*Genet. Res., Camb.* (1967), **10**, *pp.* 13–20
*With* 1 *text-figure*
*Printed in Great Britain*

# Polarized exclusion of bacteriophage T2 in crosses with T4*

By B. DE GROOT

*Department of Radiation Genetics, University of Leiden, and Institute for Radiopathology and Radiation Protection, The Netherlands*

(*Received* 8 August 1966)

## 1. INTRODUCTION

Bacteriophage T2 is partially excluded from the progeny of crosses with page T4 (Streisinger & Weigle, 1956). The T4 gene for exclusion ($ex^{+4}$) is located near the *r*II locus, and the results of crosses between T4 *am* 122 and T2 showed that it is expressed as an early function (De Groot, 1966). A phage strain with intermediate properties was found among recombinants between T2 and ultraviolet-irradiated T4. This T2-like recombinant T2 $exr^{+4}$ does not exclude standard-type T2, it is still partially excluded by T4, but to a much lesser extent than standard-type T2, and it has the T4 glucosylation properties (De Groot, 1967).

This partial non-excludability which was rescued from irradiated T4, is possibly related to T4 glucosylation. A protective effect of glucosylation has been demonstrated previously: Shedlovsky & Brenner (1963) and Hattman & Fukasawa (1963) found that unglucosylated T-even phage is not capable of surviving infection of *E. coli* B. Richardson (1966) established that glucoseless T2 and T6 DNA is much more sensitive to *in vitro* breakdown by the bacterial exonuclease III than the glucosylated DNA. Whatever the mechanism of exclusion and excludability might be, the partially non-excludable T2 $exr^{+4}$ can be used for genetic studies on the relationship between phages T4 and T2. Preliminary experiments have indicated that in crosses between T4 and T2 $exr^{+4}$ there was an unequal distribution of the various T2 genes. In this paper the results are presented from four multifactorial crosses between T4 and T2 $exr^{+4}$. The frequencies of the T2 genes in the progeny show polarity over the map segment that contains most of the genes for the early functions (Edgar, Denhardt & Epstein, 1964).

## 2. MATERIALS AND METHODS

### (i) *Phage genes and bacterial strains*

The phage gene symbols are provided with a superscript 4 or 2 to designate the parental origin T4 or T2 respectively, as proposed by Streisinger (1956*b*). The homologous genes of phage T4 used as markers, as well as the indicator bacteria used to distinguish them are listed below in the order of their position on the map.

* This work was carried out within the frame of the association between Euratom and the University of Leiden, contract number 052-64-1-BIAN.

The position of $exr^{+4}$ is unknown. Most of the bacterial strains and their origin have been referred to previously; in the following, they will be designated without their species name.

| Origin | | Property of the standard gene | Indicator *E. coli* |
|---|---|---|---|
| T4 | T2 | | |
| $h^{+4}$ | $h^{+2}$ | Adsorption | B/2 |
| $ex^{+4}$ | $ex^{+2}$ | Exclusion of T2 by $ex^{+4}$ | — |
| $r^{4}$B17 | $r^{+2}$B | Lysis inhibition, second linkage group, cistron B | K($\lambda h$) |
| $r^{4}$A8 | $r^{+2}$A | Item, cistron A | K($\lambda h$) |
| $am^{4}122$ | $am^{+2}$ | Induction of deoxycytidylate hydroxymethylase | B |
| $v^{+4}$ | $v^{+2}$ | Dark repair of ultraviolet lesions by $v^{+4}$ | CR63 and B$phr^{-}$ |
| $r^{4}$48 | $r^{+2}$I | Lysis inhibition, first linkage group | CR63 and KS112–12 |
| $o^{4}$ | $o^{+2}$ | Osmotic-shock sensitivity | — |
| $exr^{+4}$ | — | Partially non-excludable by T4 | — |

The recombination frequencies between the markers are presented in Fig. 1; the data are based on those of Edgar, Denhardt & Epstein (1964), Streisinger (1956*a*), De Groot (1966) and unpublished results.

