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An Analysis of the Clone Size Distribution of ΦX174 Mutants and Recombinants

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In a quasi-single-burst experiment the clone size distribution of the number of wild-type bacteriophage ΦX174 appearing after a single cycle of growth of ΦX amber mutants in the permissive host *Escherichia coli* CR34/C416 was determined by plating on the nonpermissive host *E. coli* C. The distribution of revertant phage (mutants) was almost random, not clonal as found for T2. This result is consistent with a theory that the progeny single-stranded DNA molecules are synthesized off one or a few templates in a "stamping machine" fashion; however, other explanations for the random distribution are not excluded.

The distribution of recombinants approximates a clonal distribution like the distribution of recombinants between loosely linked markers of T4. This result can perhaps be considered to indicate a geometric replication process; however, it is argued that the distribution is also explainable by the stamping machine model for ΦX replication.

INTRODUCTION

A hypothesis detailing the mechanism of replication of ΦX174 has been proposed (Denhardt and Sinsheimer, 1965d) to explain a number of experimental observations (Denhardt and Sinsheimer, 1965b, c). Some facts about the ΦX replication process and the suggested mechanism are outlined in Fig. 1; the attribute of the model relevant to this paper is the hypothesis that only the parental replicative form (RF) serves as the template for the synthesis of all the progeny single-stranded DNA.[1]

[1] The data of Matsubara *et al.* (1963a) indicate that in each infected cell about 1500 single-stranded DNA molecules were synthesized in 13 minutes; since there are 5500 nucleotides in ΦX DNA, this fact implies that if all the single-stranded DNA molecules are synthesized on the parental RF molecules, then about 5 nucleotide links are formed each millisecond. The same figure obtains for *E. coli*, which contains about 6×10^6 nucleotides and replicates every 20 minutes. This comparison suggests that it is possible for all the progeny ΦX DNA to be synthesized on a unique template.

Luria (1945) proposed three possible ways in which the viral genome could reproduce. The descriptions of these ways as (a) "exponential" (or "geometric"), (b) "follow-the-leader," and (c) "stamping machine" are self-evident (see Stent, 1963, page 192). Luria (1951) subsequently showed that the distribution of T2 mutants was clonal, in agreement with the prediction derived from case (a) and incompatible with the predictions of cases (b) and (c). Luria's analysis was subsequently refined by Steinberg and Stahl (1961) with the same conclusions.

A prediction of the scheme proposed for ΦX replication is that the clone size distribution of ΦX mutants should be in accord with case (c), the "stamping machine," since it is suggested that the parental RF "stamps out" all the progeny single-stranded DNA molecules. This paper presents data to test this prediction; in addition, the clone size distribution of ΦX recombinants will be discussed as it relates to the various replication mechanisms.

The clone size distribution of T2 and T4 recombinants has been studied by Hershey

