was successful, all of the flowers pollinated set fruit. Examinations of styles showed that all crosses that failed were characterized by pollen tube growth stoppage similar in appearance to that of the self-incompatibility reaction. However, the length of pollen tubes before inhibition varied in the different unsuccessful crosses.

The crossing results contrast in several respects with those reviewed by Lewis and Crowe.[2] First, unilateral hybridization occurs between the two self-incompatible forms, Chillon I and Cajamarca, a situation neither anticipated nor previously described. Secondly, crossing behavior is of more than two kinds. In fact, each collection demonstrates a unique response in pattern of crosses to the other four. However, the patterns are not haphazard. The five entities are arranged along the top of the table in such order that each entity will cross as male parent only to the entities to the right and as female only to the entities to the left. Thirdly, the occurrence of three levels of unilateral behavior among self-fertile forms, as found here, has not been previously demonstrated and cannot be readily reconciled with the Lewis and Crowe[2] model of the evolution of self-fertility from self-incompatibility.

It is apparent from these results that unilateral crossing behavior is more complex, at least in *L. hirsutum*, than has been suspected. The relationship of this behavior to the genetic system controlling self-incompatibility is not clear-cut. The genetics and the physiology of the two systems badly need study, and this can be done best in species such as *L. hirsutum* where closely related forms show drastically different behavior.

Summary.—Unilateral hybridization occurs among *L. hirsutum* lines and between these and lines of *L. esculentum*. The restriction of crosses follows an orderly pattern not previously found in the plant kingdom and not in accordance with present theories of the origin and evolution of self-incompatibility and self-fertility.

The aid and advice of Dr. C. M. Rick is gratefully acknowledged.

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*CHROMOSOME BREAKAGE ACCOMPANYING GENETIC RECOMBINATION IN BACTERIOPHAGE**,†

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Genetic recombination in bacteriophage was first observed by Delbrück and Bailey[1] and by Hershey and Rotman,[2] who showed that cells simultaneously infected with two different mutants of phage T2 yield, in addition to the two infecting types, both doubly mutant and wild type progeny phages. Subsequently, ex-

tensive studies have been made of bacteriophage recombination, especially with the phages T1, T2, T4, and λ. Hundreds of mutants have been crossed, and for each phage type the observed recombination frequencies may be represented on a single unbranched genetic map.[3-6]

The genetic material of these bacteriophages is known to be DNA and it is commonly assumed that the genetic information resides in the linear sequence of nucleotides which comprise the DNA molecule. Under this assumption, genetic recombination in bacteriophage corresponds to the production of a nucleotide sequence derived partly from one parental line and partly from another. This may be imagined to occur in either of two ways. (1) *Copy choice:* The recombinant sequence is synthesized *de novo* by copying first one parental sequence, then another. (2) *Breakage:* The recombinant sequence is formed by the association of DNA fragments from different parental lines.

Recombinant phages produced by copy choice would be free of parental DNA, whereas breakage could result in the appearance of portions of parental DNA in recombinant phages.

In this paper, we describe experiments with the bacteriophage λ designed to see whether there is parental DNA in recombinant phages. Two-factor crosses were made between λ and λ heavily labeled with the isotopes C^{13} and N^{15}. Then density-gradient centrifugation[7, 8] was used to determine the distribution of labeled parental DNA among both parental and recombinant genotypes of the progeny. Our results demonstrate the presence of discrete portions of parental DNA in recombinant phages.

Materials and Methods.—Preparation of $C^{13}N^{15}$ media: Algal hydrolysates: Carbon-13 of 93% isotopic purity supplied in the form of methane[9] was oxidized to CO_2 by repeated passage over CuO at 850°C. The CO_2 was absorbed in saturated $Ba(OH)_2$ solution. A 6.8 gram portion of the resulting $BaC^{13}O_3$ was used along with $N^{15}H_4Cl$ of 99% isotopic purity[10] for the growth of *Ankistrodesmus* following the procedure of Davern.[11] After harvest by centrifugation, the algae were suspended in 15 ml of *N* HCl and kept at 100° for 6 hours. The mixture was centrifuged to yield a supernatant called H-1 and a pellet which was resuspended in 15 ml of 6 *N* HCl and refluxed for 20 hr. After cooling, the mixture was centrifuged to yield the supernatant H-2 and a pellet which was discarded. Hydrolysates H-1 and H-2 were separately evaporated to dryness *in vacuo* at room temperature and each residue was taken up in 5 ml of water. After three repetitions of this process, the final solutions were brought to pH 6 by the addition of carbonate-free NaOH solution. To H-2 was added 50 mg of HCl-washed decolorizing charcoal, and the mixture was shaken for 1 hr. Then both H-1 and H-2 were filtered through fine sintered-glass disks and sterilized by passage through type HA millipore filters. Both H-1 and H-2 were made up to 10 ml with sterile water and kept frozen until used.