The two directions in the circular genome will be used in the discussion of the results. Starting at any point, the genes on either side can be referred to as *distal* or *proximal*, depending on whether they are found in a clockwise or anticlockwise direction, respectively. Thus the terminology conforms to that employed in Hfr crosses between bacteria (Hayes, 1964).

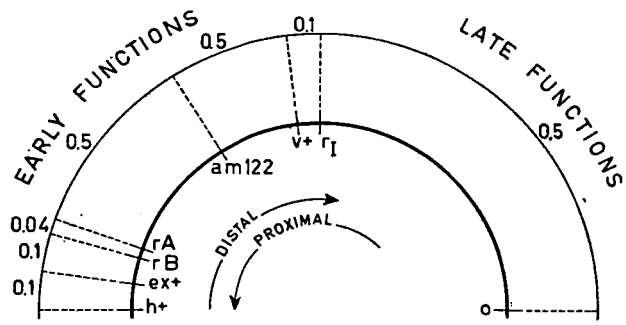


Fig. 1. Segment of the circular map of bacteriophages T2 $exr^{+4}$ and T4 with the markers used in the crosses. The joint symbols are presented on the map. The directions are explained in the text.

### (ii) *Determination of the genes*

$h^{+2}$ gives rise to turbid plaques in lawns of mixed B and B/2 (1:1). $Ex^{+4}$ and $ex^{+2}$ are recognized by crossing the phage strain considered with standard type T2 L $h^{+2}$ or with a host-range mutant T2 L $h^{2}$; the yields of $h^{+2}$ characterize the loci $ex^{+4}$ or $ex^{+2}$ as shown previously (De Groot, 1966). Cistrons $r^{4}$B17 and $r^{4}$A8 were determined in complementation tests by spotting $10^{7}$ phage on lawns seeded with mixed

https://doi.org/10.1017/S0016672300010727 Published online by Cambridge University Press

indicator K($\lambda h$) and B($10^3$:1) and $2 \times 10^8$ of the T4, mutant in the complementary cistron (Benzer, 1957). $Am^4$ was spotted on *E. coli* B, a 'non-permissive' host for *amber* mutants (Edgar & Epstein, 1963). The determinations of $v^{+4}$ and $v^{+2}$ were carried out by irradiation with a dose of ultraviolet light that inactivated $v^{+4}$ strains to $10^{-2}$ and $v^{+2}$ strains to $10^{-4}$ survival. An unirradiated $10^{-2}$ dilution of the phage was plated as a control (Harm, 1963). If the strain considered possessed the $am^4122$ locus, CR63 was used under dim sodium light to avoid photoreactivation. In the case of $am^{+2}$ strains, the non-photoreactivable bacterium B$phr^-$ was used (Harm & Hillebrandt, 1962). This was kindly supplied by Dr A. Rörsch. T4$o^4$ was isolated from standard type T4 D by means of propagation at a low multiplicity of infection to avoid phenotypic mixing with the head membrane proteins from the standard-type gene, followed by osmotic shock as described by Doermann & Boehner (1964). The mutant was isolated after three of these cycles. The method was also used for recognition of osmotic-shock resistance ($o^4$) or sensitivity ($o^{+2}$).

## 3. RESULTS

Crosses between phages T4 and T2 $exr^{+4}$ were made in the hosts CR63 and B as listed in Table 1. The frequencies of T2 genes in the progeny of the crosses are