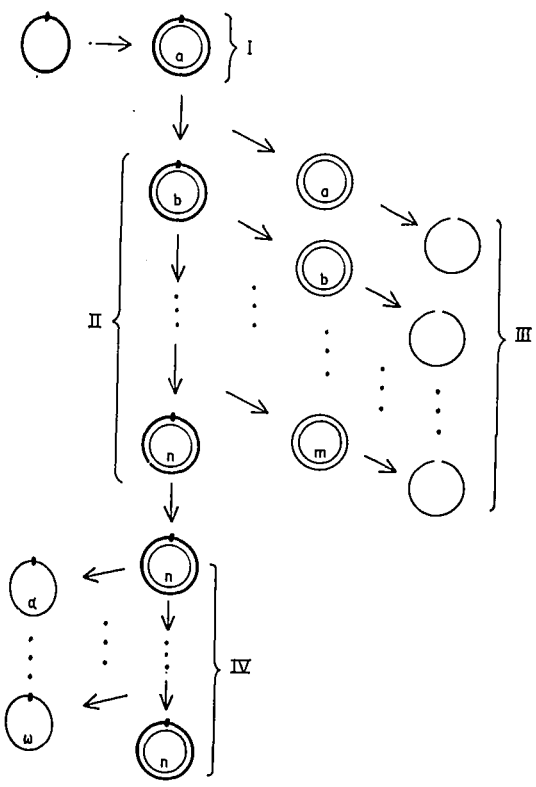

FIG. 1. The replication of ΦX174. Stage I: The infecting single-stranded DNA is converted into a double-stranded intracellular replicating form (RF) by *de novo* synthesis of the complementary strand of DNA by a host enzyme (Sinsheimer *et al.*, 1962; Denhardt and Sinsheimer, 1965b). The nature of the linker, represented by a dot, is not known. Stage II: The RF molecule containing the parental single-stranded DNA (the parental RF) is replicated semiconservatively by a coli enzyme (Denhardt and Sinsheimer, 1965b). There is weak evidence (Denhardt and Sinsheimer, 1965b; K. Matsubara, personal communication) that the progeny RF usually do not replicate. Stage III: The progeny RF may synthesize messenger RNA. The data of Hayashi *et al.* (1963) indicate that the base sequence of all the messenger RNA molecules is identical to the base sequence of single-stranded ΦX DNA. Stage IV: Because (1) the infected complex remains sensitive to the decay of ^{32}P carried in the parental single-stranded DNA up to the end of the eclipse period (Denhardt and Sinsheimer, 1965c), (2) there is no parent to progeny transfer of DNA (Kozinski, 1961; Sinsheimer, 1961), and (3) the progeny single-stranded DNA is synthesized *de novo* (Matsubara *et al.*, 1963b; Denhardt and Sinsheimer, 1965b), only the parental RF is assumed to serve as template of the polymerase for the conservative synthesis of progeny single-stranded DNA. Thus, all the progeny single-stranded DNA molecules (α to ω) are synthesized off the same "minus" strand in the parental RF in a "stamping machine" fashion. This synthesis is assumed to be performed by a DNA polymerase coded for by a ΦX gene resembling in function the DNA-directed RNA-polymerase, except that it polymerizes deoxyribonucleotides. In addition to biochemical evidence (Sinsheimer, 1959; Sinsheimer *et al.*, 1962), there is genetic evidence that all the ΦX particles have identical single-stranded DNA molecules (I. Tessman *et al.*, 1964) and that the replication of ΦX174 involves a double-stranded DNA molecule (Howard and Tessman, 1964).

and Rotman (1949), Stahl (1956), and Steinberg and Stahl (1961). For closely linked markers (0.1% recombination frequency) the distribution of recombinants is random (Stahl, 1956). This result can be explained if the assumption is made that recombinants between closely linked markers tend to be formed late in the latent period and hence have little chance to multiply (Steinberg and Stahl, 1961). Recombinants between more loosely linked markers could be formed earlier in the latent period and should have a chance to multiply to some extent. This seems to be the case.

The frequency of recombination between genetic markers in ΦX (and the related phage S13) is of the order of 0.05–0.005% (E. S. Tessman and I. Tessman, 1959; Pfeiffer, 1961; E. S. Tessman, 1965), lower than the recombination frequency observed between even the most closely linked T4 markers discussed above. Because of this low recombination frequency it is necessary to use a selective technique to detect the recombinants. To this end wild-type recombinants between conditionally lethal amber mutants (Stent, 1963, page 218) were selected by plating on the nonpermissive host.

MATERIALS AND METHODS

Phage and bacteria. The three ΦX174 amber mutants used were *am6*, *am8*, and *am9*; they were isolated from a nitrous acid-inactivated stock by C. Hutchinson in R. Sinsheimer's laboratory. The three mutations are in three separate cistrons as judged by complementation tests. Mutants *am8*

and *am9* are unable to grow at 40°; mutant *am6* can grow at 40°. From a nitrous acid-inactivated stock (I. Tessman, 1959) of *am6* a temperature-sensitive phage (*am6-25*) unable to grow at 40° was isolated. Reversions could occur independently at either locus of all the temperature-sensitive amber phage. The reversion index for the amber mutants ranged from about 10^{-6} up to as high as 10^{-4} for *am6* and *am8*; for *am9* it was around 10^{-8}.

The permissive host for the amber mutants is *Escherichia coli* CR 34/C416. This strain (referred to as CR) derives its permissiveness from the CR34 parent, which is related to *E. coli* CR63 and presumbly carries the same *am* suppressor. CR requires thymine or thymidine (Denhardt and Sinsheimer, 1965b). The nonpermissive host is *E. coli* C (BTCC No. 122); it will be referred to as C.

