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The effect of homozygous deletions upon heterozygote formation in bacteriophage T4D†

BY INGERID KVELLAND‡
Department of Genetics, University of Washington, Seattle, Washington

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1. INTRODUCTION

In 1951 Hershey & Chase discovered mottled plaques in the progeny from a cross $T2r^+ \times T2r$ that were shown to be mottled due to segregation of r and r^+. The importance of such heterozygous phage particles (HETs) in the process of genetic recombination has been discussed by many workers. Levinthal (1954, 1959) focused attention on HETs as basic intermediates in genetic recombination. The model Levinthal proposed for T2 HETs pictures a haploid double-stranded genome with an internal region where the two strands are genetically different (a heteroduplex HET).

Nomura & Benzer (1961) found a higher frequency of *r*II-HETs when using *r*II point mutants than when using *r*II-deletion mutants. In a cross involving a deletion mutant: $r^+ \times r1272$ they found 0·53 % HETs compared to a frequency of 1·1 % HETs in a cross involving point mutants: $r^+ \times r205r287$.

DNA synthesis is known to be inhibited by 5-fluorodeoxyuridine (FUDR) (Cohen *et al.* 1958), and when Séchaud *et al.* (1965) used FUDR in phage crosses they found that *r*II point mutant HETs increased five- to tenfold, while *r*II-deletion mutant HETs showed no increase. These results, together with those obtained by Nomura & Benzer (1961), were taken to indicate that there are two classes of HETs. One is the heteroduplex HETs already mentioned and the other is a terminal redundancy structure described by Streisinger, Edgar & Denhardt (1964). The latter type of HET is the result of a terminal repetition of nucleotide sequences. If the redundant region is derived from genetically different parents, the region will be heterozygous for the marked loci included in the redundancy. Terminal redundancy HETs are assumed to be formed and lost by recombination, while heteroduplex HETs are assumed to be formed by recombination and lost by semiconservative replication (Séchaud *et al.* 1965). Terminal redundancy HETs may include both point mutants and deletion mutants, while heteroduplex HETs supposedly cannot include deletion mutants because of mismatching of bases. The proposed structures of HETs are pictured in Fig. 1.

Streisinger, Emrich & Stahl (1967) suggest that some factor(s) extrinsic to the

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‡ Present address: Institute of General Genetics, University of Oslo, Oslo 3, Norway.

chromosome itself, such as the amount of DNA that fits into the phage head, is responsible for the length of the phage chromosome. If this is correct, introduction of a deletion should be compensated for by an increase in the length of the redundancy corresponding to the length of the deletion in order to maintain the complete length of the chromosome. This is thought feasible through giant chromosome formation by breakage and reunion within homologous regions present in head to tail pairing of DNA molecules with terminal repetitions.

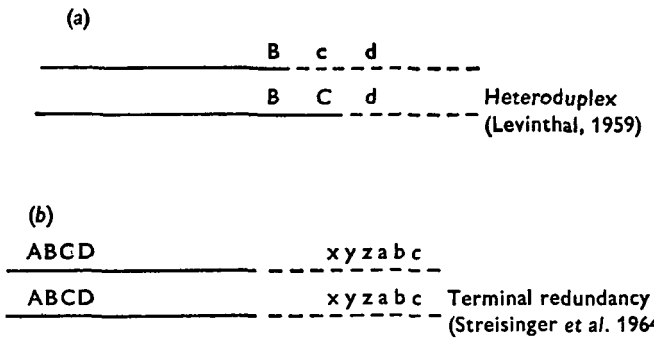

Fig. 1. Assumed HET structures.

Streisinger *et al.* (1967) tested the hypothesis that a deletion is compensated for by an increase of the region of redundancy equal to the length of the deletion. They performed the following crosses: (1) rH23$h^{2+} \times r$H23h^{4+}, where rH23 is a long deletion that covers the entire rII region; (2) rH88$h^{2+} \times r$H88h^{4+}, where rH88 is a somewhat shorter deletion; (3) $r168h^{2+} \times r168h^{4+}$, where r168 is an even shorter deletion; and (4) $r607h^{2+} \times r607h^{4+}$, where r607 is a point mutant. The authors found that the frequency of h^{2+}/h^{4+} heterozygotes was highest in the cross with the longest deletion and decreased as the length of the deletion decreased.

The markers h^{2+} and h^{4+} are allelic and Séchaud *et al.* (1965) found that the frequency of h^{2+}/h^{4+} HETs did not increase in FUDR; that is, h^{2+} and h^{4+} behaved as if they were deletion mutants. The markers h^{2+} and h^{4+} would therefore be expected to form HETs only within the region of terminal redundancy.

MacHattie, Ritchie, Thomas & Richardson (1967) showed that when intact T*2 (T2 phage grown on non-glucosylating host *E. coli* $B/4_0$) DNA molecules were partially degraded (1–3·5%) by *Escherichia coli* exonuclease III, an enzyme which specifically removes nucleotides stepwise from the 3′ end of a duplex DNA molecule (Richardson, Lehman & Kornberg, 1964), the resulting 5′-ended single chains at each end joined to form circular DNA molecules which were observed in the electron microscope. No circular forms were observed using T*2 fragments. This was taken as physical evidence for the existence of complementary nucleotide sequences at the terminal 5′-ended single chains of the T*2 molecule. The length of the terminal repetition was estimated to be from 1% to 3% of the intact molecule.

Streisinger *et al.* (1964) suggested that after several rounds of T4 replication,

the result would be a population of chromosomes that are circular permutations of a common sequence of genetic information. Physical evidence for circular permutation in T2 was provided by Thomas & MacHattie (1964), who examined electron-microscope grids prepared from DNA solutions of unbroken T2 DNA that had been denatured with NaOH followed by reannealing at low pH at 65 °C. An abundancy of circular forms was found. They had all nearly the same contour length which was found to correspond to one complete genetic map. No circular forms were found in preparations of T2 half-molecules or T5 whole molecules.

In the present paper the effect of homozygous deletions upon HET frequency and average HET length is investigated. The main results from the experiments support Streisinger's hypothesis that the T4 chromosome contains a redundancy, and that introduction of a deletion is compensated for by increasing the length of the redundancy.