Yeast extract: The hydrolysate H-1 was used to support the growth of yeast[12] for approximately ten generations in a medium of the following composition: $N^{15}H_4Cl$, 0.10 gm; Hydrolysate H1, 10 ml; NaCl, 0.4 gm; K_2HPO_4, 0.075 gm; $MgSO_4$, 0.30 gm; $CaCl_2$, 0.016 gm; $FeCl_3$, 7×10^{-5} gm; H_2O, 150 ml.

The culture was gently aerated at 30°C until the yeast attained a concentration of 4×10^7/ml and had just stopped budding. After harvest by centrifugation, the yeast was suspended in 5 ml of H_2O and brought to 100°C for 1 min and kept at 37°C overnight. The suspension was then centrifuged and the supernatant, called yeast infusion, was frozen. The pellet was suspended in 10 ml of 6 *N* HCl and refluxed 20 hours, after which the hydrolysate was repeatedly evaporated and then adjusted to pH 6 as described above. Finally, the hydrolysate was added to the yeast infusion, and the combined solution was filtered through a fine sintered-glass disk and sterilized by passage through a type HA millipore filter. This yeast extract was made up to 10 ml with sterile water and was kept frozen.

The isotopic purity of the $C^{13}N^{15}$ media was verified by measurements of the buoyant density of DNA prepared from *Escherichia coli* grown for many generations in a medium containing $N^{15}H_4Cl$ and either H2 or $C^{13}N^{15}$ yeast extract as described below. In both cases, the $C^{13}N^{15}$ DNA was found to be 0.047 gm cm^{-3} more dense than unlabeled DNA from the same organism. The calculated increment for the Cs salt of *E. coli* DNA with 99% N^{15} and 93% C^{13} is 0.050 gm cm^{-3} in the absence of selective solvation.

Bacteriophages: Phage λ++ is the "wild type" of Kaiser.[13] Phage λ*h* was derived from λ++ and has extended host range enabling it to plate on CR63. Phage λ*cmib*$_2^+$ is described by Kellenberger *et al.*[14] and is designated here as λ*cmi*.

Bacteria: Strain 3110, a λ-sensitive derivative of *E. coli* K12, was used for the preparation of λ*cmi* stocks and as host for crosses. Strain C 600[15] was used for plating as was the λ-resistant strain CR63.[16] Strain K12S made lysogenic for λ++ was used for the production of λ++ stocks by induction.

Media: Tryptone broth and suspension medium were prepared according to Weigle *et al.*,[8] and A medium is 7 gm Na_2HPO_4, 3 gm KH_2PO_4, 0.6 gm $MgSO_4$, 0.5 gm NaCl, 5 × 10^{-4} gm $FeCl_3$, and 1 liter H_2O, to which carbon and nitrogen sources were added separately.

Phage stocks: Phage λ++ was prepared by UV induction of K(λ) grown in A medium containing either 1 mg/ml $N^{15}H_4Cl$ and 0.05 ml/ml each of H-2 and yeast extract or 1 mg/ml $N^{14}H_4Cl$, 3 mg/ml charcoal-treated casamino acids and 1 mg/ml Difco yeast extract. The bacteria were grown approximately 7 generations to 2 × 10^8/ml, centrifuged, suspended at 10^8/ml in A medium and irradiated. Carbon and nitrogen sources were then added as above and the induced culture was aerated for 100 minutes, after which the culture was lysed by saturation with chloroform. The burst size was approximately 100 for both λ$C^{13}N^{15}$ and $C^{12}N^{14}$ λ++. The lysates were centrifuged at 50,000 × *g* for 120 minutes to sediment the phage. The pellet was resuspended by standing overnight in suspension medium.