Table 1. *Crosses between bacteriophages T4 and T2* exr$^{+4}$. *Only homologous genes used as markers are listed*

| Cross No. | T4 parent | T2 $exr^{+4}$ parent | Host strain of *E. coli* | Number of progeny tested |
|---|---|---|---|---|
| 1 | T4$h^{+4}ex^{+4}am^4122\ v^{+4}$ | T2$h^{+2}ex^{+2}am^{+2}v^{+2}$ | CR63 | 29 |
| 2 | T4$h^{+4}ex^{+4}r^4$A8 $v^{+4}$ | T2$h^{+2}ex^{+2}r^{+2}$A $v^{+2}$ | B | 62 |
| 3 | T4$h^{+4}am^4122\ v^{+4}o^4$ | T2$h^{+2}am^{+2}v^{+2}o^{+2}$ | CR63 | 121 |
| 4 | T4$h^{+4}r^4$B17 $r^4$A8 $r^4$48 | T2$h^{+2}r^{+2}$B $r^{+2}$A $r^{+2}$I | B | 81 |
| | | | Total | 293 |

presented in Table 2. The following conclusions can be drawn: first, partial exclusion of $h^{+2}$ is more effective in crosses 1 and 3 with CR63 as the bacterial host

Table 2. *Frequencies of T2 markers in the progeny of the four crosses of T4 with T2* exr$^{+4}$

| Cross 1 | | Cross 2 | | Cross 3 | | Cross 4 | |
|---|---|---|---|---|---|---|---|
| Marker | Freq. | Marker | Freq. | Marker | Freq. | Marker | Freq. |
| $h^{+2}$ | 0·1 | $h^{+2}$ | 0·30 | $h^{+2}$ | 0·12 | $h^{+2}$ | 0·27 |
| $ex^{+2}$ | 0·3 | $ex^{+2}$ | 0·34 | | | $r^{+2}$B | 0·37 |
| | | $r^{+2}$A | 0·42 | | | $r^{+2}$A | 0·41 |
| $am^{+2}$ | 0·4 | | | $am^{+2}$ | 0·15 | | |
| $v^{+2}$ | 0·4 | $v^{+2}$ | 0·47 | $v^{+2}$ | 0·28 | $r^{+2}$I | 0·55 |
| | | | | $o^{+2}$ | 0·45 | | |

The markers are arranged from $h^{+2}$ to $o^{+2}$ in the distal direction from the top downwards. The genes are spaced according to their relative positions on the linkage map to facilitate comparisons between the crosses (see Fig. 1).

https://doi.org/10.1017/S0016672300010727 Published online by Cambridge University Press

than in crosses 2 and 4 with B as host. The yields of $h^{+2}$ in the progeny from the crosses with B as host are within the range of 0·25 to 0·43 reported in another context (De Groot, 1967). Second, all crosses show polarity of increasing frequencies of T2 markers from $h^{+2}$ in the distal direction irrespective of the marker considered. Third, there is a considerable variation in the extent of the genome over which polarity is expressed. In cross 4, equilibrium for the T2 gene frequency (0·5) has already been reached between $r^{+2}$A and $r^{+2}$I. By contrast, exclusion in cross 3 is exerted as distal as beyond the $v^{+2}$ gene.

A first approach to understanding the mechanism of polarized exclusion of T2 gene frequencies can be made by examining the frequencies of recombinants in the various crosses. Table 3 presents the numbers of parental combinations and recombinants between pairs of adjacent genes, symbolized as $a$–$b$. The numbers of T2 parental combinations ($a^2$–$b^2$) are smaller than those of T4 ($a^4$–$b^4$) and the numbers of $a^2$–$a^4$ recombinants are smaller than those of the $a^4$–$b^2$ type. Both differences contribute to partial exclusion. The two recombinant types $a^2$–$b^4$ and $a^4$–$b^2$ show unequal numbers within each gene pair and the differences may be considerable, for example, in cross 3.

