant for two closely linked markers, a third, freely-segregating marker will achieve genetic equilibrium immediately if the mating group is very large but will show a drift toward equilibrium if the mating group is small. Data are presented for T4 crosses which are in good agreement with the qualitative predictions for small group (pairwise) mating.

Mating parameters have been measured which permit calculation of the expected kinetics of recombination for various mating group sizes. The predictions are calculated using Steinberg and Stahl's theory of formal phage genetics. The data do not conform precisely to the theory, but it is argued that the disagreement cannot alter the conclusion that the T4 mating group is small.

The significance, or insignificance, of this finding is discussed in the light of recent discoveries concerning the geometry of recombination in T4.

Erratum: For $\overline{xy}^2/\overline{xy}$ read $\overline{xy}^2 / \overline{xy}$. This error in notation has been made throughout the text.

I. INTRODUCTION

1. The Phage Cross.

The definition of "mating" in bacteriophages derives from our concept of a phage cross (1). In a phage cross, we add to a culture of bacteria large numbers of two (or three) genetically dissimilar parental phage particles. We add the parents in equal numbers in an equal-input cross; in unequal numbers in an unequal-input cross. A phage, upon colliding with a bacterium, attaches to the bacterial surface and injects its genetic material into the cell. The average number of phage adsorbed per bacterium is the multiplicity of infection, usually 5-10 of each parent in an equal-input cross.

Following infection there is a period, the eclipse period, during which no infective phage can be found in the host cell. We say that the phage are in the vegetative state. The vegetative phage, after a short delay, begin to increase in number at a constant rate. This replication establishes a pool of vegetative phages which can recombine genetically. When the pool contains about 30 or 40 vegetative phages, maturation of the vegetative phage into infective phage begins. The rate of maturation balances that of replication so that a vegetative pool of constant size is maintained. Phage once matured can neither revert to the vegetative state

nor participate in recombinational processes. We assume that the phage matured at any moment represent a random sample of the vegetative phage present at that moment. The end of the latent period, which begins at the time of infection, is marked by the lysis of the host cells and the release of the mature phage. In T4, the burst size at the time of normal lysis is about 200-400 mature phages per cell. Premature lysis experiments involve artificial lysis of the cells before the end of the latent period.

Among the mature phage which issue from cells mixedly infected with two types of parental phages are individuals recombinant for the genetic markers of the parents. The earliest mature phage formed contains some recombinant individuals. The frequency of recombinant phages increases as the number of mature phages increases (2). This is called drift. In triparental crosses, progeny phages carrying markers from all three parents are found (3). Furthermore, in three-factor crosses involving two parents, exchanges in the regions flanking the central marker are positively correlated (4). This correlation of recombinational events is called negative interference. Taken together, these facts about phage crosses are sufficient to demonstrate that a phage cross is not directly analogous to a cross in higher organisms; the facts are, however, easily explainable in

terms of population genetics. We imagine that the vegetative phages constitute a well-mixed population in which recombination occurs repeatedly in time and at random with respect to partners. We consider that the mature phage population in any premature lysate represents the sum of random samples of the vegetative pool withdrawn continuously from the onset of maturation until the time of lysis.

2. Mating.

We can now define "mating" in the sense in which it is applied to phage crosses. By "mating" we shall designate those interactions between phages which give rise to recombinant progeny. Since mating between phages occurs behind drawn curtains, a more precise definition of the term requires a statement of what we do not mean by mating:

a. We do not mean that during a mating two (or more) vegetative phages synapse either throughout their entire length or at discrete points in their structure. We do not specify the number of phages participating in a mating and specify the topography of the interaction only to the extent that we require it to produce a linear genetic map.

b. We do not specify a mechanism of recombination. A copy-choice mechanism of recombination, in which recombination is a consequence of the synthesis

of deoxyribonucleic acid (DNA), can involve either pairs of vegetative phages (5) or large numbers of phages (1). The model assumes that recombination occurs through "switching" from one parental DNA template to another during the synthesis of a progeny phage genome. A second model, for which there is chemical evidence (6), supposes that recombination reflects breakage and reunion of phage genomes. Visconti and Delbrück (7) assumed that mating occurred by reciprocal exchange of genetic material between pairs of vegetative phages. The partial replica hypothesis (8) assumes that vegetative phages either replicate by producing fragmentary genomes or break into autonomously replicating fragments and that recombination involves a reassembly of complete phages from fragments selected at random from the vegetative pool. For such a mechanism of recombination, we expect that all of the vegetative phages which have fragmented will be able to participate in each mating (reassembly) event.

3. Measuring the Mating Group.

Two attempts to determine the number of phage participating in a mating have been reported. Hausmann and Bresch (9) studied the kinetics of recombination in a triparental, three-factor cross in T1. Hershey (1) has studied recombination between ultraviolet (UV) inactivated T2 and T4 phages.

Hausmann and Bresch performed a cross between three parental phages of the types 111, 222, and 213. They determined in premature lysates the relative frequencies of doubly recombinant phages of the types 121 and 123. If matings were pairwise, it would be expected that in early lysates 121 progeny should occur in excess of 123 progeny since the former recombinant type could arise in a single mating and the latter could arise only through successive matings. For matings involving large groups of phages, the frequencies of the two recombinant types would be expected to be about equal even in the earliest lysates. The results of these experiments were consistent with small group mating (about three phages per mating), but technical difficulties rendered interpretation of the experiments uncertain. The authors favored the intuitive conclusion that the small apparent size of the mating group reflected topographic limitations on the availability of mating partners rather than an intrinsic property of the mating process.

Hershey's experiments involve studying the genetics of phages undergoing multiplicity reactivation (MR, the appearance of viable phage in cells infected with several UV-inactivated phages). Hershey assumes that a UV damage to a phage is equivalent to a localized lethal mutation and that MR involves the elimination of these

damages by normal recombination processes. He performs a linked marker cross of the type 11 x 22 and shortly after infection heavily irradiates the infected cells so as to introduce at least one UV lesion between the two marked loci of each vegetative phage. This introduction of randomly placed lethal "mutations" into the genetically marked region of the genome should unlink the markers flanking the region in the sense that phage progeny which have not experienced at least one exchange between the flanking markers are inviable. As a limiting case, we assume that single mating events are sufficient to produce viable progeny. Then pairwise mating should result in the appearance of about 25 percent recombinant phages, because 11 x 11 and 22 x 22 matings would produce viable progeny as often as 11 x 22 matings but could not produce recombinants of the type 12. Large group interactions, on the other hand, would result in 50% recombination because all matings would involve phage of both parental types. Hershey's observation is that under the specified conditions of MR, markers normally giving only 5 percent recombination emerge at genetic equilibrium among the progeny. This result suggests that mating occurs between large groups of vegetative phages. The validity of this conclusion, however, depends upon the validity of the mechanism postulated for MR.

Our attempt to measure the size of the mating group utilizes the following type of cross: Bacteria are infected with unequal numbers of parental phage of the genotypes abc and +++. The genes a and b are fairly closely linked to each other; neither is linked to c. We lyse the bacteria at various times during the latent period and examine the mature phage present at each of these times. We select from these progeny phage those recombinant for a and b and score them for the allele that they carry at c. Recombination between a and b is used as a "label" for identifying phage which have mated, and the frequency within this selected class of the majority parent's allele at c is used as a measure of the number of phage which participated in each mating.

If it were possible to examine vegetative phage directly without waiting for maturation to begin, we could make the following predictions: If mating were pairwise, the earliest a-b recombinant phage to appear should have an equal probability of carrying c or + at the unlinked third locus. As mating proceeds, the frequency of the majority allele at the third locus should approach its frequency in the gene pool; that is, its input frequency. If, on the other hand, each mating event involved an infinite number of phage (all of the vegetative phage in the cell), we would expect the independently segregating marker at c to appear at its

input frequency among the earliest a-b recombinants and to remain at this equilibrium frequency independent of subsequent mating. For mating groups of intermediate sizes, we expect results which lie between these extremes.

Since maturation begins well after the onset of mating, we cannot determine the number of phage participating in a mating by direct examination of the earliest recombinant phage to appear. Since the maturation process is cumulative, we expect any drift toward genetic equilibrium to be slower in the population of mature phage which we examine than in the population of vegetative phage from which they originate. In order to make quantitative predictions of the expected results of this experiment as a function of hypothetical mating group sizes, it is necessary to apply a theory of formal genetics which takes into account these restrictions on sampling imposed by normal phage development.