2. MATERIAL AND METHODS

All phage mutants and wild-type phage employed are derived from T4D. Figure 2(*a*) gives the approximate location of the *amber* mutants in relation to *r*48 and the *r*II-region, and the map distances between markers. Figure 2(*b*) gives the location of the *r*II-deletions employed, and the legend gives the names and sources of the mutants. Phage stocks were prepared according to Chase & Doerman (1958) using *Escherichia coli* strain *CR*63 as host. A culture of *CR*63 in exponential growth was used as plating bacteria for progeny from all crosses. *E. coli* strain *CR*63 and strain *B* were used as hosts for crosses. Identification of phage genotypes in multi-amber crosses was achieved by the methods of Doermann & Boehner (in preparation). The streptomycin-resistant strains of *E. coli*, *B*/s and *CR*63/s were used for this purpose.

Media. H-broth (Hershey & Rotman, 1949) was used for all normal crosses and for preparation of stocks. M-9 medium prepared according to Adams (1959) and supplemented with 0·5% Difco Casamino-Acid (M-9$^+$) was used for FUDR-crosses and for stocks used in these crosses. The plate agar and soft agar used are described by Chase & Doermann (1958).

Cross procedure. All normal crosses were performed according to the methods of Chase & Doermann (1958). For FUDR-crosses the procedure was as follows. A host culture of *E. coli* strain *B* was prepared by adding 0·2 ml of an over-night culture grown in M-9$^+$ to 100 ml fresh M-9$^+$ and incubating with aeration for 245 min to give a titre of 5×10^8 viable cells per ml. Three minutes prior to phage infection, 2·5 ml of a solution containing FUDR, uracil and DL-tryptophan was added to 2·5 ml of bacterial culture. At time '0', 10 ml of phage suspension, containing 1×10^9 phages per ml of each parent, was added to this mixture giving an input multiplicity of about 8 of each parent, and a concentration of the three reagents: FUDR, uracil and tryptophan of respectively 4×10^{-5} M, 2×10^{-4} M and 20 μg/ml. Eight and a half minutes after infection, chloramphenicol was added to give a concentration of 250 μg/ml. The culture was then incubated at 37 °C for 120 min, at which time the cells were centrifuged in the cold, washed in chilled

M-9+ containing FUDR but no chloramphenicol, and finally resuspended in 15 ml of this solution. The cells were then incubated at 37 °C for 80 min to allow maturation. At this time chloroform was added to lyse the cells, and 10 min later deoxyribonuclease was added to the lysate which was then incubated for 30 min at 37 °C.

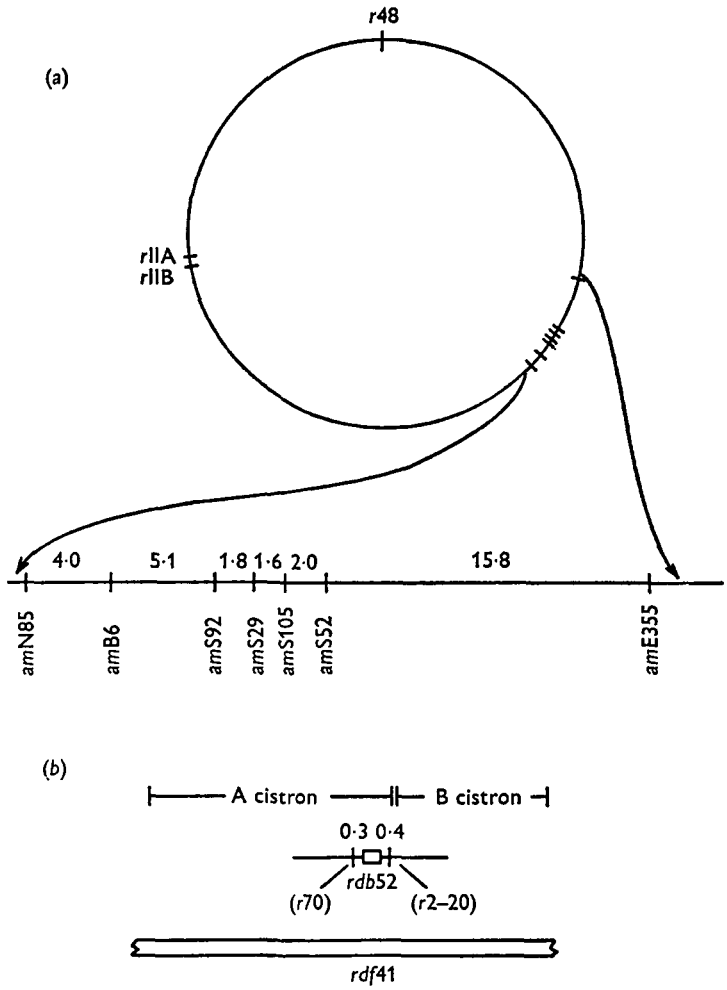

Fig. 2. (*a*) Approximate location of *amber* mutants in relation to *r*48 and the *r*II-region, and map distance (in recombination units) between the seven *ambers*. *Amber* mutants with capital S were obtained from Dr A. H. Doermann. The other *ambers* are described by Epstein *et al.* (1963) and *r*48 is described by Doermann & Hill (1953). (*b*) Approximate location of the *r*II-deletions employed, *rdf*41 is described by Edgar *et al.* (1962) and *rdb*52 by Berger (1965).

The lysate was then centrifuged to remove bacterial debris, diluted in H-broth, filtered and stored under refrigeration. Control, non-FUDR, crosses were performed at the same time using the same bacterial culture, omitting FUDR and chloramphenicol. These crosses were lysed with chloroform at 25 min after infection and otherwise treated in the same manner as described for the FUDR-crosses.