Phage λ*cmi* was prepared by one cycle of lytic multiplication in 3110 grown in A medium with either $C^{13}N^{15}$ or $C^{12}N^{14}$ as above. The bacteria were grown approximately 8 generations to 5 × 10^8/ml and λ*cmi* in suspension medium was added to a multiplicity of 3. Ten minutes after infection, a 3-fold dilution with fresh medium was made and aeration was continued for 130 minutes. The culture was then lysed by saturation with $CHCl_3$. For the preparation of the λ*cmi* stocks used in cross II, the culture was made 0.003 *M* in ethylene diamine tetra-acetate, and 0.1% in lysozyme 1 min before the addition of $CHCl_3$. The burst size was approximately 60 for both labeled and unlabeled λ*cmi*. The phages were purified by low- and high-speed centrifugation and suspended in suspension medium.

The frequency of all morphology mutants was below 0.01% in the stocks used in these experiments.

Density-gradient centrifugation: The technique of preparative density-gradient centrifugation described by Weigle *et al.*[8] was employed with several modifications. Centrifugation was at 23,000 rpm for 24 hours at 24°C. The cesium chloride solution was overlayered with mineral oil to fill the lusteroid tube in order to prevent its collapse. The initial density was chosen so that all bands formed in the lower half of the gradient. This step, recommended to us by Dr. F. Stahl, prevents the occurrence of an artifact of preparative density-gradient centrifugation which previously has caused us much difficulty. When bands formed in the upper part of the gradient are collected, one invariably finds a satellite preceding the main band. The satellite contains approximately 1% as much material as the main band and precedes it by as many as 6 drops. Phage λ*h* was added in all centrifugations in order to serve as a density marker and to establish, by its characteristic narrow unimodal distribution, that no artifacts due to deceleration or drop collection had disturbed the distribution of the various other phage types.

In all experiments, the recovery of each genotype from the CsCl gradient was essentially complete.

Results.—Two crosses were performed with labeled phages: cross I, λ ++ $C^{13}N^{15}$ × λ*cmi* and cross II, λ ++ × λ*cmi* C^{13}/N^{15}. In both cases, parallel crosses were made using unlabeled parents. These are referred to as dummy crosses I and II. The multiplicities of infection and yields for the various crosses are recorded in Table 1. The dummy crosses were made at approximately the same multiplicities

TABLE 1

Crosses	Multiplicities ++	Multiplicities *cmi*	Burst sizes ++	Burst sizes *cmi*	Recombinant frequencies (%) *c*	Recombinant frequencies (%) *mi*
I λ++ $C^{13}N^{15}$ × *cmi*	2	3	19	46	0.88	0.56
II λ++ × *cmi* $C^{13}N^{15}$	5	2.2	33	12	0.74	0.46

and gave comparable yields.