The qualitative aspects of the predictions still hold. We expect the fraction of mature a-b recombinant phage which carry the majority marker at c to rise with time if the mating group is small and to remain constant at the input frequency of the majority marker if the mating group is very large.

In the following sections of this thesis, we shall present evidence that the mating group in T4 is small and discuss the significance of these results.

II. MATERIALS AND METHODS

1. Media.

The liquid nutrient medium used in these experiments was H-broth: Bacto Nutrient Broth, 8 g; Bacto Peptone, 5 g; sodium chloride, 5 g; glucose, 1 g; distilled water 1 l. Each plate contained about 30 ml of EHA-bottom layer: Bacto agar, 10 g; Bacto Tryptone, 13g; sodium chloride, 8 g; sodium citrate (dihydrate), 2 g; glucose, 1.3 g; distilled water, 1 l. For the agar-layer plating technique (10) each plate was overlayed with bacteria and phage contained in about 2 ml of EHA-top layer: Bacto agar, 6.5 g; Bacto Tryptone, 13 g; sodium chloride, 8 g; sodium citrate (dihydrate), 2 g; glucose, 3 g; distilled water, 1 l. Dilutions were made in T-broth: Bacto Tryptone, 10 g; sodium chloride, 5 g; distilled water, 1 l.

2. Bacteria.

Strain B of Escherichia coli was used as host for all crosses. Strain S/6 (11), a derivative of B, was the standard non-selective indicator strain.

K/4 is the lambda-lysogenic strain K12S(λ1)/4 (12), and F is a hybrid between K12 and B produced by Fredericq (13) and lysogenized with phage lambda by R. S. Edgar. K/4 will support the growth of turbid "ghost" plaques of wild type T4 and of clear plaques of extended host

range (h) mutants of T4. Neither of the lysogenic strains will support growth of rII mutants (14) of T4, but both will support growth of rII$^+$ phage. A two to one mixture of F and K/4 was used as a selective indicator for distinguishing h from h$^+$ phage among the rII$^+$ progeny of the crosses. On the mixed indicator, h plaques are clear and h$^+$ plaques turbid. On this mixture h and h$^+$ grow with equal high efficiency.

CR63 (15) was used as a non-selective indicator strain for amber (am) mutants (16), which do not grow on B or its derivatives. S/6 was used as a selective indicator for am$^+$ recombinants in the cross involving am mutants.

3. Phage.

These experiments were done with the wild type strain T4D (11) and several mutant derivatives of that strain.

T4D r61 and r73 are non-reverting rII mutants described by Edgar (12). The former is in the A cistron; the latter is in the B cistron.

T4D h48 is a host range mutant described by Edgar (12). It was chosen from four of the Edgar h mutants tested because it was the most stable to heat inactivation. Jinks (17) has found that all of the h mutants in the "third linkage group" of T4, including h 48, are heat labile and that in mixed infections of h and

wild type a large fraction of the progeny phage are stabilized to heat inactivation by phenotypic mixing (18). The stable fraction of the progeny contains phage of genotypes h and h^+ in equal numbers in an equal-input cross. In the unequal-input crosses described in this thesis, h48 was always introduced in the minority parent in order to obtain lysates of the highest possible stability.

T4D r48 is an rI mutant described by Doermann and Hill (11).

The amber mutants T4D am54 and am85 were isolated and mapped by Epstein et al. (16) in this laboratory.

A linkage map of these mutants is presented in Figure 1.

4. Preparation of Phage Stocks.

All phage stocks were grown by inoculating liquid cultures of rapidly growing host bacteria with clonal populations of the appropriate phage. The phage inoculum is an agar plug containing a single, well-isolated plaque removed from a plate with a sterile capillary tube after the plate has incubated 4 hours at 30°C. The host suspension is prepared by diluting a saturated H-broth culture of appropriate host bacteria 1:1000 into fresh H-broth and aerating the culture at 30°C. for 2½ hours. The phage plaque is then inoculated into about 25 ml of this suspension.

Aeration is continued for 4-8 hours after infection until the culture appears clear and lysis is essentially complete. Chloroform is added to open any unlysed cells, and the culture is centrifuged in the cold (about 4000 x g for 20 minutes) and filtered through a Mandler candle to remove bacterial debris. CR63 is the host for plating and culturing am stocks; S/6 is the host for all other stocks.

5. Cross Procedures.

For equal-input crosses, the procedure is essentially that of Chase and Doermann (19); for unequal-input crosses, the method is essentially that described by Steinberg (20) for premature lysis experiments.

a. Equal-input crosses. Host cells in the exponential growth phase are prepared by diluting a saturated culture of B 1:1000 into H-broth and incubating 2½ hours at 30°C. with aeration. The cells are centrifuged in the cold, resuspended in fresh chilled H-broth, counted in a Petroff-Hausser counting chamber under a phase contrast microscope, and adjusted to a titer of 4×10^8. A few minutes before the addition of phage, potassium cyanide is added to the bacteria to give a concentration of 0.004 M. Equal volumes of phage and bacteria are then mixed and agitated by gentle aeration at 30°C. to promote adsorption. Each parental phage is added at a multiplicity of about 7 phage per bacterium.

After a 10 minute adsorption period, an aliquot is removed from the adsorption tube, treated with chloroform to kill infected bacteria, and plated to determine the titer of unadsorbed phage. Immediately after removal of this aliquot, antiserum is added to the adsorption tube to inactivate the unadsorbed phage, which in these experiments usually represent less than one percent of the phage input. After a 5 minute serum treatment, the adsorption tube is diluted 10^4-fold into a growth tube of H-broth prewarmed to 30^0C. The growth tube is incubated at 30^0C. with aeration. An aliquot of the growth tube is plated to determine the titer of infected cells. Multiplicity of infection is determined from this titer of infected cells and the titer of the input phage corrected for unadsorbed phage.

Premature lysis samples may be collected at any time during the latent period by shaking aliquots of the growth tube with chloroform (21).

b. Unequal-input crosses. In the unequal-input crosses described in this thesis, the multiplicity of the majority parent is 10-20, and that of the minority parent is usually about 0.1. The low input of the minority parent ensures that essentially none of the mixedly-infected bacteria will receive more than one minority parent and minimizes the statistical corrections of multiplicity values required for finite input

of parental phage (22). On the other hand, the low input of the minority parent requires alterations in the standard cross described above in order to satisfy the requirements that cyanide be sufficiently dilute in the growth tube to permit normal phage development (20) and that infected cells be sufficiently concentrated to yield statistically useful samples of recombinent progeny in early lysates.

Host bacteria and adsorption tube are prepared as described above. Aliquots of the adsorption tube are removed after a ten minute adsorption period for treatment with chloroform to determine the titer of unadsorbed phage and with serum to determine the titer of infected cells. From these titrations and the titrations of the parental phage, the multiplicities of infection are determined.

One ml of the adsorption tube is diluted into 39 ml of ice-cold H-broth and centrifuged for ten minutes in the cold (about 4000 x g at 0-4°C.). The supernatant is decanted, and the lip and sides of the centrifuge tube are wiped dry with a sterile cotton swab. The pellet is resuspended in a small volume of ice-cold H-broth and diluted into a 100-ml growth tube containing H-broth pre-warmed to 30°C. The growth tube is incubated at 30°C. with aeration. A chloroform treated

aliquot of the growth tube is plated for enumeration of unadsorbed phage. An aliquot is plated directly for titration of total infective centers. The titer of infected cells is determined from these assays by subtraction. Under these conditions, unadsorbed phage may constitute 50 percent of the infective centers in the growth tube. These unadsorbed phage, in no way affect data on selected recombinant phage in early lysates but render uncertain the determinations of total phage yield in these lysates. The recovery of infected cells after centrifugation ranged from 10-20 percent. Most of the loss probably arises from decantation of cells still in the supernatant or washed from the pellet during decantation. But the possibility remains that a significant fraction of cells is killed by the procedure.

Lysis samples are taken at various times by adding chloroform to aliquots of the growth tube. Adsorption of phage to bacterial debris is prevented by addition to each lysate of 1/10 its volume of a saturated solution of ammonium sulfate in distilled water (23).

6. Plating Conditions.

Plating bacteria are prepared by diluting saturated cultures of indicator strains 100-fold into fresh H-broth and incubating with aeration at 30°C. for 2½ hours, at which time the cells are in late exponential

growth. The bacteria are then centrifuged in the cold and resuspended in fresh broth to give a 10-fold concentration. The plating cultures remain usable for several days if stored in the refrigerator. Two to four drops of such a suspension are added to the EHA-top layer before plating. All plates are incubated at 30°C. for 16-24 hours.