Determination of phage genotype. Mottled plaques segregating *r*48 and r^+ were scored by plating the progeny from the respective crosses on *CR*63 to give about 20 plaques a plate. The plates were incubated at 37 °C for 14–16 h and coded before scoring to avoid personal bias. In order to determine the length of a heterozygous area, the progeny from the multi-amber crosses was plated on *CR*63 to give about 20 plaques per plate. The individual plaques were resuspended in H-broth containing chloroform. After storing over-night in the refrigerator the plaque-samples were plated to give no more than 50–80 plaques per plate. Ten of these plaques, representing the segregants of a single progeny plaque, were tested to identify each of the markers present in the progeny plaque. In all cases where both alleles of a given marker were found among the ten segregants, the original resuspended plaque was classified as a HET for that marker. In such cases, from 30 to 200 more segregants were tested from the respective plaque suspensions. HETs for *r*48 in the multi-amber crosses were scored by inspecting the platings from the individual resuspended plaques to see whether they segregated both *r*48 and wild type.

3. EXPERIMENTAL RESULTS

The following crosses were performed for scoring of mottled plaques: *rdf*41 *r*48 × *rdf*41, *rdb*52 *r*48 × *rdb*52, and *r*48 × wild type (a multiplicity of about seven of each parent was used.) The crosses were repeated twice or three times using different parental lysates for the last cross in a series. The crosses were done both in *E. coli* strain *CR*63 and in strain *B*. The results from all crosses are summarized in Table 1. It can be seen from the table that introduction of a long homozygous deletion covering the whole *r*II region does increase the frequency of HETs for *r*48 in the *r*I region. The short homozygous deletion had no detectable effect. The increase

Table 1. *Frequency of mottled plaques given as percentage and as number of mottled plaques observed over number of plaques examined*

(All three types of cross in a series were done at the same time using the same bacterial culture.)

Cross	Host bacteria	No. of repeated crosses 1	2	3	Total
Homozygous long deletion: *rdf*41 *r*48 × *rdf*41	*CR*63	1·25 178/14209	1·26 170/13487	1·09 121/11011	1·21 469/38707
	B	1·61 263/16285	1·74 237/13582	—	1·67 500/29867
Homozygous short deletion: *rdb*52 *r*48 × *rdb*52	*CR*63	0·91 175/19185	0·83 121/14484	0·88 95/10749	0·88 391/44418
	B	1·04 214/20562	1·10 223/20143	—	1·07 437/40705
No deletion: *r*48 × wild type	*CR*63	0·91 193/21100	0·96 123/12697	0·99 115/11530	0·95 431/45327
	B	0·90 217/24094	1·04 188/17938	—	0·96 405/42032

in HET frequency for *r*48 was more pronounced in *B* bacteria than in *CR*63. All crosses within a series showed about the same frequency of mottled plaques.

These results are in agreement with the data of Streisinger *et al.* (1967) who found a higher frequency of h^{2+}/h^{4+} heterozygotes in phage crosses containing long homozygous deletions than in phage crosses containing short homozygous deletions or point mutants. Streisinger *et al.* (1967) think their results indicate that a factor extrinsic to the phage DNA molecule itself determines the length of DNA contained in an infectious phage particle, and that a deletion in one region would be compensated for by an equivalent increase in the terminal redundant portion of the molecule. This idea predicts not only an increase in frequency of heterozygosity for a particular marker but also an increase in average length of the redundancy heterozygotes. To test this hypothesis six closely linked *amber* mutants were chosen that map in different genes. The gene numbers and amber mutants were: 25 (*am*S52), 26 (*am*S105), 51 (*am*S29), 27 (*am*S92), 29 (*am*B6) and 48(*am*N85). This region is located far from the *r*II region. Another *amber*, *am*E355, that maps in gene number 24, 16 recombination units apart from *am*S52, was chosen to see if some extremely long HETs exist in T4D. All crosses were made in *E. coli* strain *B*, since this host showed the highest increase in mottled plaque frequency.

The following crosses were performed using a multiplicity of about seven of each parent:

*rd*f41/*am*E355/*am*S52/*am*S105/*am*S29/*am*S92 × *rd*f41/*r*48/*am*B6/*am*N85;
*am*E355/*am*S52/*am*S105/*am*S29/*am*S92 × *r*48/*am*B6/*am*N85

The progeny from each cross was plated on *CR*63 and 630 phages from each normal cross and 441 phages from each FUDR cross were tested for heterozygosity as described. Figures 3–5 show the frequency of HETs which contain a particular marker and simultaneously one or more of the consecutive markers (*am*E355-HETs are not included in these figures). The data are consistent with the assumption that the distribution of HET lengths is roughly exponential, as indicated by the shape of the curves on semilog paper (see Figs. 3–5, and, also, Doermann & Boehner, 1963).

It can be seen from Fig. 3 that of all HETs collected after normal lysis and containing a particular marker, 50 % are heterozygous for a marked site 17 map units distant in the homozygous deletion cross and 3·5 map units distant in the cross with no deletion (map units are corrected for high negative interference according to Barricelli & Doermann, 1960).

A rough evaluation of the mean length of a HET is obtained by assuming that heterozygous areas are distributed randomly over the genome. In the present experiments the probability of obtaining various HET lengths as shown in Figs. 3–5 seems to fit an exponential distribution, i.e. the probability $P_h(x)$ that the distance from a marker A to the right end of a HET area is longer than x (h being the mean length of the HET area) is approximately:

$$P_h(x) = \int_x^\infty \frac{1}{h} e^{-x/h} dx = e^{-x/h}.$$

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The same expression fits also for the distance from a marker A to the left end of a HET area.