Dummy crosses: As may be seen in Figure 1, λ*h* and both parental and recom-

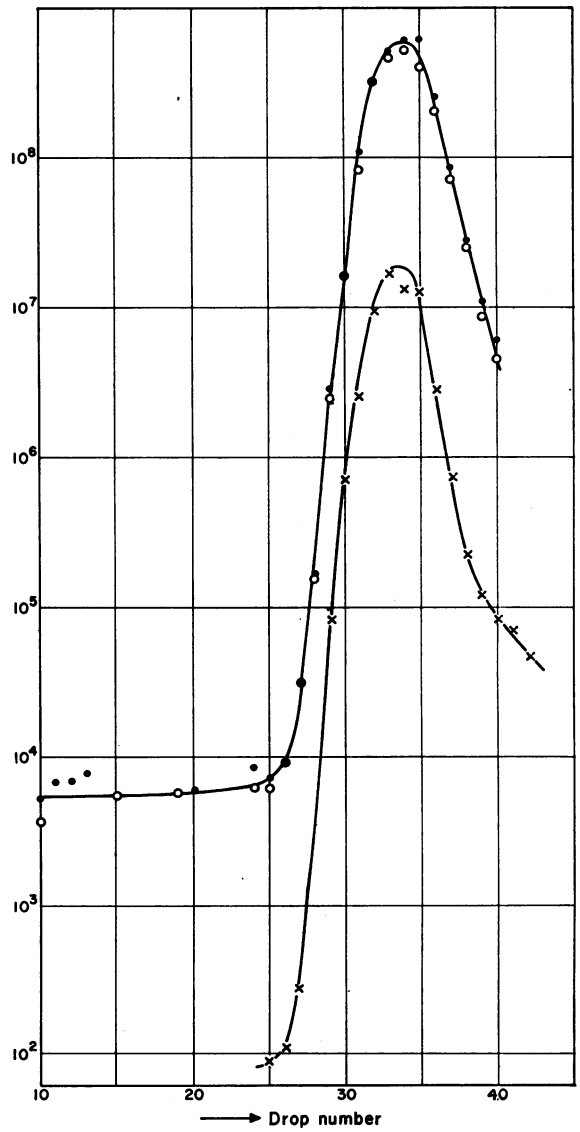

FIG. 1.—Cross λ*cmi* × λ++. Titer of the progeny phages of different genotypes in the drops collected after centrifugation. —●—●—, *cmi*; —○—○—, ++; —x—x—, *h*. λ*h* was added as density and band shape reference. The titers of the recombinants λ + *mi* and λ*c*+ follow exactly the curve of λ*h*, and the points representing the measurements have been omitted to avoid crowding the picture.

binant types emerging from a dummy cross exhibit the same narrow unimodal distribution in the density gradient. This finding allows us to attribute any deviation of the distributions found in crosses I and II from the distribution of the λh control to the presence of the density labels C^{13} and N^{15}.

Heavy parental stocks: The density distributions of the $C^{13}N^{15}\lambda$ ++ stock used in cross I and of the $C^{13}N^{15}\lambda cmi$ stock used in cross II are shown in Figures 2 and 3.

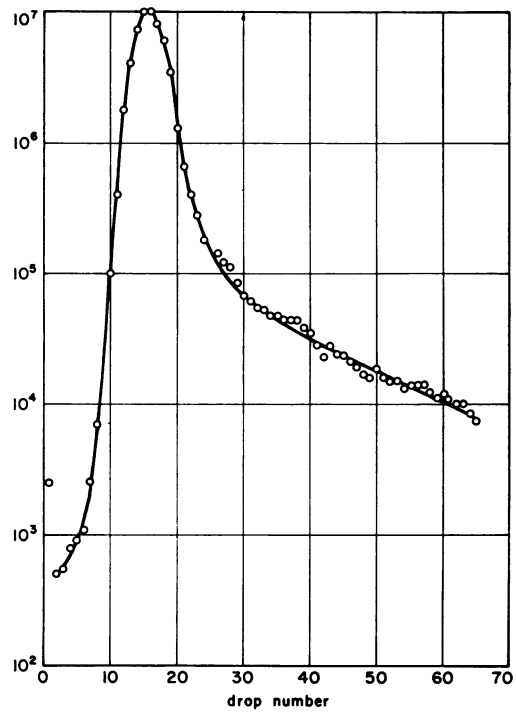

FIG. 2.—Density distribution of $C^{13}N^{15}$ λ++ used in cross I. The λ++ phages were induced from a lysogenic culture which had grown for many generations in heavy medium. The peak of the $C^{12}N^{14}.\lambda h$, not shown in the figure, came at drop 78.

The distribution of the heavy λ++ stock is essentially the same as that of λ *h* displaced to higher density by 0.05 gm cm^{-3}. This high uniformity of labeling is to be expected of the heavy λ++ phages, for they were induced from a culture which had grown many generations in heavy medium.