For selective scoring of gII$^+$ recombinant progeny a mixture of F and K/4 is used. On this mixture, only gII$^+$ phage will produce plaques, and clear h plaques can be distinguished from turbid h$^+$ plaques. Control platings show that h and h$^+$ plate on the mixed indicator with equal plating efficiencies of 0.75-1.0 as compared to an S/6 standard. The low titer of recombinant phage in unequal-input crosses requires that large numbers of parental phage be plated on selective indicator plates. Control platings of the cross mixture on the mixed indicator were made to determine the background of plaques produced by recombinant phage arising from mixed infections of plating bacteria with parental particles. This background is negligible (smaller than the calculated sampling error) at the highest concentrations of parents plated from the lysates. Control platings of phage of the expected recombinant types on a background of the cross mixture indicate that no confusion in the

scoring of h and h^+ arises from contamination of gII^+ recombinant plaques by the large excess of parental phage in the background.

III. EXPERIMENTAL RESULTS

1. Unequal-input Crosses.

Data from eight unequal-input crosses involving the linked gII markers, g^{73} and g^{61}, and a freely segregating marker, h^{48}, are presented graphically in Figure 2. In each cross, h^{48} was introduced in the minority parent. In three crosses, the minority parent carried g^{73}; in the other five, the minority parent carried g^{61}. Plotted as a function of time of lysis is the fraction of gII^{+} recombinant progeny which carry the majority marker (h^{+}) at the third locus. This fraction, in all experiments, rises as a function of time of lysis. The data are in qualitative agreement with our predictions for the kinetics of small-group mating.

2. The Quantitative Predictions.

In order to make numerical predictions of the effects of mating group size on the kinetics of recombination, we use the theory of formal phage genetics presented by Steinberg and Stahl (24). Their symbols will be used throughout. The model giving rise to the equations assumes a panmictic population of phages mating at random with respect to partner. Matings are assumed to be randomly (Poisson) distributed among phages.

For a cross of the type abc x +++, we derive from the theory the following expression for b(a++), the frequency in the vegetative pool of the recombinant type a++ after n rounds of mating:

$$b(a++) = xy\left(e^{-nF(c_2+c_3+c_4)} - e^{-nF(c_1+c_2+c_3+c_4)}\right) + xy^2\left(1 - e^{-nF(c_1+c_2+c_4)} - e^{-nF(c_1+c_3+c_4)} - e^{-nF(c_2+c_3+c_4)} + 2e^{-nF(c_1+c_2+c_3+c_4)}\right). \quad (1)$$

The corresponding expression for the frequency of the type a+c is:

$$b(a+c) = xy\left(e^{-nF(c_1+c_3+c_4)} - e^{-nF(c_1+c_2+c_3+c_4)}\right) + x^2y\left(1 - e^{-nF(c_1+c_2+c_4)} - e^{-nF(c_1+c_3+c_4)} - e^{-nF(c_2+c_3+c_4)} + 2e^{-nF(c_1+c_2+c_3+c_4)}\right). \quad (2)$$

Where:

x is the relative multiplicity of abc.

y is the relative multiplicity of +++.

$(x + y = 1)$

m is the average number of matings per phage lineage.

F is the fraction of phage emerging from a mating which have a finite probability of being recombinant.

c_1 is the probability per mating of an exchange in region a-b only.

c_2 is the probability per mating of simultaneous pairwise exchanges in regions a-b and b-c (i.e. two-strand doubles).

c_3 is the probability per mating of an exchange in region b-c only.

c_4 is the probability per mating of simultaneous exchanges in regions a-b and b-c involving more than two parents (i.e. three-strand doubles).

Where p_1 is the probability of an exchange in region a-b. p_2 is the probability of an exchange in region b-c. and S is the coefficient of coincidence:

$$c_1 = p_1(1 - p_2 s)$$

$$c_2 = \alpha p_1 p_2 s \qquad \alpha + \beta = 1$$

$$c_3 = p_2(1 - p_1 s)$$

$$c_4 = \beta p_1 p_2 s$$

For matings in which exactly M phage participate equally:

$$\alpha = \frac{1}{M - 1} \qquad \beta = \frac{M - 2}{M - 1}$$

and p varies from zero to $(M - 1)/M$.*

We determine the total frequency of recombinants for markers a and b by addition of equations 1 and 2:

$$b(\underline{a}+\underline{+}) = xy\left(1 - e^{-mP(c_1+c_2+c_4)}\right) \qquad (3)$$

From the definition of the c's, it is apparent that:

$$c_1+c_2+c_4 = p_1$$

We can compute from the expressions derived above hypothetical predictions for the kinetics of recombination in the pool of vegetative phage. We now consider

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*It should be noted that $c_1=c_2$ when marker c segregates freely from markers a and b. Equations 1 and 2 then become symmetrical with respect to x and y. This is the criterion used in these experiments for testing the non-linkage of h^{48} to the rII region.

the effects of maturation on our predictions. We assume that maturation is a cumulative random sampling of the vegetative phage pool. Thus, the mature phage are heterogeneous with respect to mating experience both because mating is random in time and because maturation operates over a long period of time. To correct equations 1, 2, and 3 for the spread in maturation times, we average the equations over the range m_1F to m_2F *, where m_1 is the average number of matings per lineage for the earliest mature particles formed and m_2 for the last mature particles formed before lysis. In the following, m_1F and m_2F are designated m' and m". Then:

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*The constant F always appears in the product mF in these equations. It is therefore impossible to measure m and F independently in two- or three-factor crosses conforming to the model to which the equations are applicable. For this reason, the product mF is treated as the time-dependent variable.

$$b(\underline{a}+\underline{s}) = \frac{1}{m''-m'}\left[xy\left(\frac{e^{-m'(c_2+c_3+c_4)} - e^{-m''(c_2+c_3+c_4)}}{(c_2+c_3+c_4)} - \right.\right.$$

$$\left. \frac{e^{-m'(c_1+c_2+c_3+c_4)} - e^{-m''(c_1+c_2+c_3+c_4)}}{(c_1+c_2+c_3+c_4)} \right) +$$

$$xy^2\left(m''-m' - \frac{e^{-m'(c_1+c_2+c_4)} - e^{-m''(c_1+c_2+c_4)}}{(c_1+c_2+c_4)} - \right.$$

$$\frac{e^{-m'(c_1+c_3+c_4)} - e^{-m''(c_1+c_3+c_4)}}{(c_1+c_3+c_4)} - \qquad (1a)$$

$$\frac{e^{-m'(c_2+c_3+c_4)} - e^{-m''(c_2+c_3+c_4)}}{(c_2+c_3+c_4)} +$$

$$\left.\left. 2\,\frac{e^{-m'(c_1+c_2+c_3+c_4)} - e^{-m''(c_1+c_2+c_3+c_4)}}{(c_1+c_2+c_3+c_4)} \right)\right]$$

$$b(\underline{a}+\bullet) = \frac{xy}{m''-m'}\left(m'' - m' - \frac{e^{-m'(c_1+c_2+c_4)} - e^{-m''(c_1+c_2+c_4)}}{(c_1+c_2+c_4)} \right) \qquad (3a)$$

and equation 2a is symmetrical in x and y to 1a, provided that one marker segregates freely from the other two. If rounds of mating are linearly distributed, the average matings per lineage $\bar{m}$ for the mature phage sample is $(m_1 + m_2)/2$.

For the cross described, the fraction of mature phage recombinant for markers a and b which carry the majority marker at locus c is the ratio of equation 1a to 3a if +++ is the majority parent and the ratio of 2a to 3a if abc is the majority parent.

3. Determination of Mating Parameters.

In calculating hypothetical curves from these equations, we use values of x and y in the range of those measured directly in the unequal-input crosses. We assume that p_2 corresponds to the value required for non-linkage for any hypothetical α and β. The justification for this assumption of non-linkage is the absence of a convincing difference in the kinetics of recombination for crosses reciprocal with respect to the input multiplicities of g61 and g73 (Figure 2, A, B, C). That is, the data are consistent with the assumption that equations 1a and 2a are symmetrical in x and y.