Media. Modified 3XD is the medium described by Guthrie and Sinsheimer (1960). Starvation buffer (SB), tryptone-KCl broth (TKB), bottom agar, and top agar were described by Denhardt and Sinsheimer (1965a). Thymidine was obtained from Calbiochem and stored in the cold as a sterile solution in water at 2 mg/ml.

Experimental procedure. Log phase CR was grown to 1 to 2×10^8 cells/ml in modified 3XD supplemented with thymidine, 20 μg/ml. The cells were pelleted, washed once with SB, resuspended in 5 ml of SB at 1 to 2×10^8 cells/ml and starved by aeration at 30° for 1–2 hours. The starved cells were assayed for viable cell titer, and phage in SB were added to a multiplicity of 3–6 phage/cell when only a single genotype was involved. For the recombination experiments, the cells were infected simultaneously with two amber mutants, each at a multiplicity of 5. This was achieved by mixing aliquots of two recently assayed phage stocks and using a sample of this mixture to infect the cells.

Aeration at 30° was continued for 10 minutes to allow phage adsorption. The culture was filtered through a 0.45 μ filter, washed 2 times with 5 ml of ice-cold SB, and

TABLE 1

SUMMARY OF THE MUTANT CLONE SIZE DISTRIBUTION DATA

	am6	*am8*	*am9*	Total	Random dist.[c]	Cum. dist.[d]	
						ΦX	T2
Total[a]	740	778	602	2120	—	—	—
$\mu = 0$[b]	646	679	510	1835	—	—	—
1	78	76	74	228	237	264	183
2	9	8	13	30	23	36	90
3	1	1	2	4	3	6	63
4	1	—	—	1	0	2	41
5	—	—	1	1	—	1	35
6	—	—	—	0	—	0	32
7	—	1	—	1	—	—	25
8	—	1	—	1	—	—	21
9	1	—	—	1	—	—	19
10	1	3	1	5	—	—	19
10+	3	9	1	13	—	—	16

[a] The total number of single-burst tubes examined for each mutant separately and all together.

[b] The number of single-burst tubes with 0, 1, 2, etc., revertants.

[c] The number of tubes with 1, 2, 3, and 4 revertants expected from a completely random distribution, calculated from the proportion of tubes with 0 revertants using the Poisson distribution.

[d] The cumulative distribution of clone sizes of mutants of T2 and ΦX. The number shown on each line is the number of clones that contained μ or more revertants. The data for T2 are taken from Table 3 of Luria (1951). We believe that the 21 ΦX clones with 7 or more revertants originated from infections initiated by (preexisting?) revertants (see Table 2), and hence we have excluded them from the cumulative distribution; even if they were all included the interpretation of the data would not be altered.

resuspended in 5 ml of cold SB. The resuspended culture was assayed for infective centers on C and CR and for surviving bacteria. The surviving bacteria were measured by plating at 40° so that the temperature-sensitive phage on the plate were unable to grow; usually between 10% and 50% of the bacteria survived.

A sample of the resuspended culture was lysed with lysozyme-Versene (Denhardt and Sinsheimer, 1965a), and the phage titer was determined. Since the infected cells had had no time to develop, the phage observed were uneclipsed phage carried over from the original stock.

The infected cells were diluted in TKB, and appropriate numbers were distributed in 0.25-ml volumes into small test tubes and incubated for 3–5 hours at 30°. Usually immediately following the incubation period the contents of each tube were plated at 30° with about 1×10^8 TKB-grown log-phase C in 1.2 ml of top agar on small plastic petri plates containing 8 ml of bottom agar. The average burst size of the amber phage was measured by assaying an aliquot of several of the single burst tubes on CR.

Reconstruction experiments showed that during the plating for mutants (i.e., revertants of the amber mutants) all the non-amber phage could be detected among the 10^3 to 10^6 amber phage on the plate, and that during the plating for recombinants recombination between the amber mutants on the plate did not occur.