It follows that for $x = h$, $P_h(x) = e^{-1}$: the average x value is in each diagram the point in which the value $e^{-1} = 0{\cdot}37$ is reached by the diagram. In the present

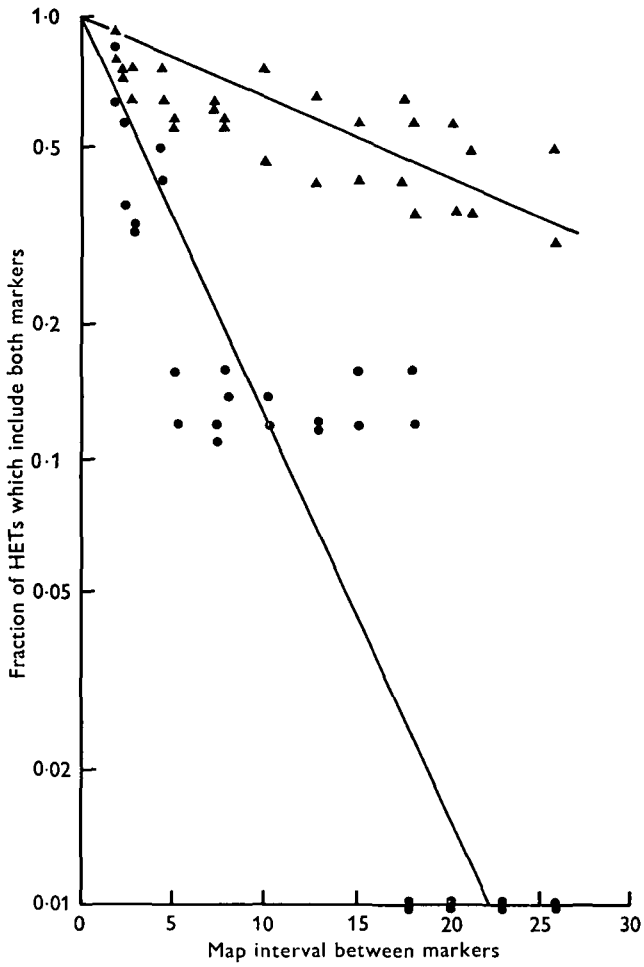

Fig. 3. Length distribution of HETs obtained from normal crosses. ▲—▲, Homozygous deletion cross (38 HETs); ●—●, no deletion in either parent (31 HETs). The lines drawn in this figure and in Figs. 4 and 5 are the best fit by eye. In the deletion crosses (normal lysis and premature lysis) several HETs are long and they participate in the data pool for calculation of several HET frequency points.

paper, instead of the mean, we prefer to use the median value M of the HET length. The median M is defined as the point at which the probability $P_h(x)$ given above has the value $\frac{1}{2}$ (or $P_h(M) = 0{\cdot}5$). The median value M is related to the mean h by the formula $M = h \ln 2$ (see Barricelli, 1960, appendix, formula C, and also Doermann & Boehner, 1963). Using this method in calculating the average HET length it is found to be 24 map units for the homozygous deletion cross

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(Fig. 3) and 5 map units for the cross with no deletion. The difference in average HET length between the two crosses is obviously significant and fairly close to the length of the *r*II region which is a minimum estimate of the long deletion, *rdf*41 (Edgar *et al.* 1962, found the *r*II region to be about 17 map units long). These results agree qualitatively with the assumption that a deletion of the phage DNA molecule is compensated for by additional redundant DNA.

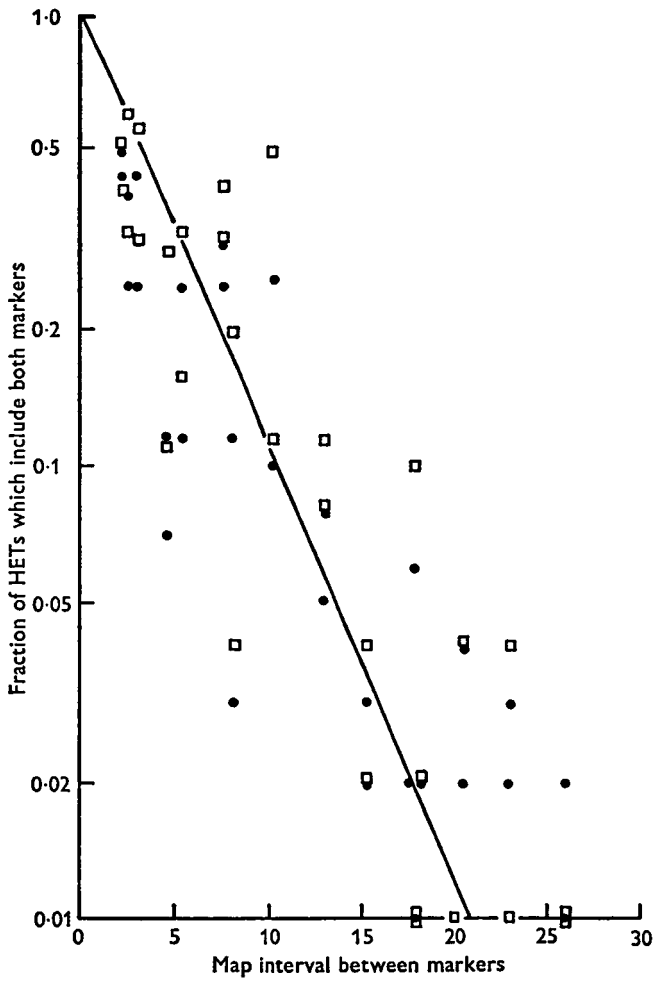

Fig. 4. Length distribution of HETs from FUDR crosses. □—□, Homozygous deletion cross (134 HETs); ●—●, no deletion in either parent (156 HETs).

The HETs found to accumulate in FUDR are assumed to have a heteroduplex structure (see Séchaud *et al.* 1965). If these HETs are on the average shorter than redundancy HETs, a reduction in average HET length is to be expected in FUDR. The distribution of HET lengths found in the FUDR crosses is given in Fig. 4. In the homozygous deletion cross the median value is reduced from 17 to 5 map units corresponding to a reduction in the average HET length from 24 to 5 map

units. This result indicates that the type of HET that does accumulate in FUDR (referred to as heteroduplex HETs) is on the average much shorter than the long HETs of the normal homozygous deletion cross, which presumably have a redundancy structure. No reduction in average HET length was found for the cross with no deletion in either parent. This indicates that redundancy HETs are short when no long deletion is present in the parental genome.