The values of p_1, m', and m" are specified by the application of the theoretical model described above

to data from two-factor crosses. For a two-factor cross the frequency of recombination for phage in the vegetative pool is:

$$R = 2xy(1 - e^{-mFp}) \tag{4}$$

and for mature phage:

$$R = 2xy\left(1 - \frac{e^{-m'p} - e^{-m''p}}{(m''-m')p}\right) \tag{5}$$

For crosses in which the frequency of recombination is very low, equation 5 is approximately:

$$R = 2xy\ \bar{m}Fp \tag{5a}$$

In a two-factor cross with freely recombining markers, we can specify the value of p for any hypothetical mating group size. Measuring the recombination frequency among the first mature phage to appear in such a cross eliminates the effects of spread in maturation times and permits us to calculate m' from equation 4. In an equal-input cross of g61 and h48, the frequency of recombination at 23 minutes after infection (which corresponds in all of these experiments to about 0.5 plaque-forming particles per cell) was 0.356 ± 0.018.

The product of the input frequencies xy, corrected for finite input, was 0.233. From equation 4, m' is 2.9 for pairwise mating or 1.44M/(M - 1) for M-wise mating. This estimate is higher than Hershey's (1) estimate of 2.2, but Hershey points out that his "unlinked" markers are linked. The estimate does not differ significantly from that of 2.55 based on Doermann's r47 x tu43 cross (2).

For a cross between two closely linked markers, we expect from equation 5a that the recombination frequency among mature phage will be linear with rounds of mating. The drift in recombination frequency experimentally observed is linear with time (25), indicating that m is linear with time. The data from such crosses permit us to determine the ratio $\bar{m}P/m'$ at any time as the ratio R_t/R_1, where R_t is the recombination frequency at time t and R_1 is the recombination frequency among the earliest mature phage to be formed.

The linked markers (r61 and r73) used in the unequal-input experiments were used also in crosses to determine the time dependence of $\bar{m}P$. Wild type recombinants are scored selectively on the indicator strain F. It should be noted that on the selective indicator both wild type recombinants and r/r^+ recombinant

heterozygotes* give rise to plaques and are scored as r^+ recombinant progeny (26). This is true also of the selective platings of the unequal input crosses. Implicit in the choice of this selective technique is the assumption that recombinant heterozygotes are in

*A phage heterozygote is a single phage particle which gives rise to a genetically mixed progeny. The segregating markers are confined to a relatively short "overlap" region in which the phage appears to be heterozygous, on either side of which it appears to be homozygous. Heterozygous phages appearing among the progeny of a cross involving two closely linked markers are of two types: nonrecombinant heterozygotes (NRH) segregate principally the genotypes of the two parents used in the cross; recombinant heterozygotes (RH) segregate one parental genotype and one recombinant genotype. In a cross of r61 x r73, NRH constitute a negligible fraction of the selected class of progeny; RH constitute about 10-20 percent of the selected class (26). Levinthal (5) and Edgar (27) have proposed that heterozygous vegetative phages are intermediates in phage recombination. According to Edgar's model, markers flanking the overlap region recombine as a consequence of heterozygote formation; markers within the overlap region might undergo further recombination when the heterozygote segregates upon replication. The latter recombination step would be without genetic consequence in the segregation of RH, which have only one marker in the overlap region. Thus, a RH is in essence two phages which differ by only one marker. Whether such a heterozygote will be scored as recombinant or parental by a given selective technique depends, of course, on the markers and the selective technique.

fact equivalent to recombinant phage. It should also be noted that g61 and g73 are sufficiently far apart to give an error of about 10 percent in equation 5a for values of $\bar{m}F$ on the order of 6 or 7. We have ignored this deviation from linearity since it is within the limits of the experimental error.

Data from two crosses, one equal-input and one unequal-input, are presented in Table 1 and plotted in Figure 3. To simplify comparison of the crosses, the curves have been superimposed in the figure by plotting the R values for each cross as fractions of the R value at 55 minutes. Assuming, as we did above, that the 23 minute sample represents the earliest mature phage to be formed, we determine values of $\bar{m}F/m'$ from the curve in Figure 3. From these determinations and from the value of $1.44M/(M - 1)$ for m', we calculate that mF increases with time at the rate of $0.115M/(M - 1)$ per minute for M-wise mating or 0.23 per minute for pairwise mating. The post-lysis (90 minute) values of R give estimates of 2.67 m' and 2.33 m' for the final value of $\bar{m}F$. These values lie between Hershey's (1) estimate of 3 m' and an estimate of 2.0 m' based on Doermann's data (2). Final lysis values of m" based on our estimates of the final value of $\bar{m}F$ are $6.3M/(M - 1)$ and $5.3M/(M - 1)$ for M-wise mating.

Finally, we must specify the value of p_1, the linkage parameter in an $\frac{a}{+}61 \times \frac{+}{a}73$ cross. In nine crosses of $\frac{a}{+}61$ by $\frac{+}{a}73$, the recombination frequencies, corrected for unequal and finite input, were: 11.0, 8.9, 9.0, 8.3, 7.7, 7.9, 7.5, 9.6, and 7.6 percent. We shall use the average, 8.6 map units, as the distance between the two markers. Assuming that at the time of lysis $\bar{m}F$ equals 2.5 m', (which is the average of our two determinations of the final value of $\bar{m}F$), we find from equation 5a that p_1 is about $0.0125\ M/(M - 1)$.

4. Comparison of Data with Theory.

In Figure 4, we have plotted, for pairwise and 4-wise mating models, the fraction $b(\underline{a}+)/b(\underline{a}\cdot)$ for mature phage as a function of m" and time. For each model, we have calculated theoretical curves for three values of $\overline{xy}^2/\overline{xy}$*, where the bar signifies correction for finite input. (From equations 1a and 3a, we see that in the expression for $b(\underline{a}+)/b(\underline{a}\cdot)$ the relative multiplicities of the parents appear only in the fraction xy^2/xy.) Also plotted are the collected data from Figure 2.

It is evident at a glance that the data do not conform to our predictions. The data lie well below even the pairwise mating curves calculated for the range of multiplicities used in the experiments. Furthermore,

* See erratum, page 56.

the observed curves appear to rise somewhat more steeply than the theoretical curves. To the extent that the data can be said to fit any theoretical curve, they fit the curve calculated for pairwise mating with $\overline{xy^2}/\overline{xy}$ equal to 0.85. For most of the experiments, this implies an error of about a factor of 3 in the measurement of the multiplicity of the majority parent.

a. Effects of Errors in the Measurement of Mating Parameters. Before drawing conclusions from the failure of the data to conform to our predictions, we must reevaluate our predictions. The calculations of the theoretical curves are based on experimental measurements of three parameters and on an assumption concerning one of them. The equations consider two routes of formation of recombinants in three-factor crosses: direct production of recombinants in single matings and assembly-line production of recombinants through successive matings. Our operational definition of small-group mating is that with such mating we can distinguish between recombinants arising from these two routes. Among x^+ recombinants arising from single matings, the frequency of the freely segregating h^+ will be lower than it is in the gene pool because its frequency in the mating group is lower than its frequency in the gene pool. On the other hand, we expect x^+ recombinants which have had two or more mating

experiences to carry h$^+$ at equilibrium frequency. If in assigning values to the mating parameters we have made any errors which underestimate the contribution of the direct route to the production of r$^+$ progeny, our predicted kinetic curves will approach equilibrium too rapidly. We shall therefore consider the effects of altering within reasonable limits our estimates of the values of the mating parameters to see whether it is possible to reconcile our observations and our predictions. To avoid a cumbersome, if not an impossible, exposition we shall consider each estimate separately.

If we underestimate p_1, the probability per mating of an exchange between r61 and r73, we increase the probability of an r$^+$ recombinant having more than one mating in its lineage. The contribution of such an error, however, is very small. A two-fold increase in p_1 lowers the calculated curves less than one percent.

We have assumed that p_2 corresponds to the value required for non-linkage of h to the rII region and have supported the assumption by pointing to the identical kinetics of recombination in unequal-input crosses reciprocal with respect to r61 and r73. By assuming non-linkage, we have specified a value of one for S, the interference coefficient. It is possible to generate

identical theoretical curves for the kinetics of recombination in these reciprocal crosses even if h is linked to the gII region. The necessary condition is that we assign a value to S such that h is freely segregating in matings in which exchanges occur in the region between the gII markers. Although this has the effect of increasing for any value of mF the number of once-mated r^+ recombinants relative to the number of more-than-once-mated r^+ recombinants, it does not alter our predictions. We have assumed non-linkage of the gII and h regions in estimating m', which measures the mating experience of the earliest phage matured. The increase in m' resulting from the assumption of linkage between these regions exactly balances the effects of the linkage on the relative weights of the direct and sequential routes of recombinant production. Thus, our predictions based on the assumption of linkage of the "unlinked" marker are identical to those which we have already computed.