RESULTS

Mutant Clone Size Distribution

The results of the experiments on the clone size distribution of ΦX mutants are presented in Tables 1 and 2. The total cumulative distribution is plotted in Fig. 2 together with Luria's (1951) data for T2. Clearly, the distribution of ΦX mutants is very different from the distribution of T2

TABLE 2

ANALYSIS OF THE MUTANT CLONE SIZE DISTRIBUTION DATA

Parameter*	*am6*	*am8*	*am9*
Expected carry-over[a]	0.72	5.3	0.3
Expected full bursts[b]	2.1	13.1	0.58
Observed full bursts[c]	5	14	2
Average size of full revertant bursts[d]	112	38	90
Expected fertile bursts[e]	270	74	90
Observed fertile bursts[f]	94	99	92
$\sum_1^5$ wild[g]	103	95	111
$\sum$ ambers[h]	9.0×10^7	5.5×10^7	2.6×10^9
Mutation frequency[i]	1.1×10^{-6}	1.7×10^{-6}	4.3×10^{-8}
Average burst size of revertants[j]	1.16	1.12	1.23
Average burst size of amber phage[k]	167	28	51

* (*a*) The expected carry-over of revertants is calculated from the reversion index of the phage stock used and the fraction of total phage carried over as determined after lysozyme-Versene treatment. (*b*) The expected number of full bursts of revertants is calculated from the reversion index of the phage stock and the total number of infected cells examined. (*c*) The observed number of full bursts is considered to be the number of bursts with 7 or more revertants (Table 1). It is not unreasonable that the observed number of full bursts is larger than the expected number because more than one phage particle (probably 3 or 4) can grow in an infected cell. (*d*) The average burst size of the observed full revertant bursts indicated in (*c*). (*e*) The number of bursts expected to yield revertants is calculated from the proportion of infective centers which yield revertants when plated before distribution into the single-burst tubes. (*f*) From Table 1. (*g*) The total number of revertants in tubes with 5 or fewer revertants. (*h*) The total number of amber phage particles tested. (*i*) The ratio of (*g*) to (*h*) of this table. Luria (1951) found that the mutation frequencies of the two mutants he looked at were 1.2×10^{-4} and 5×10^{-5}. (*j*) The average burst size of the revertants, calculated as the ratio of (*g*) to (*f*) − (*c*) of this table. The data given by Luria (1951, Table 3) indicate that the average burst size of the two mutants he looked at was about 4. (*k*) The average burst size of the amber mutants in the experiments presented.

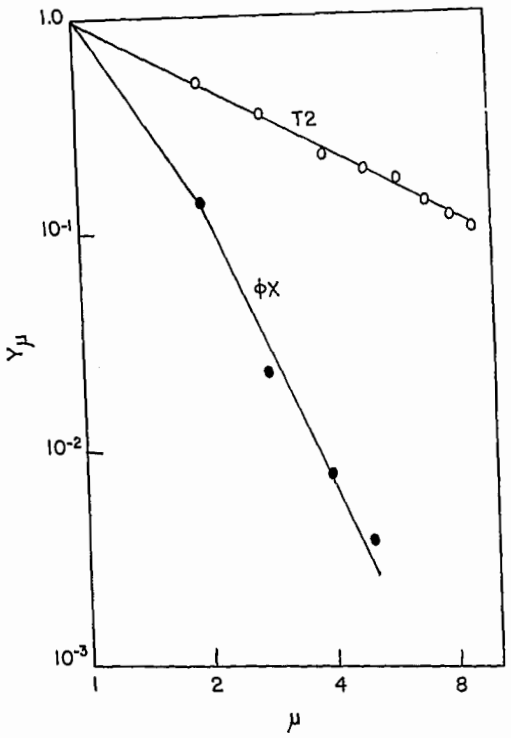

FIG. 2. Double-logarithmic plots of the cumulative clone size frequency distribution of ΦX and T2 mutants. The abscissa, μ, is the clone size; the ordinate, Y_μ, is the frequency with which clones of size μ or more are found. The cumulative clone size frequencies are calculated from the data in the last two columns of Table 1.

mutants and is almost random (Table 1) as expected for a "stamping machine" replication mechanism (Stent, 1963, page 193).

Because the ΦX results are represented essentially by a few plates with one or a few plaques on them among many plates with no plaques, it is important to be sure that the plaques observed really represent occasional revertant phage present in a burst rather than contamination or carry-over of revertants from the original stock. The temperature-sensitive amber phage were used as controls against contamination; a sample of phage was picked from the non-amber plaques and tested on C for temperature sensitivity. In most experiments fewer than 10% of the plaques yielded phage able to grow at 40°. These plaques were assumed to result from contamination and were eliminated from the data.