Because the heavy λcmi stock was prepared by only one cycle of growth in uniformly labeled cells infected by unlabeled phages, its density distribution provides information regarding the distribution of parental carbon and nitrogen among progeny phages. An important feature of the λcmi distribution shown in Figure 3 is the existence of two modes in addition to the main component which is fully labeled. According to their position in the density gradient, these two modes correspond to phages with 56 per cent and 78 per cent heavy label. The light parental atoms are presumably harbored in their DNA and not in their protein, which is not injected upon phage infection.[17] If all of the DNA were parental and all the protein new, it may be estimated from the composition of λ and the

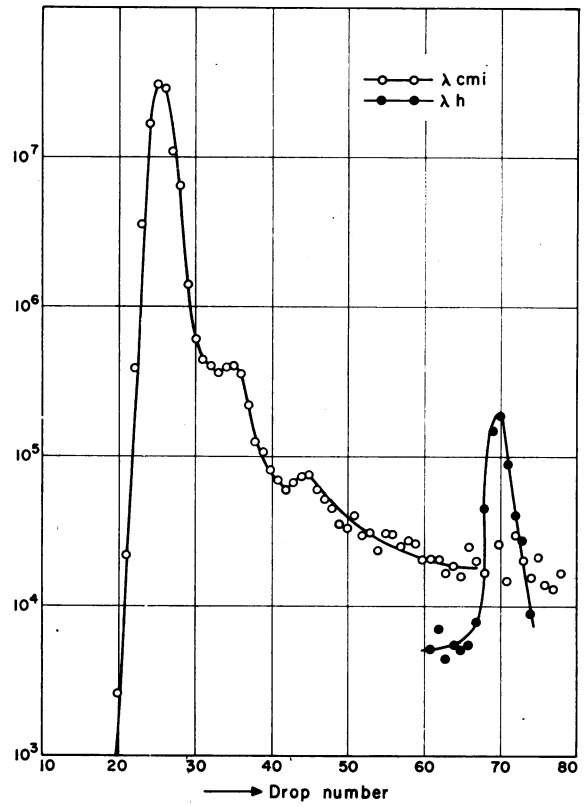

FIG. 3.—Transfer experiment with λ*cmi*. λ*cmi* grown in bacteria $C^{12}N^{14}$ were used to infect bacteria $C^{13}N^{15}$ in a $C^{13}N^{15}$ medium. After one cycle of growth, the λ*cmi* labeled with $C^{13}N^{15}$ were centrifuged. The figure gives the titers of the phages in the different drops collected after centrifugation. Phage λ*h*$C^{12}N^{14}$ was added as a density reference.

purity of the isotopes used that the phage would contain 54 per cent heavy label. We therefore presume that the phages with 56 per cent heavy label are composed of new (heavy) protein and parental (light) DNA and that the phages with 78 per cent label are composed of new protein and DNA which is half-parental. We shall refer to phages with only parental DNA as "conserved" and to those with half-parental DNA as "semiconserved." Semiconserved phages have appeared in each of the nine experiments in which we have infected labeled cells with unlabeled phages or *vice versa*. However, only when the multiplicity of infection was high have conserved phages also appeared. The yield of infectious hybrid phages has ranged between 1 and 4 per cent of the progeny phages while yields of infectious conserved phage have been found as high as 1 per cent (when $m = 7$) and less than 0.001 per cent (when $m = 0.1$).

Crosses: As may be seen in Figure 4, the density distributions of the progeny of cross I exhibit the following principal features:

(1) The distribution of the originally labeled parental genotype λ++ resembles that found when labeled phages alone are allowed to multiply in unlabeled cells. That is, modes of conserved and semiconserved phages are found in addition to unabsorbed and to essentially unlabeled phages.