We have measured in two-factor crosses of closely linked markers the variation of $\bar{m}_F$ with time. An error in this measurement will expand or contract the m" scale which we have superimposed on the abscissa in Figure 4. Since this will not produce a vertical displacement in our theoretical curves but will change

only their slopes, we conclude that our chief problem does not arise from errors in this estimate.

Finally, we consider the estimate of m'. Since the pool of vegetative phage drifts rapidly toward genetic equilibrium during the first few rounds of mating, we might expect that small errors in the estimate of m' will produce large errors in our predictions of the early lysate results. As a minimum estimate of m', we use Hershey's value of 2.2 and compute pairwise mating curves corresponding to those shown in Figure 4. These curves are plotted, along with the data from the unequal input crosses, in Figure 5. We observe that these curves show a rise more or less parallel to the data but that the data still fit best a pairwise mating curve for $\overline{xy}^2/\overline{xy}$ equal to about 0.85-0.90. Within 95 percent confidence limits, our own estimate of m' is $2.3 < m' < 3.7$. We conclude therefore that an error in the estimation of m' does not wholly account for the lack of fit between our observations and our predictions.

b. Effects of Errors in the Model. The preceding discussion indicates that the vertical discrepancies between our data and our theoretical curves cannot arise from errors in our measurement of any of the mating parameters. We are compelled therefore to

examine the assumptions on which the theory is based for errors which might explain the discrepancies.

The model we have set up includes the following assumptions:

1) Adsorption is a random process leading to a Poisson distribution of adsorbed phage among the host bacteria.

2) All of the phage which adsorb to a bacterium enter its vegetative pool.

3) Mating is random with respect to partner.

4) Mating is random in time (Poisson distributed among phages).

5) The vegetative pool is a panmictic population.

We shall consider first the sorts of changes in these assumptions which are trivial in the sense that they bring the numerical predictions into agreement with the data without affecting our qualitative conclusion that the T4 mating group must be small.

We expect adsorption of phage to bacteria to be nonrandom because the population of host cells is heterogeneous with respect to size (28). Without assuming some specific non-Poisson distribution, we cannot speculate on the absolute effects of nonrandom adsorption

on the value of $\overline{xy^2}/\overline{xy}$, but such a skewed adsorption distribution is required to produce the observed deviation from the predicted curves that we doubt that nonrandom adsorption can account for much of the deviation. For example, if we assume that a population of bacteria adsorbs an average of 20 phages per cell and that all bacteria yield equal numbers of progeny phages, then 20 percent of the cells would have to adsorb 67 percent of the phage in order to lower $\overline{xy^2}/\overline{xy}$ from 0.95 to 0.85.

Only 10-20 percent of the infected cells in the unequal-input crosses are recovered after centrifugation. It is therefore possible that nonrandom adsorption and selective killing of cells which have adsorbed large numbers of phage conspire to reduce the value of $\overline{xy^2}/\overline{xy}$ among the survivors of centrifugation. Platings of post-lysis progeny of uncentrifuged control cells in every experiment yield results which are identical within experimental error to those for the latest lysis samples of the centrifuged cells. If selective killing occurred during centrifugation, we would expect the values for the control cells to be much higher than those for the centrifuged cells. It seems therefore that such selective killing does not occur.

If only part of the phage adsorbed to a cell could enter into the vegetative pool of the cell, then our measured multiplicities would be in excess of the actual multiplicities of infection and our estimate of $\overline{xy^2}/\overline{xy}$ would be correspondingly too high. Edgar and Steinberg (29) have shown that at least 30 infecting phages can replicate with equal efficiency in B, and this rules out the possibility of "limited participation" as an explanation of our results. If, however, we imagine that the vegetative pool of phages is compartmentalized such that the progeny of any one infecting phage can enter into genetic interactions with the progeny of only a part of the other infecting phages, then the measured relative multiplicities would not describe the intracellular matrimonial opportunities from the phage's point of view. If the population within each compartment were panmictic, the effect of such subdivision of the vegetative pool would be equivalent to increasing the titer of the host bacteria in direct proportion to the average number of subdivisions per bacterium. Clearly the experiments reported here are subject to too many uncertainties to constitute a critical test of this notion. Within the precision of these experiments, we are justified in concluding that the kinetic curves saturate at $\overline{xy^2}/\overline{xy}$ about equal to 0.85

for multiplicities of the majority parent on the order of 15-25. More conclusive support for the hypothesis would be the demonstration of a similar saturation of recombination frequencies in two-factor unequal-input crosses of freely segregating markers.

The assumption that mating is random in time requires that the duration of a mating be short as compared to the average time between matings and that no long rest period be required subsequent to mating. If the time required for mating is not negligibly short, a phage, each time it mated, would lose courting time available to its less-mated fellows. The consequences of this would be a surplus of phage which had mated only once, a deficit of phage which had not mated at all, and a lowering of our theoretical curve. Visconti and Delbrück (7) have shown, however, that the rate of disappearance of the minority parent in an unequal-input cross is in good agreement with random-in-time mating.

Any of the above-mentioned alterations in our model will lower the predicted curves without altering our qualitative prediction that small-group mating will result in a drift in time toward genetic equilibrium. We now consider a nontrivial hypothesis relating to panmixia and mating random with respect to partner.

Trautner (30) and Hausmann and Bresch (9) have proposed, in order to explain multiplicity-dependent variations in recombination frequencies in T1 crosses, that the vegetative pool becomes better mixed as the number of phage in the pool increases. Thus, the first mature phage to be formed might appear to arise from pairwise matings not because mating is pairwise, but rather because the recombinant phage arose from a sub-pool consisting of the progeny of only two infecting phages. As mixing improves with time, we expect a rise toward equilibrium which follows a curve dependent on the mixing model. This curve could conform fairly closely to a pairwise mating curve.

This sort of model is probably not applicable to studies of the recombination kinetics of T4. Hershey (1) points out that the close approach to genetic equilibrium observed in crosses of freely segregating markers implies that virtually perfect mixing must be achieved at some time during the latent period. Edgar and Steinberg (31) have done experiments to determine that time. They measured the frequency of phage heterozygotes in premature lysis samples and found a fifty percent increase in that frequency between the appearance of one phage per cell and ten phages per cell. From that time on, the frequency remained constant. Since phage heterozygotes are unstable products of pairwise interactions,

Edgar and Steinberg conclude that the attainment of an equilibrium frequency must reflect the attainment of a maximum degree of mixing. Hershey argues that that degree corresponds to panmixia.

In our experiments, as in Edgar and Steinberg's, a titer of 10 phage per cell is reached between 25 and 29 minutes after infection. We observe that all of the experimental curves continue to rise after this time and conclude that such a rise must result from small-group mating.

Edgar and Steinberg's observations establish that mixing is as perfect as it will be by about 27 minutes but leave open estimates of the degree of mixing before that time. It is evident from Figure 4 that many of our experimental points representing lysates collected between 23 and 25 minutes appear to be disproportionately low. Incomplete mixing before 25 minutes could account for this observation.

We have tested an alternative explanation of the lowness of these early points. Hershey and Chase (32), in two-factor T2 crosses, found that of the two percent of the progeny heterozygous for one marker six percent are heterozygous for a second, independently-segregating marker. If phage selected for having experienced recent genetic interactions in one region have an increased

probability of being heterozygous for a second region, it seemed reasonable to suppose that early recombinant progeny in premature lysis experiments might show increased heterozygosity for regions independent of the crossover region. In these experiments, h is always in the minority parent. Since a heterozygote for h and h^+ will yield phage capable of lysing K/4, it would probably be scored as an h plaque on mixed indicator. A significant number of such heterozygotes among early r^+ recombinants would cause an apparent surplus of r^+h recombinants in early lysates.

To test this notion, an equal-input cross of $rm54r48$ by $rm35$ was performed. Lysates were collected at various times, and rm^+ recombinant progeny were scored on the selective indicator S/6. The frequency of $r48$ heterozygotes among these recombinant progeny was measured in each lysate. The results of this experiment are shown in Table 2. The counts, although small, indicate that there is no significant excess of heterozygotes in early lysates. We conclude that the low values measured for early lysates in the unequal-input crosses do not arise from an excess of h heterozygotes. It is therefore possible that poor mixing accounts for these low values.

IV. DISCUSSION

We have presented data which indicate that recombinant progeny phages from T4 crosses do not derive freely segregating markers at random from all the phages in the vegetative pool, but tend to carry markers derived from only two vegetative phages. Mating, in the sense in which we have defined it, is pairwise.