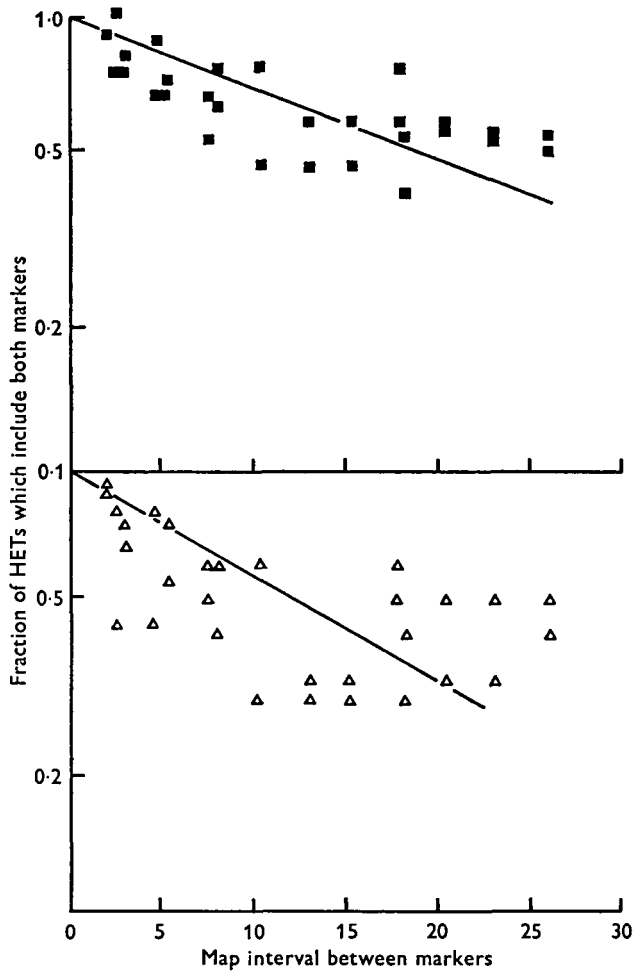

Fig. 5. Length distribution of HETs found after premature lysis. ■—■, Homozygous deletion cross (27 HETs); △—△, no deletion in either parent (16 HETs).

Control, non-FUDR crosses were performed at the same time as the FUDR-crosses, using the same bacterial culture, omitting FUDR and chloramphenicol. These crosses were lysed 25 min after infection but otherwise treated in the same manner as the FUDR-crosses. The average yield was low (burst size 18 in the deletion cross and 26 in the cross with no deletion), indicating that lysis was premature. The length distribution of HETs found in these crosses is given in

Fig. 5, which shows that the homozygous deletion cross gave a median value of 19 map units, and the cross with no deletion a median value of 12 map units. This corresponds to an average HET length of 27 map units in the deletion cross and 17 map units in the cross with no deletion. These results, especially the increase in average HET length found in the cross with no deletion, indicate that premature lysis HETs are on the average longer than normal lysis HETs. This could mean that redundancy HETs are made earlier in the latent period than heteroduplex HETs.

Table 2. *Number of HETs that covered from one to six closely linked* amber *markers*

	Number of *amber* markers covered					
Cross	1	2	3	4	5	6
Homozygous deletions						
Normal lysis	20	8	2	0	2	6
Premature lysis	13	5	1	1	1	6
FUDR	93	31	8	1	0	1
No deletion						
Normal lysis	22	6	2	0	1	0
Premature lysis	8	2	3	0	0	3
FUDR	120	28	6	1	0	1

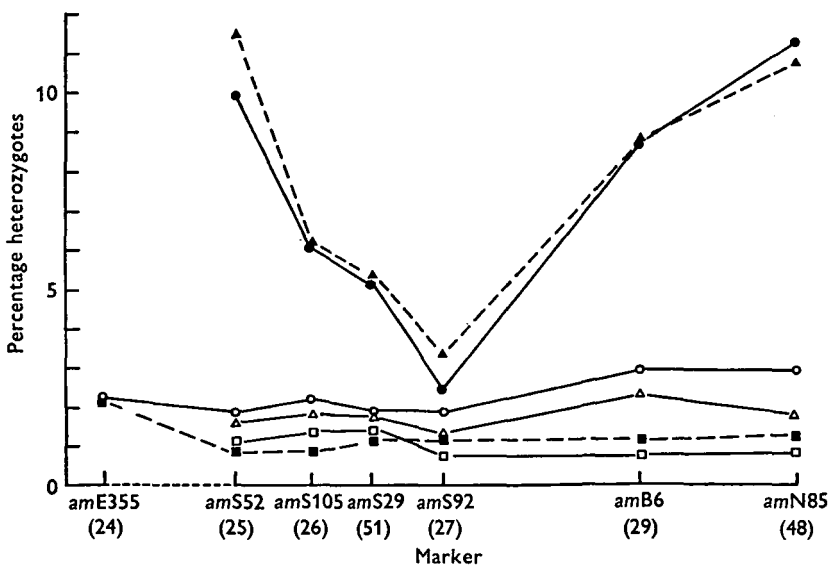

Fig. 6. HET frequency for *amber* markers in normal crosses; normal lysis and premature lysis, and in FUDR crosses. ○—○, Homozygous deletion, normal lysis; △—△, homozygous deletion, premature lysis; ■---■, no deletion, normal lysis; □—□, no deletion, premature lysis; ●—●, homozygous deletion, FUDR; ▲---▲, no deletion, FUDR, Numbers in parenthesis are gene numbers wherein the appropriate marker is located. 630 phages were tested for each of the non-FUDR crosses and 441 for each of the FUDR crosses.

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Table 2 shows that after normal lysis, HETs from the deletion cross covered all six linked markers in 6 out of 38 cases while in the control cross none were found to be that long among 31 HETs examined. In premature lysis, six-marker HETs were found 6 times among 27 HETs in the deletion cross and 3 times among 16 HETs in the control cross. These results, which also can be deduced from Fig. 5, indicate that long HETs are more frequent in early lysates.

Table 3

(*a*) From each cross the progeny particles were scored for heterozygosity. Phages that were HET for *r*48 were scored for heterozygosity for one or more of the seven *amber* markers. Phages that were HET for *am*E355 were scored for heterozygosity for one, five or six of the remaining *amber* markers. 630 phages were tested from each normal and premature lysis cross and 441 from each FUDR cross.