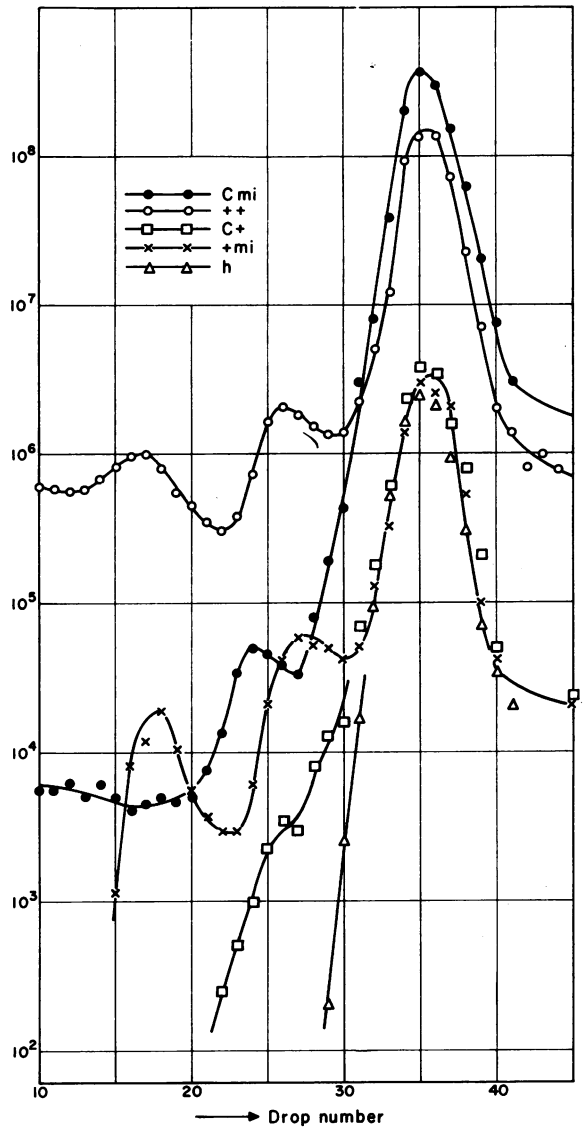

FIG. 4.—Cross λ++ $C^{13}N^{15}$ × λ*cmi* $C^{12}N^{14}$. Titer of the progeny phages of different genotypes in the drops collected after centrifugation. λ*h* was added as density and band shape reference.

(2) The originally unlabeled parental genotype *cmi* emerges from cross I with a very different distribution of label from that of the type ++. Neither conserved nor semiconserved modes are found. The λ*cmi* is essentially unlabeled but with a definite skewness toward higher density.

(3) The recombinant type +*mi* and *c*+ are distributed in strikingly different fashion from each other. The type +*mi* is found in three modes, one essentially unlabeled, one with slightly less label than the semiconserved λ++, and another with slightly less label than conserved λ++. The reciprocal type *c*+ is found

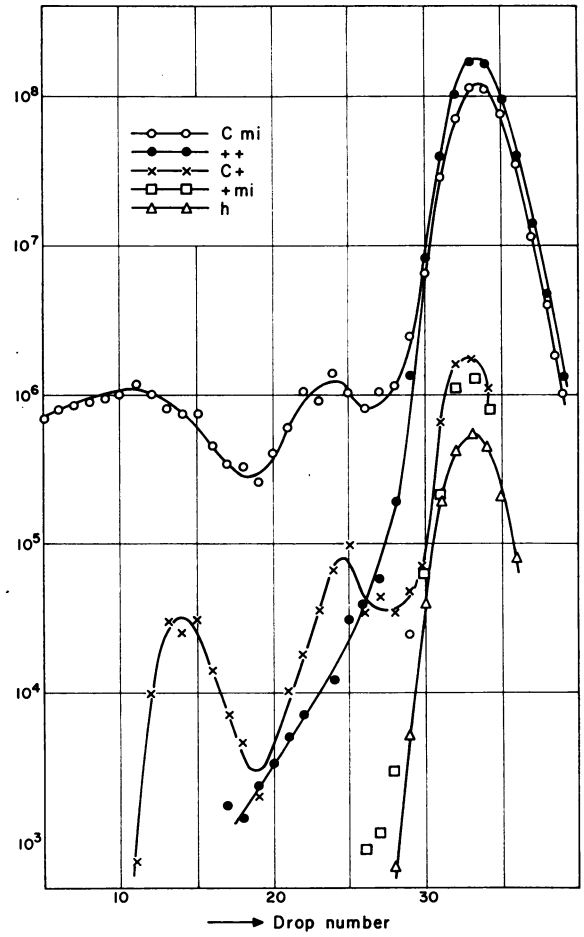

FIG. 5.—Cross λ*cmi* $C^{13}N^{15}$ × λ++ $C^{12}N^{14}$. Titer of the progeny phages of different genotypes in the drops collected after centrifugation. λ*h* was added as density and band shape reference.

in a single band which has a shoulder at a density between that of semiconserved and unlabeled phage.