This observation indicates that recombination in phage is not directly analogous to recombination between the chromosomes of higher organisms. In polyploid cells, multivalent synapsis of chromosomes is observed at meiosis. Bridges and Anderson (33) observed in triploid Drosophila that all three X chromosomes could participate in the production of one recombinant chromosome. If vegetative phages behaved like chromosomes and an infected bacterium were analogous to a highly polyploid cell, we would expect a synaptic pattern which would lead to large group mating. Our experiments do not fulfill this expectation.

Nor is the observation of pairwise mating consistent with the notion that recombination in phage is mediated by a large pool of fragments or partial replicas which are reassembled at random into complete genomes. Extensive fragmentation of vegetative phages would necessarily lead to large group mating.

The observation of pairwise mating in phage implies that recombination events are subject to restrictions which are not inherent properties either of copy-choice or breakage-reunion models of recombination. For a copy-choice model, pairwise synapsis would provide the necessary restriction. We can limit a breakage-reunion model to a pairwise mating system in three ways:

1. Mating is synaptic and involves either the reciprocal exchange of genetic material or cooperative construction of one genome which contains overlaps and several fragments which are discarded.

2. Vegetative genomes never break into more than two fragments. Thus, each mating (reassembly) can involve at most two phages.

3. Vegetative genomes break into more than two fragments, but the fragment pool is so small that it never contains fragments of more than two genomes.

The first of these models is entirely consistent with our observations. It is distinguishable from pairwise copy-choice recombination only by chemical studies.

The second model is not acceptable if the genome is linear. It requires that doubly recombinant phage arise through successive cycles of fragmentation and reassembly. The coincidence coefficient in a three

factor cross is zero. The model is inconsistent with the observation of negative interference in phage crosses because it requires that exchanges be completely independent events. Furthermore, instead of predicting identical kinetics for reciprocal crosses in the unequal-input experiments we have described, this model predicts that the fraction plotted in Figure 4 will rise toward equilibrium if two exchanges are required to produce the $\underline{r}^{+}\underline{h}^{+}$ recombinant and fall toward equilibrium if only one exchange is required.

The third model differs from the first only in that it lacks the requirement for synapsis. It assumes that the kinetics of fragmentation and reassembly are so balanced that the pool can never contain fragments of more than two genomes at one time. To avoid the objections we have just raised to the second model, we must assume that fragmentation of a genome arises from simultaneous random breaks at two or more places in the genome. We thus gain negative interference at the expense of accepting an unpalatable assumption. It is difficult to imagine a mechanism for producing simultaneous random breaks in a genome.

Before the recent discovery that the T4 linkage map may be circular (34), we would have concluded that our results strongly favored a synaptic pairing model

of recombination. A circular linkage map, however, opens new possibilities if it reflects a structural circularity of the phage genome. Two breaks are required to fragment a circle. Thus, two exchanges will be involved in the reassembly of a circle from the fragments of two circles. Stahl (35) points out that this might remove our objections to the second model described above because it builds negative interference into the fragmentation model. The requirement for two breaks per fragmentation establishes a structural basis for correlated recombination events. Stahl proposes that a cyclic fragmentation-reassembly process would simulate synaptic pairwise mating if the rates of fragmentation and reassembly were so balanced that the vegetative phage genomes never broke into more than two fragments. On the basis of this assumption, he has derived a mapping function which reduces the recombination frequencies measured in two-factor T4 crosses to a satisfactorily additive genetic map. He obtains essentially identical results with a mapping function derived on the assumption of synaptic circular mating. This might mean that synaptic and asynaptic pairwise mating are indistinguishable by genetic tests.

We have argued that pairwiseness is an intrinsic property of the T4 recombination mechanism. We find,

however, that the determination of this mating parameter restricts very little the sorts of models which might describe the recombination mechanism in phage. Meselson and Weigle (6) have presented evidence that the lambda genome is linear. If studies like those described here showed that mating was pairwise in lambda as well as in T4, we might rule out Stahl's model for asynaptic mating. At present, we can conclude only that the identification of the source of pairwise mating in T4 will require further study.

TABLE 1

Results of equal- and unequal-input crosses of $r^{61} \times r^{73}$ to determine the time dependence of m". For the equal-input cross, the input multiplicities were 16 and 12.8. For the unequal-input cross, they were 2.3 and 25.6. The frequency of r^+ is calculated as the number of plaques on F divided by the number on S/6.

Time of lysis	Frequency r^+ Equal-Input	Unequal-Input	Phage per cell
23	2.07×10^{-2}	5.75×10^{-3}	0.4
24	2.18	5.43×10^{-3}	1.1
29	2.63	———	18
33	2.92	8.09×10^{-3}	37
40	3.33	1.0×10^{-2}	95
45	4.02	9.71×10^{-3}	145
55	4.72	1.24×10^{-2}	178
90	5.63	1.30×10^{-2}	248

TABLE 2

The frequency of g48 heterozygotes among am+ recombinant progeny in premature lysates from an equal-input cross of am54g48 x am85. Only clearly mottled plaques are scored as heterozygotes. Ninety-five percent fiducial limits of expectation are calculated according to Stevens (36).

Time of lysis	No. am+ plaques examined	No. mottled plaques	Frequency of mottled plaques
22	2921	7	$0.00096 < f < 0.0049$
23	1026	4	$0.0011 < f < 0.0100$
33	954	7	$0.0029 < f < 0.0149$
71	1003	3	$0.00062 < f < 0.0088$

FIGURE 1

Linkage map of the markers used in the experiments described in the text. The map is derived from data presented in this thesis and from data of Doermann and Hill (11) and Epstein *et al.* (16).

FIGURE 1

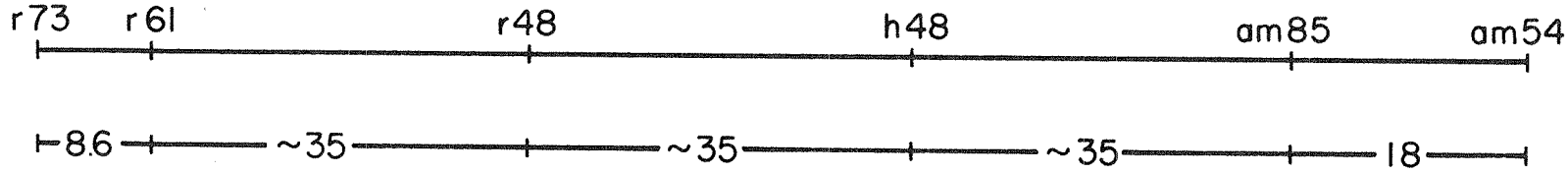


FIGURE 2

The results of the eight unequal-input crosses tabulated below. The abscissa is the time after the initiation of growth at which premature lysates were collected. The ordinate is the fraction of the majority recombinant r^+h^+ among the total r^+ recombinant progeny. The errors indicated around each point correspond to the standard deviation of the determination. A, B, and C each represent data from a pair of crosses in which the same preperation of host cells was used.

Symbol	Majority Parent	Minority Parent	Y	X	$\overline{xy^2}/\overline{xy}$	% Recovery
●	r^{73}	$r^{61}h^{48}$	17.2	0.15	.93	9
○	r^{61}	$r^{73}h^{48}$	25.4	0.075	.96	9
■	r^{73}	$r^{61}h^{48}$	10.0	0.08	.88	14
□	r^{61}	$r^{73}h^{48}$	13.8	0.08	.92	18
▼	r^{73}	$r^{61}h^{48}$	22.0	0.11	.95	10
▽	r^{61}	$r^{73}h^{48}$	19.0	0.11	.94	15
◆	r^{73}	$r^{61}h^{48}$	16.3	0.12	.93	24
△	r^{73}	$r^{61}h^{48}$	25.6	2.3	.89	21

Y is the multiplicity of the majority parent.
X is the multiplicity of the minority parent.
$\overline{xy^2}/\overline{xy}$ is the value of xy^2/xy corrected for finite input (22), where y is the relative multiplicity of the majority parent and x is the relative multiplicity of the minority parent (equations 1a and 3a).
The recovery of infected cells after centrifugation to remove cyanide is indicated in the last column.