	Normal lysis		Premature lysis		FUDR	
Type of HET	D*	No D	D	No D	D	No D
HETs containing *r*48 and one or more *ambers*	1	1	3	2	14	12
HETs containing *am*E355 and:						
Six other *ambers*	1	0	—	—	—	—
Five other *ambers*	0	1	—	—	—	—
One other *amber*	1	0	—	—	—	—

(*b*) Progeny phages heterozygous for the region marked with six closely linked *ambers* were classified according to whether the heterozygous region was continuous (one HET area), was interrupted by one homozygous region (two HET areas) or by two homozygous regions (three HET areas). 630 phages from each normal and premature lysis cross and 441 from each FUDR cross were tested.

	Normal lysis		Premature lysis		FUDR	
No. of HET areas	D	No D	D	No D	D	No D
1	37	31	26	16	99	104
2	1	0	1	0	16	23
3	0	0	0	0	1	2

* D = deletion.

Figure 6 gives the percentage of HETs found for different markers; after normal lysis, premature lysis, and in FUDR. The cross with no deletion in either parent gave after normal lysis about 1 % HETs for all six closely linked markers and about 2 % HETs for *am*E355. The homozygous deletion cross gave about 2 % HETs for all seven markers. In FUDR, the average HET frequency was found to be about 7 %. However, in FUDR, different markers showed a great variation in HET frequency. About 10 % HETs was found for *am*S52 and *am*N85, while *am*S92 showed only about 3 %. In fact, in the homozygous deletion cross hardly any increase in HET frequency was found for the *am*S92 marker.

The progeny from the multi-amber crosses were tested for heterozygosity for *r*48 in addition to the *amber* markers. This was done by inspecting the platings

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from the resuspended plaques to see whether they segregated both *r*48 and r^+. Table 3(*a*) shows that in the non-FUDR crosses a total of seven phages out of 2520 examined showed heterozygosity for *r*48 in addition to being HET for one or more of the *ambers*. (In these crosses a total of 135 phage particles were found that showed heterozygosity, including those which were HETs for *am*E355). Thus, about 5% of all phages which were HET for one or more of the *ambers* were also HET for *r*48. Table 3(*a*) also shows that, after normal lysis, one phage particle was found that showed heterozygosity for *am*E355 in addition to being HET for all of the six linked markers, and one which was HET for *am*E355 and five of the linked markers. If there is no discontinuity, these two phage particles should have a heterozygous region 42 and 34 map units long respectively. A third

Table 4. *Number of parental and recombinant single-marker and multi-marker HETs in normal crosses and in FUDR crosses* (am*E355 used as outside marker in normal lysis*)

	Normal lysis		Premature lysis		Total		FUDR	
	D*	No D	D	No D	D	No D	D	No D
Parental HETs								
Single-marker HETs	10	3	2	4	12	7	12	27
Multi-marker HETs	1	4	2	0	3	4	7	4
Recombinant HETs								
Single-marker HETs	3	11	5	0	8	11	19	20
Multi-marker HETs	5	3	1	2	6	5	4	7
Zero-ended and one-ended HETs	19	10	17	10	36	20	92	98

* D = deletion.

phage was found showing heterozygosity for *am*E355 and *am*N85. Table 3(*b*) shows that in non-FUDR crosses two phage particles out of a total of 2520 examined showed discontinuity of heterozygosity within the region marked with six closely linked *ambers*. In FUDR, 42 phages out of a total of 882 tested showed discontinuity of heterozygosity within this same region.

HETs with two ends within the marked region were analysed to see whether they were parental or recombinant for outside markers (*am*E355 used as outside marker in normal lysis). These HETs and zero-ended and one-ended HETs are listed in Table 4. This table shows that both in normal crosses and in FUDR crosses about half of the HETs with two ends within the marked region were parental for outside markers. If these HETs can be said to be representative, it would be reasonable to assume that also half of the zero-ended and one-ended HETs are parental for outside markers.

The segregation pattern of all HETs was determined from data obtained by testing from 40 to 210 segregants for individual progeny phages. Table 5 illustrates that in normal crosses 12 out of 32 multi-marker HETs showed polarity in the homozygous deletion crosses and 5 out of 17 multi-marker HETs in the crosses

with no deletion. In the FUDR crosses, a total of 24 out of 77 multi-marker HETs showed polarity.

Table 6 pictures the marker incidence of parental multi-marker HETs. It can be seen from this table that 12 out of 17 such HETs exhibit very few copies of one of the two DNA strands.

Table 5. *Number of polarized, and non-polarized HETs in normal crosses and in FUDR crosses: 40–210 segregants tested for each HET* (am*E355–HETs not included*)

Cross	Total phages tested	Polarized HETs	Non-polarized HETs
Homozygous deletion			
Normal lysis	630	9	9
Premature lysis	630	3	11
FUDR	441	16	25
No deletion			
Normal lysis	630	3	6
Premature lysis	630	2	6
FUDR	441	8	28

Table 6. *Marker incidence in parental multi-marker HETs in normal and in FUDR crosses*

	Markers						
Het no.	*am*E355/+	*am*S52/+	*am*S105/+	*am*S29/+	*am*S92/+	+/*am*B6	+/*am*N85
			Deletion, normal lysis				
343	40/0	5/35	5/35	40/0	40/0	40/0	40/0
			Deletion, premature lysis				
5	—	0/180	8/172	11/169	0/180	163/17	180/0
245	—	0/40	0/40	5/35	5/35	0/40	0/40
			No deletion, normal lysis				
32	0/40	40/0	4/36	4/36	40/0	40/0	40/0
188	0/40	15/25	15/25	0/40	0/40	0/40	0/40
523	3/37	0/40	0/40	2/38	1/39	0/40	0/40
604	0/40	0/40	3/37	4/36	0/40	0/40	0/40
			Deletion, FUDR				
122	—	127/13	0/140	8/132	6/134	12/128	0/140
190	—	0/40	5/35	5/35	6/34	0/40	0/40
234	—	0/70	0/70	9/61	8/62	0/70	62/8
242	—	40/0	7/33	8/32	40/0	40/0	40/0
349	—	0/110	2/108	2/108	0/110	34/76	73/37
391	—	40/0	40/0	40/0	5/35	20/20	40/0
			No deletion, FUDR				
24	—	13/127	0/140	3/137	4/136	0/140	0/140
89	—	40/0	7/33	8/32	40/0	40/0	40/0
102	—	40/0	40/0	16/24	12/28	15/25	40/0
429	—	0/110	46/64	47/63	0/110	49/61	0/110