Maximum recombination frequencies between genes will only be obtained if each bacterium produces large and equal numbers of genes originating from each parent. Unequal numbers will reduce these maximum values (Lennox *et al.*, 1953). The recombination frequencies observed between the pairs of unlinked genes in the four crosses are lower than the maximum value 0·50 (Table 3). However, it is unlikely that such low values as 0·04 and 0·20 in cross 3 would merely have resulted from recombination between unequal numbers of genes. Estimates of recombination values for unlinked pairs of genes can be made, if the recombinational events between the genes $a$ and $b$ are non-reciprocal. The great differences between the recombinant types $a^2$–$b^4$ and $a^4$–$b^2$ of each gene pair suggest this assumption. This means that the probability of a recombinational event in an unlinked pair of genes is the compound probability of the frequencies of the participating genes (Table 3, footnote). The expected values are presented in Table 3 under the observed values and the deviations suggest that additional restriction of recombination has occurred under the conditions of this experiment. Interaction $\chi^2$'s for unlinked pairs are significant in cross 3 and suspiciously large in cross 2. The results of premature-lysis experiments (unpublished) also lead to the conclusion that recombination between T2 $exr^{+4}$ and T4 is restricted.

No expected recombination frequencies were calculated for linked genes since the use of genetic linkage values obtained under standard conditions would introduce a further uncertainty.

An additional argument for reduced recombination follows from the high frequencies of progeny genomes containing only the markers from the $exr^{+4}$ parent (Table 3). The table shows that the majority of the $a^2$–$b^2$ parental combinations in crosses 2, 3 and 4 occur in conserved genomes. This means that restriction of recombination is exerted over a considerable part of the genome.

Table 3. *Analysis of recombination frequencies between adjacent gene pairs*

| Cross 1 | $h^+$–$ex^+$ | $ex^+$–$am$ | $am$–$v^+$ | T2 genomes |
|---|---|---|---|---|
| $a^2$–$b^2$ | 4 | 4 | 6 | 1 |
| $a^4$–$b^4$ | 19 | 13 | 12 | |
| $a^2$–$b^4$ | 1 | 5 | 5 | |
| $a^4$–$b^2$ | 5 | 7 | 6 | |
| Expectation* | linked | unlinked | unlinked | |
| Recombination frequency | | | | |
| Observed | 0·21 | 0·41 | 0·38 | |
| Expected** | | 0·46 | 0·48 | |
| Significance of deviation*** | | $P > 0{\cdot}2$ | $P > 0{\cdot}2$ | |
| **Cross 2** | $h^+$–$ex^+$ | $ex^+$–$r$A | $r$A–$v^+$ | T2 genomes |
| $a^2$–$b^2$ | $17\frac{1}{2}$ | 19 | 16 | 13 |
| $a^4$–$b^4$ | 40 | 34 | 23 | |
| $a^2$–$b^4$ | 1 | 2 | 10 | |
| $a^4$–$b^2$ | $3\frac{1}{2}$ | 7 | 13 | |
| Expectation* | linked | linked | unlinked | |
| Recombination frequency | | | | |
| Observed | 0·07 | 0·14 | 0·37 | |
| Expected** | | | 0·54 | |
| Significance of deviation*** | | | $P = 0{\cdot}08$ | |
| **Cross 3** | $h^+$–$am$ | $am$–$v^+$ | $v^+$–$o$ | T2 genomes |
| $a^2$–$b^2$ | 14 | 14 | 30 | 11 |
| $a^4$–$b^4$ | 102 | 83 | 63 | |
| $a^2$–$b^4$ | 1 | 4 | 4 | |
| $a^4$–$b^2$ | 4 | 20 | 24 | |
| Expectation* | unlinked | unlinked | unlinked | |
| Recombination frequency | | | | |
| Observed | 0·04 | 0·20 | 0·23 | |
| Expected** | 0·24 | 0·35 | 0·48 | |
| Significance of deviation*** | Significant | $P < 0{\cdot}001$ | $P < 0{\cdot}001$ | |
| **Cross 4** | $h^+$–$r$B | $r$B–$r$A | $r$A–$r$I | T2 genomes |
| $a^2$–$b^2$ | $16\frac{1}{2}$ | 30 | 22 | 15 |
| $a^4$–$b^4$ | 46 | 48 | $25\frac{1}{2}$ | |
| $a^2$–$b^4$ | 5 | 0 | 11 | |
| $a^4$–$b^2$ | $13\frac{1}{2}$ | 3 | $22\frac{1}{2}$ | |
| Expectation* | linked | linked | unlinked | |
| Recombination frequency | | | | |
| Observed | 0·23 | 0·04 | 0·41 | |
| Expected** | | | 0·46 | |
| Significance of deviation*** | | | $P = 0{\cdot}12$ | |