FIGURE 2

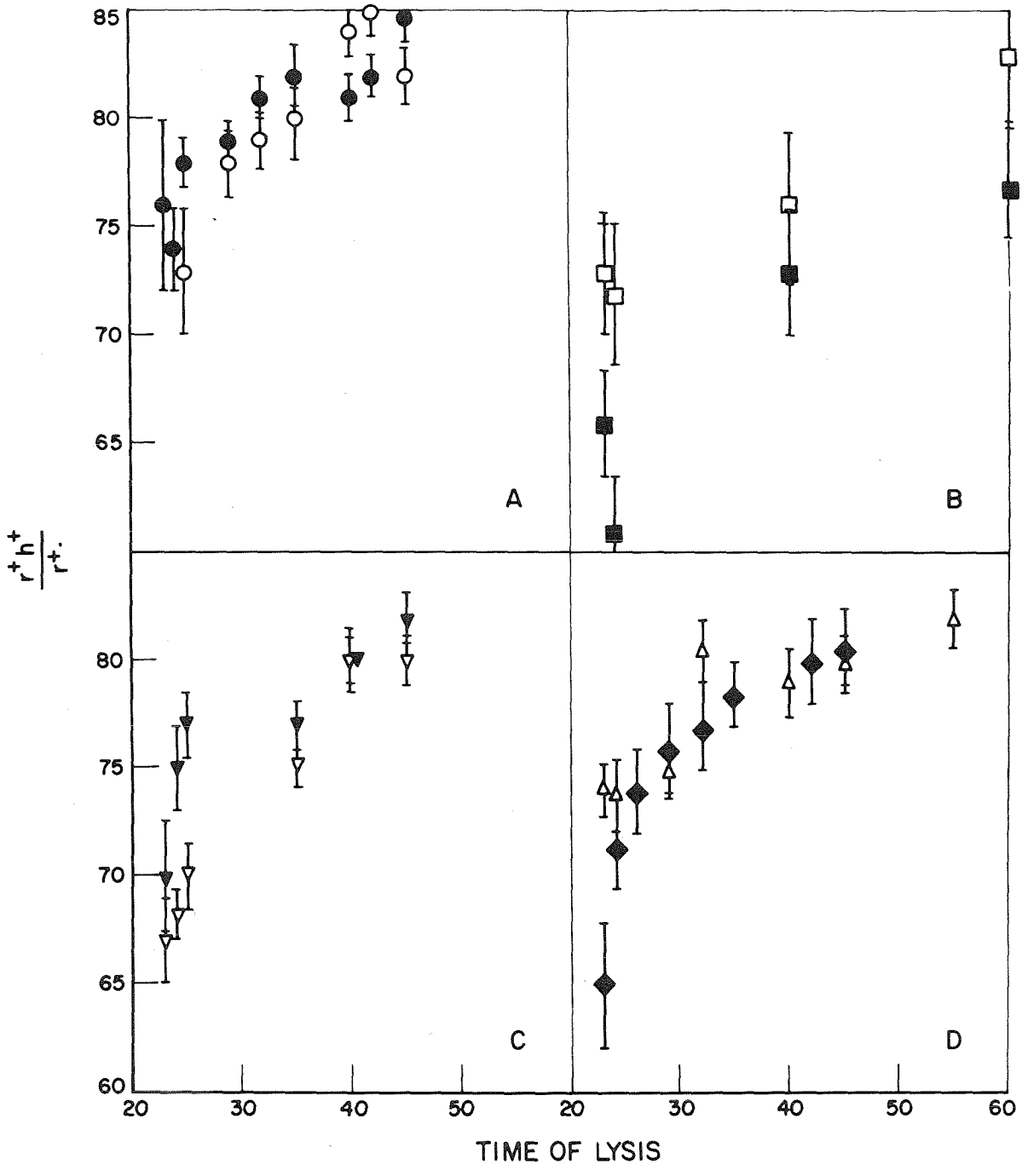

85
80
75
70
65
A
B
80
75
70
65
60
C
D
20
30
40
50
20
30
40
50
60
TIME OF LYSIS
r+h+ / r+-

FIGURE 3

The recombination data from Table 1 are plotted for comparison. The abscisse is time of lysis; the ordinate is the recombination frequency in each lysate expressed as a fraction of the recombination frequency at 55 minutes.

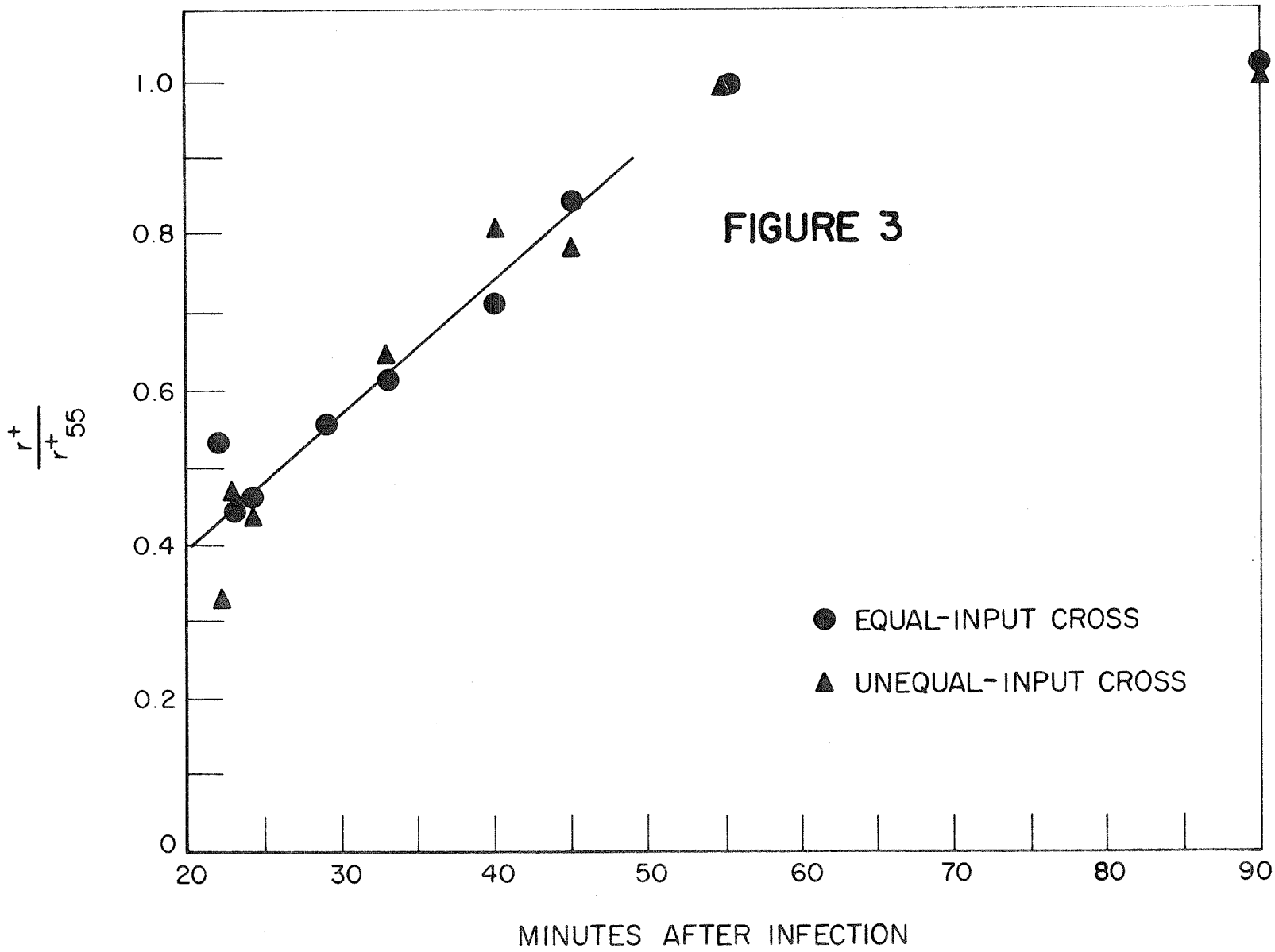

1.0
0.8
0.6
0.4
0.2
0
$\frac{r^+}{r^+_{55}}$
20
30
40
50
60
70
80
90
MINUTES AFTER INFECTION
EQUAL-INPUT CROSS
UNEQUAL-INPUT CROSS

FIGURE 3

FIGURE 4

Plots of the calculated values of the fraction of the majority recombinent r^+h^+ among the total r^+ recombinent progeny (equation 1a/equation 3a) in premature lysates for pairwise and 4-wise mating models. The solid curves are values calculated for pairwise mating; the dashed curves for 4-wise mating. From top to bottom, the curves represent predictions for unequal input crosses in which $\overline{xy}^2/\overline{xy}$ is 0.95, 0.90, and 0.85. The mating parameters used in the calculation are described in the text.

The data presented in Figure 2 are replotted for comparison with the predictions.