4. DISCUSSION

The results from the present work confirm the conclusions made by Streisinger *et al.* (1967) that the introduction of long homozygous deletions for the *r*II-region increases the frequency of heterozygosity for other regions of the phage genome among the progeny of a cross. Scoring of mottled plaques for *r*48 was used as a preliminary measurement of the effect of long and short homozygous deletions, and the crosses were done both in *E. coli* strain *CR*63 and in strain *B*. The increase in mottled plaque frequency when using the long deletion, *rdf*41, was more pronounced in *B* bacteria than in *CR*63. The reason for this is not known. The short homozygous deletion had no detectable effect on mottled plaque frequency. These results lead to the further prediction that introduction of a long homozygous deletion for the *r*II-region would also increase the average length of a heterozygous area located elsewhere in the genome. The data confirm that prediction and add additional evidence to the existence of HETs which have a physically redundant structure. Furthermore the results support the hypothesis proposed by Streisinger *et al.* (1967) that each phage particle of T4 contains on the average a 'headful' of DNA, and that introduction of a deletion is compensated for by a lengthening of the redundancy corresponding to the length of the deletion.

Doermann & Boehner (1963) found for *r*II-HETs, after normal lysis, an average length of 11 map units (HET length corrected for high negative interference). This long average HET length may be ascribed to a technique selective for redundancy HETs.

For premature lysis, Doermann & Boehner (1963) found an average HET length of 14 map units. Also in the present experiments an increase in average HET length was found after premature lysis, especially in the cross with no deletion (an increase from 5 to 17 map units). The homozygous deletion cross showed only a slight increase. HETs that covered all six closely linked markers occurred after normal lysis only in the homozygous deletion cross. After premature lysis, on the contrary, the frequency of long HETs covering all six linked markers was only slightly lower in the cross with no deletion than in the homozygous deletion cross. These results indicate that redundancy HETs are made earlier in the latent period than heteroduplex HETs, if the assumption is made that the two types of HETs can be distinguished because of their length.

Wiemann (1965) found in a cross, in which one parent was wild type and the other contained six *r*II point mutants, one type of HETs that covered from one to three markers, were parental for outside markers, and showed non-polarized segregation. (Doermann & Boehner, 1963, found that a *r*II marker near one pinpointed end of a HET area appeared less frequently among the segregants than did a *r*II marker located farther from that end. This phenomenon was referred to as polarized segregation.) Wiemann classified the above-mentioned HETs as having a heteroduplex structure and their length was estimated to vary from two to six map units. In the same cross Wiemann found another type of HET that covered up to all six *r*II markers, and showed polarized segregation. These HETs

were classified as terminal redundancy HETs since they showed the same kind of segregation as *r*II-deletion HETs found in another cross in which the marked parent contained 5 *r*II point mutants plus one *r*II-deletion mutant. By definition, *r*II-deletion HETs should have a redundancy structure. Wiemann estimated the length of the redundancy HETs to vary from 15 to 35 map units. In the present control cross with no deletion in either parent, only one long HET was found covering five out of six markers; however, this same phage particle was also heterozygous for *am*E355, located 16 map units apart from *am*S52. If there is no discontinuity, the total length of this particular HET corresponds to about 34 map units. The majority of HETs in the control cross was single-marker HETs, while in the deletion cross about half of the HETs was multi-marker HETs. This is in accordance with the prediction that introduction of a long homozygous deletion is compensated for by increasing the length of the redundancy.

Evidence for the existence of heteroduplex HETs have been provided by Hertel (1965) and Vigier (1966). Hertel investigated gene function of 'early' and 'late' *amber*-HETs and *r*II-HETs. Assuming that gene function is read from one strand only, and that the function is required before onset of DNA replication, only HETs having the wild allele on the 'active' strand should survive under restrictive conditions. If the wild-type function is required after DNA is replicated, all HETs should survive. Hertel found that about half of the 'early' *amber*-HETs and about half of the *r*II-HETs did disappear when absorbed to restrictive bacteria. These results were interpreted by Hertel to mean that the majority of HETs in T4D has a heteroduplex structure.

Vigier scored r^+ double recombinants from the following two types of crosses:

(1) $\overset{a}{\blacksquare}\!\!-\!\!-\!\!-\overset{b}{\blacksquare}\times\!\!-\!\!-\overset{c}{\blacksquare}\!\!-\!\!-$ and (2) $\overset{a}{\blacksquare}\!\!-\!\!-\!\!-\overset{b}{\blacksquare}\times\!-\overset{d\ e}{+\,+}\!-$,

where *a*, *b*, and *c* are all deletion mutants and *d* and *e* are point mutants, all located in the A cistron of the *r*II-region. Vigier used FUDR and plated either directly on *K* (λ) or pre-absorbed to *B* before plating on *K* (λ). He found in Cross 1 no significant difference between the two platings, while in Cross 2 he found a marked increase in r^+-recombinants after one cycle of growth in *B*. This increase in r^+-recombinants Vigier attributes to the formation of heteroduplex HETs in Cross 2 with the wild allele on the non-functional strand. In Cross 1, redundancy HETs are formed for the marked region which on one end of the chromosome has one deletion mutant and on the other end one or two other deletion mutants. Since all mutants are located in the same cistron this type of redundancy HET cannot grow on *K* (λ).

In FUDR, a tenfold increase has been found in HET frequency for point mutants, while deletion mutants show the same frequency as found in normal crosses (Séchaud *et al.* 1965). These results lead these authors and others to assume that the type of HETs that do accumulate in FUDR are the heteroduplex HETs since they are believed to be destroyed by DNA replication. If these HETs are on the average much shorter than redundancy HETs, a decrease in average HET length should occur when using FUDR. Such a decrease was found in the homo-

zygous deletion cross where the average HET length was reduced from 24 map units to 5 map units. No detectable reduction was found in average HET length in the cross with no deletion in either parent. These results indicate that the length of the redundancy HETs is much shorter when no long deletion is present in either parent. Berger (1965) investigated the progeny from a cross in which one parent contained six point mutants in the *r*II A cistron and the other contained four point mutants in the *r*II B cistron. He found also the same average HET length in a normal cross as in a FUDR cross.