The number of revertant phage carried over from the original stock was determined by lysing the infected cells with lysozyme-Versene and assaying for phage. In all cases the number of revertant particles carried over (Table 2) was insufficient to account for the number of wild-type plaques.

Recombinant Clone Size Distribution

Table 3 contains a summary of the data on the clone size distribution of recombinants resulting from the amber crosses. The cumulative total is given in the last column of Table 3 and is plotted in Fig. 3 together with

TABLE 3

SUMMARY OF THE RECOMBINANT CLONE SIZE DISTRIBUTION DATA

	Cross			Cumulative total[c]
	am8 × *am9*	*am6* × *am8*	*am6* × *am9*	
Total[a]	592	592	594	—
$\rho = 0$[b]	528	508	482	—
1	33	50	68	260
2	6	8	19	109
3	7	3	8	76
4	5	3	4	58
5	2	3	2	46
6	1	4	1	39
7	3		1	33
8	1	2	4	29
9	1	1	2	22
10	1	—	—	18
11	1	—	—	17
12	—	2	—	16
13	—	1	—	14
14	1	—	1	13
16	—	—	1	11
17	1	—	—	10
18	—	1	—	9
35	—	1	—	8
36	—	1	—	7
51	1	—	.	6
58	—	1	—	5
68	—	1	—	4
71	—	1	.	3
78	—	—	.	2
89	—	1	—	1

[a] The total number of single-burst tubes tested.

[b] The number of tubes with 0, 1, 2, etc., recombinant phage.

[c] The number of clones that contained ρ or more recombinants.

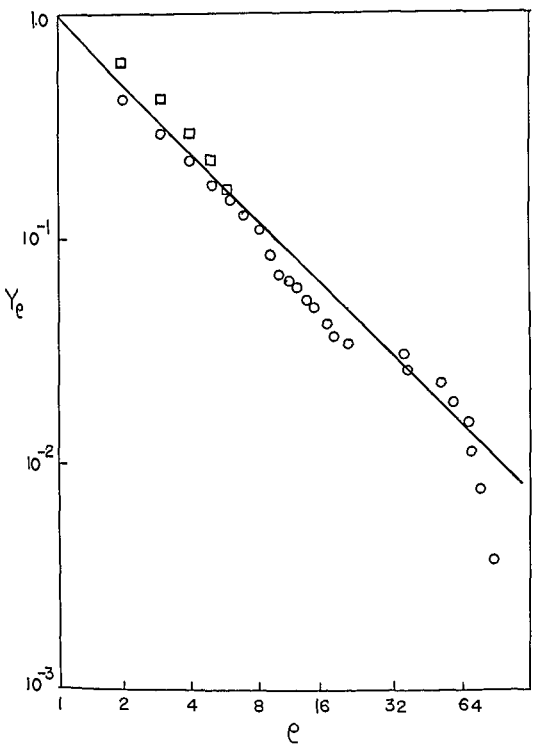

FIG. 3. Double-logarithmic plots of the cumulative clone size frequency distribution of ΦX and T4 recombinants. ρ is the number of recombinant phage observed in a clone; Y_ρ is the frequency with which clones of size ρ or more are observed. O——O is the plot for ΦX, normalized to the total number of clones and derived from the last column of Table 3. □——□ is the plot for T4, taken from data of Stahl (1956, Table 4) for recombinants between two T4 markers giving 1.1% recombination. The straight line represents the theoretical distribution expected for a geometric replication mechanism.

Stahl's (1956) data on the clone size distribution of recombinants between two T4 mutants which recombine with a frequency of 1.1%. Both sets of data are consistent with the hypothesis that the genetic material replicates in a geometric fashion.

Further details of the recombinant clone size distribution experiments are given in Table 4. The recombination values form a proper additive genetic map with *am9* midway between *am6* and *am8*. It is perhaps significant that there is an apparent correlation between the average burst size of the recombinant and the recombination frequency; however, further data are needed before this point can be considered established.