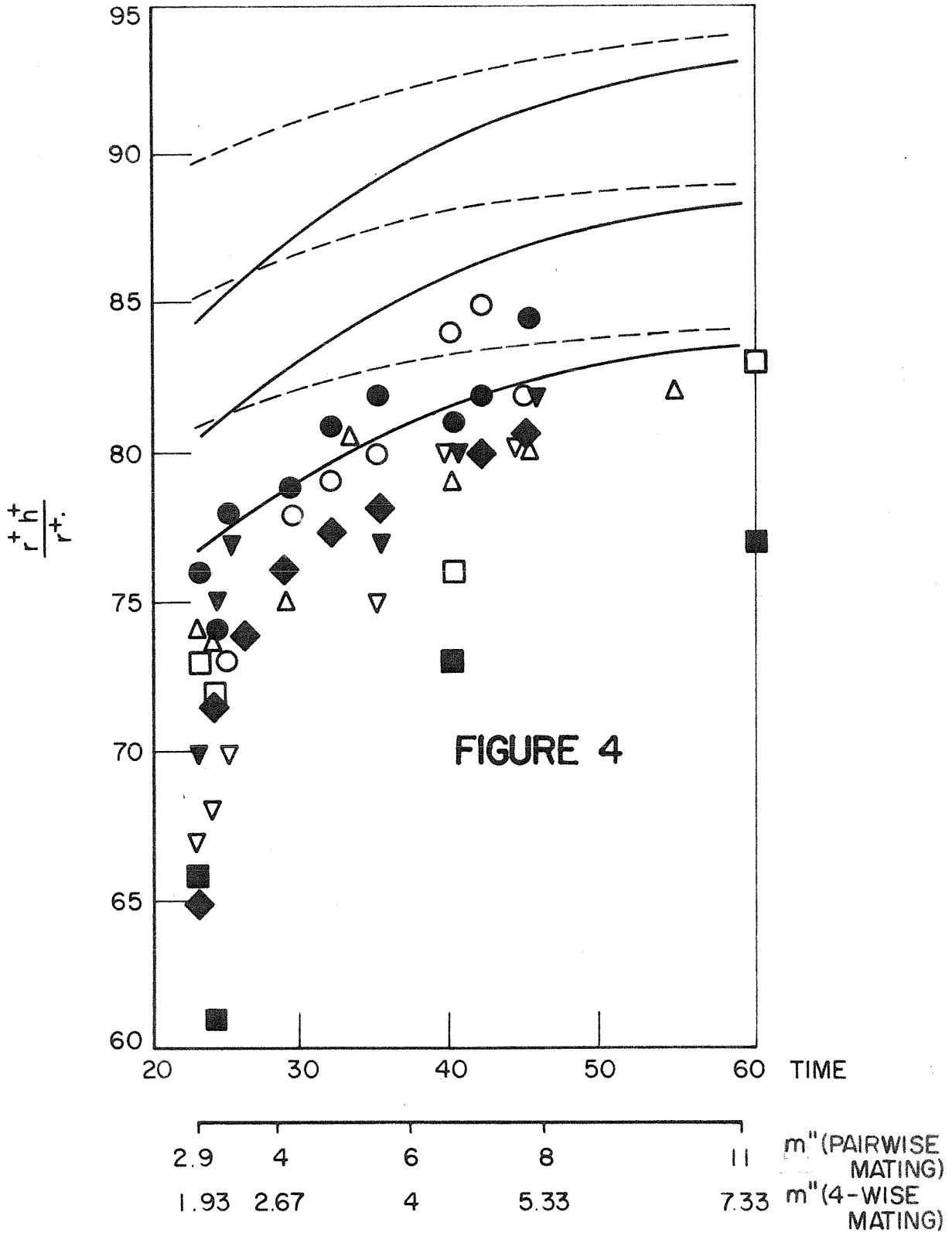
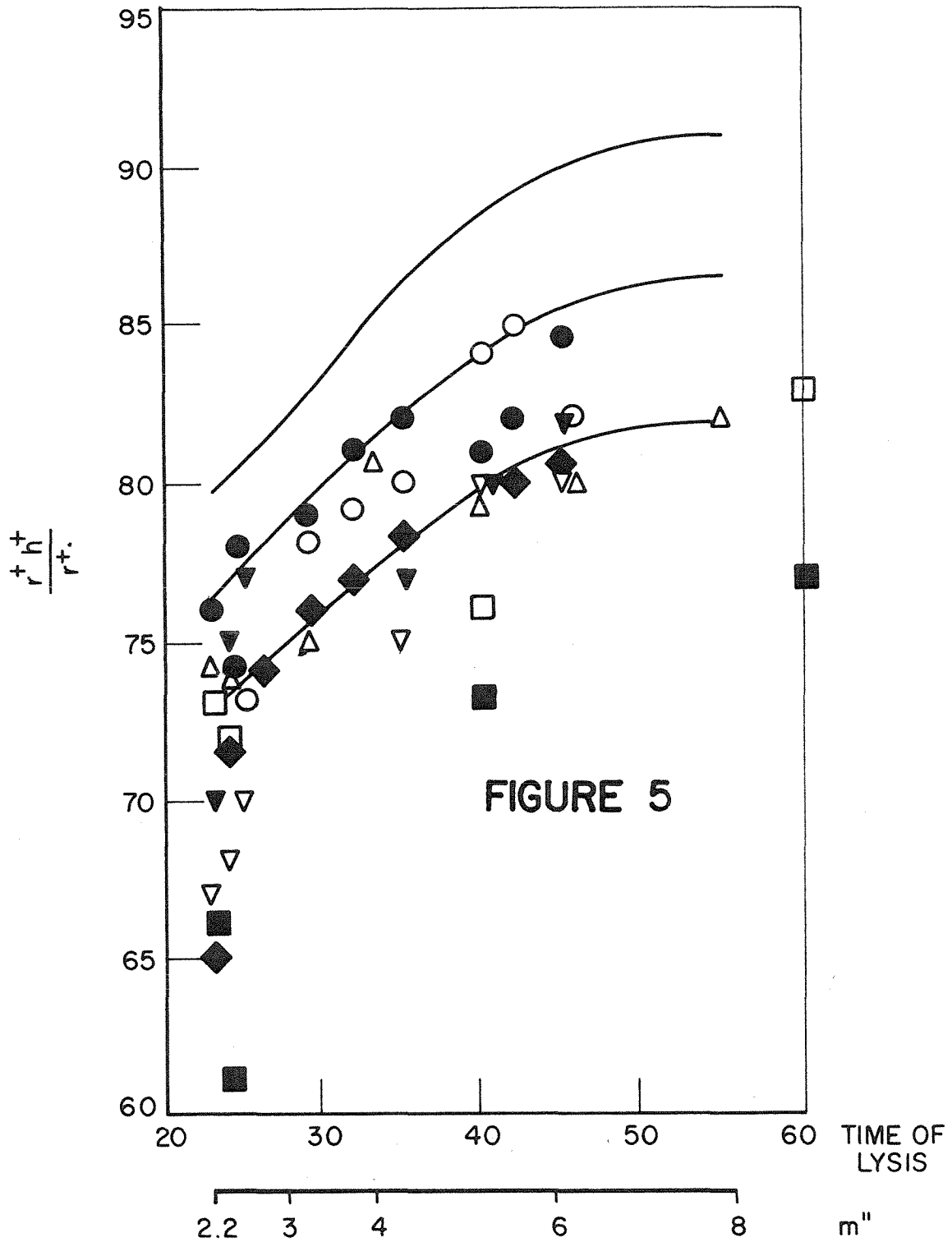

95
90
85
80
75
70
65
60
20
30
40
50
60
TIME
$\frac{+}{+}\frac{h}{r}\Big|\frac{+}{r}\cdot$
FIGURE 4
2.9
4
6
8
11
m" (PAIRWISE MATING)
1.93
2.67
4
5.33
7.33
m" (4-WISE MATING)

FIGURE 5

The curves, from top to bottom, are pairwise mating predictions for $\overline{xy^2}/\overline{xy}$ equal to 0.95, 0.90, and 0.85 and m' equal to 2.2. This value of m' is Hershey's (1); the other mating parameters are calculated from this value of m' and the data presented in the text. The data from Figure 2 are also plotted.


95
90
85
80
75
70
65
60
20
30
40
50
60
TIME OF LYSIS
2.2
3
4
6
8
m''
FIGURE 5

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Erratum: For $\overline{xy}^2/\overline{xy}$ read $\overline{xy^2}/\overline{xy}$. This error in notation has been made throughout the text.