To insure that the nonequivalent distributions of the two recombinant types from cross I were not due to peculiarities associated with the particular recombinant genotypes, a cross (cross II) was performed with $C^{13}N^{15}$ label in λ *cmi* rather than in λ++. As may be seen by comparison of Figures 4 and 5, the result of reversing the parental label is that the distributions of the two recombinants as well as the two parental types are interchanged.

Discussion.—Replication of the lambda chromosome: The maximum recombination frequency observed[6] for phage λ is only 15 per cent. This circumstance suggests that we may understand the main features of the distribution of parental DNA among progeny phages without at first considering the effects of genetic recombination. In summary, these features are:

(1) Among progeny from infections at high multiplicity, the original parental

DNA is distributed in two discrete modes corresponding to phages with DNA that is entirely original parental (conserved phages) and to phages with half original parental, half newly synthesized DNA (semiconserved phages).

(2) Among progeny from infections at multiplicity of much less than one phage per bacterium, no conserved phages are found; only semiconserved phages appear besides those with DNA entirely newly synthesized.

(3) From crosses between isotopically labeled and unlabeled phages, the genotype of the labeled parent emerges in both conserved and semiconserved modes, but these modes are essentially free of other genotypes.

The finding of semiconserved phages indicates that the DNA complement of λ is equally divided between two subunits which may separate from one another and appear in progeny along with newly synthesized subunits.

The finding of conserved phages at high but not at low multiplicities of infection may be explained in two ways. Either the two parental subunits never came completely apart or else they did so only to become reassociated by chance in the same progeny particle. The latter alternative is, however, made improbable by the finding that semi-conserved phages emerging from crosses between labeled and unlabeled parents are almost entirely of the genotype that was originally labeled. The substantial yield of labeled recombinants prevents us from ascribing this result to very poor mixing of vegetative phage in the early pool. In view of this, the hypothesis of separation and reassociation would require that the genes *c* and *mi* be restricted to only one of the two subunits and that the other never appear in progeny if the two became separated. This seems very unlikely. Accordingly we shall consider the first alternative only, in which the two parental subunits found in conserved phages never came apart.

Thus the DNA complement of λ is a single structure capable of remaining intact throughout the processes of infection and maturation. This does not imply that this structure replicates conservatively. On the contrary, the finding of conserved phages only at high multiplicities of infection suggests that the conserved phage may never have replicated; they may be "nonparticipating" phages. We are led to conclude that *the entire DNA complement of λ is a single semiconservatively replicating structure.*

In this respect, the DNA of λ behaves as a single molecule possessing the Watson-Crick structure replicating according to the scheme proposed by them.

In the remainder of this discussion, we shall refer to the DNA complement of λ as its chromosome.

The mechanism of recombination: Our main finding relevant to the mechanism of genetic recombination is that discrete amounts of original parental DNA appear in recombinant phages. This suggests that *recombination occurs by breakage of parental chromosomes followed by the reconstruction of genetically complete chromosomes from the fragments.*

Consideration of the detailed distribution of parental DNA among recombinants leads to several additional conclusions regarding the recombination process. The finding of a distinct recombinant class containing substantially more than 50 per cent original parental DNA indicates that *recombination by chromosome breakage may occur without separation of the two subunits of the parental chromosome.* If, as has been argued above, replication does not occur without separation of chromo-

somal subunits, it may also be concluded that *chromosomes need not replicate in order to recombine.*

From the positions of *c* and *mi* on the genetic map of λ (Fig. 6), it is seen that a break between these loci leaves an 85 per cent fragment of the map attached to the

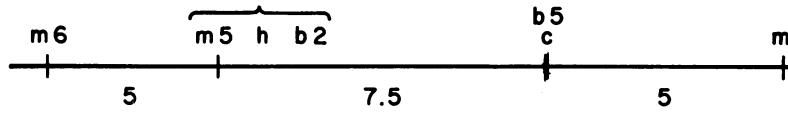

FIG. 6.—Genetic map of λ.