* The expectation (linked or unlinked) is inferred from $T_4 \times T_4$ crosses.

** The expected recombination frequency for unlinked gene pairs is calculated as follows, assuming stochastical independence between the two gene pairs: if $A_2$, $A_4$, $B_2$ and $B_4$ are the numbers of $a^2$, $a^4$, $b^2$ and $b^4$ progeny (i.e. $A_2 = a^2$–$b^2$, + $a^2$–$b^4$, etc.) and $T = A_2 + A_4 = B_2 + B_4$, the expected frequency is $(A_2B_4 + A_4B_2)/T^2$.

*** The probability that the deviation between observed and expected frequencies could be due to chance: based on $\chi^2$ for interaction with Yates' correction for continuity.

## 4. DISCUSSION

Polarized exclusion of T2 genes is probably the result of some restriction process in the vegetative pool of mixed T2 *exr*$^{+4}$ DNA and T4 DNA. For understanding polarized exclusion it is necessary to introduce the assumption of two different rates of replication for T2 *exr*$^{+4}$ DNA and T4 DNA. Even under conditions of unrestricted growth T2-infected cells may synthesize phage DNA more slowly than cells with T4; Stahl & Murray (1966) reported a lower burst size for standard-type T2 than for T4 under the same conditions. However, this difference alone does not explain the low yield of T2 genes in the progeny from crosses between standard-type T2 and T4 (Streisinger & Weigle, 1956). Even if normal differences in replication rate between T2 *exr*$^{+4}$ and T4 can explain partial exclusion to some degree, a residual exclusion and its polarization remain to be explained. Since normal differences in replication rates of the DNA's of T2 *exr*$^{+4}$ and T4 do not explain polarized restriction of T2 *exr*$^{+4}$, it is difficult to think of a mechanism for polarized exclusion that does not involve some sequential process in the DNA. This process might be sequential replication of DNA. When replication takes place in a segment of restricted T2 *exr*$^{+4}$ DNA, the replica might undergo recombination with T4 DNA and thus escape from restriction. Polarization of restriction would, consequently, result from a start of replication of DNA at the distal region of the genome (Fig. 1).

There are other observations supporting this hypothesis. Polarity in the replication of bacterial chromosomes beginning at a fixed starting point has been established by Lark, Repko & Hoffman (1963) by means of pulse labelling of auxotrophic strains. The same result was obtained by Nagata (1963) who found the point of attachment of the F$^{+}$ factor in Hfr strains to be the starting point for replication. Holliday (1965) reported sequential mitotic recombination in synchronously dividing cells of *Ustilago maydis* following ultraviolet irradiation at various times during division. This was explained by assuming sequential genetic replication, starting from the ends of the chromosome arms. There is a correlation between the polarity of T2 gene frequencies and the sequence of events determined by the genes upon infection of the bacterial host. Edgar, Denhardt & Epstein (1964) found a segment of the genome of T4, where the majority of the genes for the early functions reside (Fig. 1). Beyond that segment in the distal direction are found the genes for late functions such as the production of endolysozyme and the proteins for morphogenesis. The sequence of events during the intrabacterial growth cycle of the phage is roughly correlated with the positions on the map of the genes determining them, and the results reported here show that this correlation may be extended to polarized exclusion of T2 genes. In all four crosses exclusion is most stringent for the $h^{+2}$ locus, indicating that restriction may be initiated at a fixed starting point.