The predominant type of HETs accumulating in the present FUDR crosses covered only one or two markers, indicating that the type of HET which is assumed to have a heteroduplex structure is short and probably of the order of 2–6 map units as was found also by Wiemann (1965).

In the present non-FUDR crosses (see Table 5) 12 out of 32 multi-marker HETs showed polarized segregation in the homozygous deletion crosses and 5 out of 17 multi-marker HETs in the crosses with no deletion. The slightly higher frequency of polarized HETs in the homozygous deletion crosses is to be expected since introduction of the long deletion increases the probability of recovering multi-marker HETs in the marked region. In all crosses (normal crosses plus FUDR crosses) a total of 17 HETs were found that covered all six linked markers. These HETs are assumed to have a redundancy structure and should show polarity if the ends of the heterozygous region are close enough to the marked region. Twelve of these long HETs showed polarity, the other five showed the same marker frequency for all six markers. Berger (1965) found that the majority of his FUDR–HETs showed non-polarized segregation. This is in agreement with the results from the present FUDR-crosses, only 24 out of 77 multi-marker HETs exhibited polarity (see Table 5).

Levinthal (1954, 1959) found that most of T2 phages that were heterozygous only for the middle one of three linked markers, were recombinant for outside markers (see Fig. 1*a*). Wiemann (1965) found that the majority of T4 HETs obtained in a cross, six *r*II point mutants × wild type, was parental for outside markers. He assumed that these HETs had a heteroduplex structure and had occurred as a result of insertion of small pieces of single stranded parental DNA into an otherwise homoduplex DNA molecule. Berger (1965) found that about half of his FUDR–HETs were parental for outside markers, while the majority of HETs in a normal cross was recombinant for outside markers. However, Berger pointed out that the data for normal HETs were quite limited.

In the present experiments about half of all HETs with two ends within the marked region was parental for outside markers, both in normal crosses and in FUDR crosses (see Table 4). In the non-FUDR crosses, 19 out of 26 parental HETs were single-marker HETs. These HETs may have arisen by insertion of small pieces of parental DNA.

Table 6 shows that 12 out of 17 parental multi-marker HETs exhibit very few copies of one of the two DNA strands. If the assumption made by Wiemann (1965) is right, that parental HETs are the result of insertion of small pieces of parental

DNA in an otherwise homoduplex DNA molecule, the present segregation pattern of parental multi-marker HETs requires the assumption to be made that one of the two DNA strands is preferentially copied.

The mechanism for T4 progeny formation in FUDR has been studied by Kozinski & Kozinski (1963, 1964), who found that progeny phage particles and DNA molecules made in the presence of FUDR contained parental segments of DNA integrated as discrete single stranded subunits. Berger (1965) proposed a model for HET formation in FUDR indicating different requirement for thymidine in different regions of the genome. When no new thymidine is available, single-stranded pieces of parental DNA is integrated. This model explains Bergers finding that different markers showed great fluctuation in HET frequency in FUDR, but not in normal crosses. Such a fluctuation in HET frequency was also found in the present FUDR crosses. Some of the markers that were found to show high HET frequency in Bergers FUDR experiment were also found to show high HET frequency in the present FUDR crosses.

Womack (1965) found that different markers differed in their ability to be rescued from a u.v.-irradiated genome. A region with high cross-reactivation could mean a region where recombination occurs more frequently than in other regions—this again could mean a region with high HET frequency. Womack found low rescue values for *am*E355, *am*S29, *am*B6 and *am*N85 but a peak rescue value for *am*S52. In the present control cross all six closely linked markers showed about 1 % heterozygosity, while *am*E355 showed about 2 %. These results do not indicate any correlation between HET formation under normal conditions and high rescue values in cross-reactivation experiments. Berger (1965) suggested that the normal T4D genome contains thymidine-rich regions. Such regions should be expected to suffer high u.v. damage and therefore show low rescue values. In FUDR crosses, on the contrary, according to Bergers model, such regions should show high HET frequency. One of the *am* markers tested, *am*N85, showed a high HET frequency in the present FUDR crosses. In Womack's experiment this same marker showed a low rescue value. However, *am*S52, which showed a peak rescue value in Womack's experiment, also showed a peak HET frequency in the FUDR crosses. These results seem to indicate that there is no correlation between HET formation for a particular marker in FUDR and the requirement for thymidine in the region where the marker is located.

The variation in HET frequency found for particular markers in FUDR and partly also in normal crosses seems to depend upon which marker is tested in a specific gene. Another explanation may be that in some parental stocks the marker tested happened to be in an overlap region and therefore showed a higher frequency of heterozygosity than did other markers. (Computer data obtained by Dr N. A. Barricelli indicate than an overlap area in a phage particle does persist (personal communication).)

SUMMARY

Experiments were designed to investigate the effect of homozygous deletions upon the frequency and the average length of heterozygous regions in bacteriophage T4D. A long deletion, *rdf*41, which covers at least the whole *r*II region, was found to increase the heterozygosity for *r*48, while no increase was observed when a short deletion was employed. The long deletion was found to increase the average length of *amber*-HETs by a length approximately the size of the *r*II region.

A drastic reduction in average HET length was found in FUDR crosses homozygous for the long deletion *rdf*41, indicating that the type of HET that does increase in FUDR is very short.

In the cross with no deletion in either parent, premature lysis HETs were found to be much longer than normal lysis HETs. Assuming that redundancy HETs are long compared to heteroduplex HETs this result indicates that redundancy HETs are made earlier in the latent period than heteroduplex HETs. A fluctuation in HET frequencies was found for different markers, especially in FUDR.

About half of all HETs, both in normal crosses and in FUDR crosses, was found to be parental for outside markers.

In non-FUDR crosses, polarized segregation was shown by 12 out of 27 multi-marker HETs after normal lysis and 5 out of 22 multi-marker HETs after premature lysis. In FUDR crosses, 24 out of 77 multi-marker HETs showed polarity.

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