Stahl (1956) found that the average burst size of the wild-type recombinant was about 1.2 and 2.8 in crosses between two T4rII markers giving 0.1 and 1.1% recombinants, respectively; the total burst size was close to 200. For ΦX the average burst size of the wild-type recombinants (Table 4) is larger than this, and the difference is particularly significant when the lower average burst size of nonrecombinant phage and the lower recombination frequency are considered. For T2 and T4 no bursts of recombinants were found that even approached in size the average burst size of the nonrecombinant phage. In contrast, a significant number of bursts of ΦX recombinants were observed that exceeded in size the average burst size. It appears that although recombination occurs in only about 0.2% of the infected cells, the cells in which it does occur are likely to release more than one recombinant phage particle.

In the cross of *am6* × *am8* two distinctly different types of recombinants are formed; one makes giant plaques with fuzzy edges, the other makes small plaques with distinct edges. Although many clones were found containing many recombinant phage, in no case were the two kinds of recombinants observed to emerge from the same burst. This result suggests that when more than one recombinant appear in a burst they are all derived from the same event and that it is unlikely that two recombination events occur in the same cell.

DISCUSSION

The Random Distribution of Mutants

Reversions in the nonpermissive host. A serious complication concerning the interpretation of the random mutant distribution arises from the fact that both the process of RF formation and that of RF replication seem to be performed by host cell enzymes (Sinsheimer *et al.*, 1962). Hence, when an amber phage infects the nonpermissive host, its single-stranded DNA is incorporated into RF, and the RF is replicated. This occurs for the three amber phage studied here (Sinsheimer, personal communication).

TABLE 4

ANALYSIS OF THE RECOMBINANT CLONE SIZE DISTRIBUTION DATA

Parameter*	Cross		
	am8 × *am9*	*am6* × *am8*	*am6* × *am9*
No. fertile bursts (*a*)	64	84	112
No. wild plaques (*b*)	243	563	327
No. amber phage (*c*)	1.34×10^6	1.78×10^6	2.05×10^6
No. infected cells (*d*)	3.6×10^4	3.9×10^4	3.5×10^4
Expected fertile bursts (*e*)	0.3	0.45	0.09
Expected mutants (*f*)	2.4	5.0	2.3
Recombination frequency (*g*)	1.81×10^{-4}	3.16×10^{-4}	1.6×10^{-4}
Burst size recombinants (*h*)	3.8	6.7	2.9

* (*a*) The total number of infected cells that released recombinants. (*b*) The total number of recombinant phage observed. (*c*) The total number of amber phage tested. (*d*) The total number of infected cells examined. (*e*) The number of cells that could have been infected with a previously existing revertant. (*f*) The number of wild-type revertants expected among the amber phage listed in (*c*); this number is calculated using the mutation frequencies determined in Table 2. (*g*) The recombination frequency: the ratio of (*b*) to (*c*). (*h*) The average burst size of the recombinant phage: the ratio of (*b*) to (*a*).

The lethal (in the nonpermissive host) mutation carried by the amber phage affects some step in the growth cycle after RF formation and replication.

As a consequence of DNA synthesis occurring during the formation and replication of the RF, it is possible that a mutation could occur which would reverse or bypass the lethal amber mutation and allow the phage to complete its growth cycle in the nonpermissive host. This would be a random process and could explain the random distribution of ΦX revertants found during the plating of the amber mutants in the single-burst tubes on C. In the same way, when a stock of amber phage is assayed for revertants on C the plaques observed could arise either from previously existing revertants or from reverse mutations occurring after infection of the nonpermissive host by the amber phage. Unfortunately, we have no way to distinguish these two possibilities.

If our model for ΦX replication is correct then two independent mutations would usually be required for the amber phage to make a plaque on the nonpermissive host since both phenotypic and genotypic reversion would have to occur in the same cell; this would require mutations in both the messenger RNA synthesizing RF and the parental RF and hence would be a very rare event (see Fig. 1). Be that as it may, we shall assume for the rest of the discussion that the amber mutants are unable to make plaques on C.

Different DNA-synthesizing enzymes. Another explanation for the random distribution of mutants follows from the fact that the enzyme that synthesizes the progeny single-stranded DNA is very likely different from the enzyme that replicates the RF. If mistakes were more likely to be made during the formation of the progeny single-stranded DNA than during the formation of the RF, then a random distribution of mutants would be observed whether one or all of the RF molecules served as templates for the synthesis of single-stranded DNA. This problem can be met satisfactorily only by isolating the various enzymes and studying them separately.