Recombination between T2 *exr*$^{+4}$ and T4 is restricted. One of the factors involved in restricted recombination might be the non-homology of the parental strains. They are different in several properties such as adsorption specificity, ultraviolet sensitivity and the collar between head and midpiece, which results from different base sequences in their DNA's at several places. Consequently, base pairing between

T2 *exr*$^{+4}$ and T4 DNA might be less efficient with a restrictive effect on recombination. From this point of view, restricted recombination is not necessarily related with restriction of replication.

Large numbers of progeny containing only the markers of the parental T2 *exr*$^{+4}$ strains were observed in crosses 2, 3 and 4. These progeny cannot all be composed of parental T2 *exr*$^{+4}$ DNA because their number exceeds that of the parental T2 *exr*$^{+4}$ phage added in the cross.

The burst sizes of the crosses show that parental T2 *exr*$^{+4}$ DNA was capable of one to three replications under these conditions. The appearance of conserved parental phage is possible, since even after unrestricted growth some conservation has been demonstrated by Boyle, Ritchie & Symonds (1965); a fraction of semi-conserved standard-type T2 phage was found after transfer of parental T2 with heavy label in light bacteria. However, its frequency ($4 \times 10^{-3}$) is too low to explain the large numbers of conserved T2 genomes reported here. The unequal frequencies of the complementary types of recombinants $a^2$–$b^4$ and $a^4$–$b^2$ lead to the conclusion of non-reciprocity of crossing-over. However, this is generally considered to be an unusual way of exchange of genetic material. Non-reciprocal recombination between phages has been established by Epstein (1958) for ultraviolet-irradiated T4, while Bresch (1955) found recombination in phage T1 easier to explain with a model for non-reciprocal recombination. It is questionable whether the results described above apply to crosses between standard-type T2 and T4. In such crosses polarized exclusion was also observed (De Groot, 1967, and unpublished), but the frequencies of conserved T2 genomes were lower. The fate of the DNA's of T2 *exr*$^{+4}$ and T2 in the presence of T4 DNA is now being subjected to further investigation.

## SUMMARY

Four crosses were made between bacteriophages T4 and partially non-excludable T2 *exr*$^{+4}$. Each cross was designed as a four-factor cross: in total seven pairs of homologous genes were used as markers, extending over half the circular map from the locus for adsorption specificity ($h^+$) along the map segment for the early functions to osmotic-shock sensitivity ($o$).

The frequencies of T2 markers recovered in the progeny show polarized exclusion: the frequencies are low for $h^{+2}$ at the beginning of the map segment for the early functions and increasing to $o^{+2}$. Recombination of T2 *exr*$^{+4}$ is restricted for all loci concerned and the recombinational events are probably non-reciprocal. Restriction of sequential replication from a fixed starting point is proposed to explain polarized exclusion of T2 genes. Non-homology is dicussed in relation to restricted recombination.

*Note added in proof:* When this paper was in press, Dr. R. L. Russell, Cornell University, Ithaca, showed me the results of his extensive work (Ph.D. thesis) on exclusion of standard-type T2 by T4 *amber* mutants. These results are in general agreement with the ones described for T2 *exr*$^{+4}$ in this paper.

The author wishes to thank Professor F. H. Sobels, Miss I. Mattern and Dr G. Veldhuizen for their critical reading of the manuscript. I am indebted to Dr H. de Jonge for statistical advice.

The capable technical assistance by Miss Th. A. M. Verkley and Mrs E. van Egmond is acknowledged.

## REFERENCES

BENZER, S. (1957). The elementary units of heredity. In *The Chemical Basis of Heredity* (McElroy and Glass, eds.), pp. 70–93. Baltimore: Johns Hopkins Press.

BOYLE, J. M., RITCHIE, D. A. & SYMONDS, N. (1965). Density-gradient separation of host-modified bacteriophage T2. *J. molec. Biol.* **14**, 48–54.

BRESCH, C. (1955). Zum Paarungsmechanismus von Bakteriophagen. *Z. Naturf.* **10b**, 545–561.

DOERMANN, A. M. & BOEHNER, L. (1964). An experimental analysis of bacteriophage T4 heterozygotes. II. Distribution in a density gradient. *J. molec. Biol.* **10**, 212–222.

EDGAR, R. S., DENHARDT, G. H. & EPSTEIN, R. H. (1964). A comparative genetic study of conditional lethal mutations of bacteriophage T4 D. *Genetics*, **49**, 635–648.

EDGAR, R. S. & EPSTEIN, R. H. (1963). Conditional lethal mutations in bacteriophage T4. In *Genetics Today* (S. J. Geerts, ed.), *Proc. XI Int. Congr. Genet., The Hague*, **2**, 1–16.

EPSTEIN, R. H. (1958). A study of multiplicity-reactivation in the bacteriophage T4. I. Genetic and functional analysis of TD4-K12 (λ) complexes. *Virology* **6**, 382–404.

DE GROOT, B. (1966). Partial exclusion of bacteriophage T2 by T4: an early function in the second linkage group. *Genetica*, **37**, 37–51.

DE GROOT, B. (1967). The bar-properties, in particular glucosylation of deoxyribonucleic acid, in crosses of bacteriophages T2 and T4. *Genet. Res.* **9**, 149–158.

HARM, W. (1963). Mutants of phage T4 with increased sensitivity to ultraviolet. *Virology*, **19**, 66–71.

HARM, W. & HILLEBRANDT, B. (1962). A non-photoreactivable mutant of *E. coli* B. *Photochem. Photobiol.* **1**, 271.

HATTMAN, S. & FUKASAWA, T. (1963). Host-induced modification of T-even phages due to defective glucosylation of their DNA. *Proc. natn. Acad. Sci. U.S.A.* **50**, 297–300.

HAYES, W. (1964). *The Genetics of Bacteria and their Viruses*, p. 566. Oxford: Blackwell.

HOLLIDAY, R. (1965). Induced mitotic crossing-over in relation to genetic replication in synchronously dividing cells of *Ustilago maydis*. *Genet Res.* **6**, 104–120.

LARK, K. G., REPKO, T. & HOFFMAN, E. J. (1963). The effect of amino acid deprivation on subsequent deoxyribonucleic acid replication. *Biochim. biophys. Acta*, **76**, 9–24.

LENNOX, E. S., LEVINTHAL, C. & SMITH, F. (1953). The effect of finite input in reducing recombinant frequency. *Genetics*, **38**, 508–511.

NAGATA, T. (1963). The molecular synchrony and sequential replication of DNA in *Escherichia coli*. *Proc. natn. Acad. Sci. U.S.A.* **49**, 551–559.

RICHARDSON, C. C. (1966). Influence of glucosylation of deoxyribonucleic acid on hydrolysis by deoxyribonucleases of *Escherichia coli*. *J. biol. Chem.* **241**, 2084–2092.

SHEDLOVSKY, A. & BRENNER, S. (1963). A chemical basis for the host-induced modification of T-even bacteriophages. *Proc. natn. Acad. Sci. U.S.A.* **50**, 300–305.

STAHL, F. W. & MURRAY, N. (1966). The evolution of gene clusters and genetic circularity in microorganisms. *Genetics*, **53**, 569–576.

STREISINGER, G. (1956*a*). The genetic control of ultraviolet sensitivity levels in bacteriophages T2 and T4. *Virology*, **2**, 1–12.

STREISINGER, G. (1956*b*). The genetic control of host range and serological specificity in bacteriophages T2 and T4. *Virology*, **2**, 377–387.

STREISINGER, G. & WEIGLE, J. (1956). Properties of bacteriophages T2 and T4 with unusual inheritance. *Proc. natn. Acad. Sci. U.S.A.* **42**, 504–510.