Sampling loss of revertants. Suppose the ΦX replication process did involve a stage at which the genetic information replicated geometrically, but that only a small fraction of the replicas appeared in the progeny phage. More specifically, let the RF replicate geometrically and let only a fraction of the RF molecules each contribute a single complement of genetic information to the progeny. A large sampling loss of this kind could create an approximately random dis-

tribution from an initial clonal distribution. However, both the fact that a clonal distribution of recombinants is observed and the numerical relations indicated in the following paragraphs suggest that a sampling loss of this kind is not responsible for converting an initially clonal distribution into a random distribution.

Matsubara *et al.* (1963a) found that under their conditions about 1500 single-stranded DNA molecules were synthesized on the average per infected bacterium and that the average burst size was about 200. This indicates an apparent sampling loss of progeny *single-stranded DNA* of 80–90 %, whereas for T2 the sampling loss is only about 10–20 % (Stent, 1963, page 129).

About 50–200 RF particles are formed in a ΦX-infected cell (Sinsheimer *et al.*, 1962; Denhardt and Sinsheimer, 1965b). Hence, if each RF molecule contributes only one genetic complement to the progeny (by making 10–20 single strands, one of which appears in the progeny), then all the RF molecules must contribute in order to give a burst size of 50–200. Consequently, if all the RF molecules replicated geometrically and then contributed genetic information to progeny, we should observe a clonal distribution since there would be no sampling loss of *genetic information*.

Also, if the sampling loss were not random but instead represented DNA which had accumulated after phage maturation had stopped, then it would not affect the clone size distribution.

The Clonal Distribution of Recombinants

Geometric replication. The clonal distribution of ΦX recombinants is similar to the distribution of recombinants between loosely linked T4 markers (Stahl, 1956). A distribution of the kind observed would obtain if ΦX multiplied as T4 does and the recombinants were formed, as in T4, by events occurring with a constant probability in a steady-state pool comprising a constant number of phage genomes. Since evidence described elsewhere has led us to postulate a very different picture of ΦX multiplication, as shown in Fig. 1, it is desirable to see whether the experimentally observed distribution of recombinants can be reconciled with that picture.

"Stamping machine" replication. According to our hypothesis (Denhardt and Sinsheimer, 1965d) the parental RF "stamps out" all the progeny single-stranded DNA. A simple interpretation of this model predicts that the distribution of recombinants should resemble a "follow-the-leader" distribution such that clones of 1, 2, 3, etc., are equally frequent (Stent, 1963, page 193). Prior to the recombinational event single-stranded DNA molecules of one genotype are made; after the event DNA molecules of a recombinant genotype are made, since the recombination would alter the RF template. (In the case of the random distribution of mutants only the progeny single-stranded DNA molecule is mutant, the template is not altered.) Recombinant progeny single-stranded DNA might also be made by copying parts of two RF templates; however, this would give a random distribution of recombinants.

To rationalize the experimental findings in harmony with the scheme of Fig. 1 a mechanism is required by which the probability of producing recombinants varies in inverse proportion with 2^n where n is the order number of the phage "generation" counted from the end of phage development.

One such mechanism would involve: (1) a linearly expanding pool of RF molecules; (2) a chance of recombinant RF formation proportional to the product of the number of RF molecules of the two parental types; (3) a constant chance for any one of the RF molecules, including recombinant ones, to become (or to replace) one of the "master templates" that generate progeny single-stranded DNA.

This scheme would probably require also that the recombination in the expanding pool of RF molecules occurs concomitantly with the production of progeny "plus" strands rather than being completed before the latter process begins in order to yield the observed distribution of recombinant clone sizes.

Other schemes involving recombination between the master templates and an expanding pool of RF can also be devised.

In conclusion, whereas the observed distribution of mutants of ΦX, if it is not due to spurious effects of reversions to *am*$^+$ in the indicator hosts, is in clear agreement with the scheme of Fig. 1 for the multiplication of phage ΦX, the distribution of recombinants can be reconciled with the scheme only by making special *ad hoc* assumptions. Additional experimental evidence is required before a decision can be reached whether the recombinant clone size distribution is incompatible or not with the terms of the proposed scheme of ΦX reproduction.

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