c locus and 15 per cent to the *mi* locus. In both crosses I and II, there occurs a mode of recombinants with approximately 86 per cent labeled parental DNA, and these recombinants carry at the *c* locus the allele contributed by the labeled parent. Thus it seems that *the probability of recombination between two loci is at least approximately proportional to the amount of DNA between them.* The recombinant type which appears with 90 per cent labeled DNA is also found in another mode with approximately 45 per cent labeled DNA. This class of recombinants could have been derived by semiconservative replication from recombinant chromosomes of the type carrying 90 per cent label or they could have been produced by breakage of hybrid chromosomes of parental genotype. It seems reasonable to suppose that both processes contribute recombinants to the mode at 45 per cent. Finally, we might expect the recombinant bearing the *c* allele brought in by the unlabeled parent to be distributed in two labeled modes at approximately 14 per cent and 7 per cent. However, phages so slightly labeled could not be resolved from the large band of unlabeled phage. Only a shoulder on the dense side of the unlabeled peak would be expected. As may be seen in Figures 4 and 5, such a shoulder is in fact observed.

The findings of this experiment thus may be understood in terms of a recombination mechanism involving breakage of a semiconservatively replicating two-stranded chromosome. The fragments produced could become incorporated into complete chromosomes either by joining of appropriate fragments (break and join) or by the completion of a fragment by copying the missing region from the homologous portion of a chromosome of different parentage (break and copy). Figures 7 and 8 show structurally plausible models for recombination by these two processes. The present experiments do not distinguish between these alternatives. Neither do they rule out the possibility of some copy choice recombination.

The contribution of original parental DNA to progeny bearing the initially unlabeled genotype does not conflict with our main conclusion that recombination occurs by breakage. However this distribution is not in simple accord with the notion that the probability of recombination between loci is always proportional to the amount of DNA separating them on the chromosome. The existence of favored breaking places as well as several other complexities, either experimental or genetic, may be invoked to explain the distribution of λ*cmi* in cross I or of λ++ in cross II. However we prefer to postpone discussion of this matter until it has been investigated further. Our conclusions are strongly supported by the experiments reported in the next paper,[18] which shows that in two-factor crosses between P^{32}-labeled and unlabeled λ, more P^{32} is transferred to recombinant progeny

MODELS FOR RECOMBINATION BY BREAKAGE OF DNA MOLECULES

Solid and dashed lines represent single chains with nucleotide sequences descended from different parents.

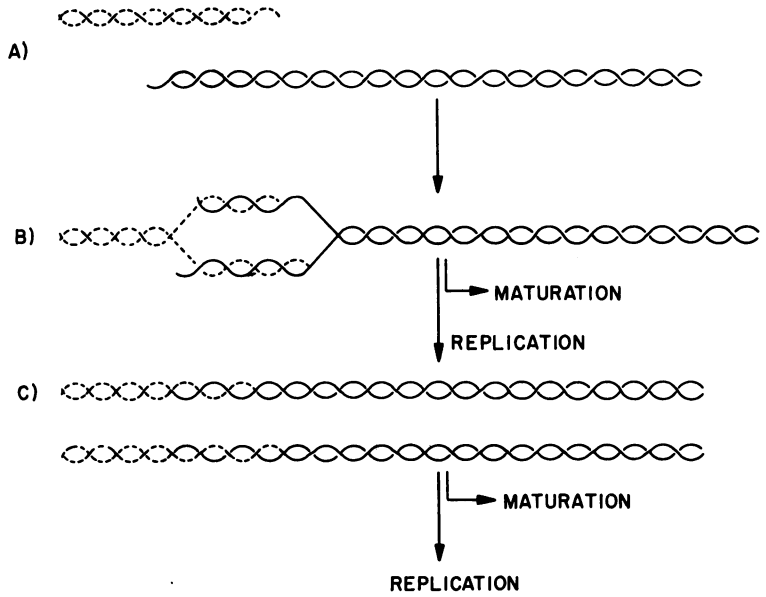

FIG. 7.—*Break and Join.* (*A*) Fragments from different parental lines possessing a region of common nucleotide sequence. (*B*) Association of fragments by complementary base-pairing in the region of common sequence. The recombinants with 90% parental DNA observed in the present experiments would contain chromosomes resembling this structure. (*C*) The two replication products of (*B*). Some of the recombinants with 45% parental DNA would have this type of structure.

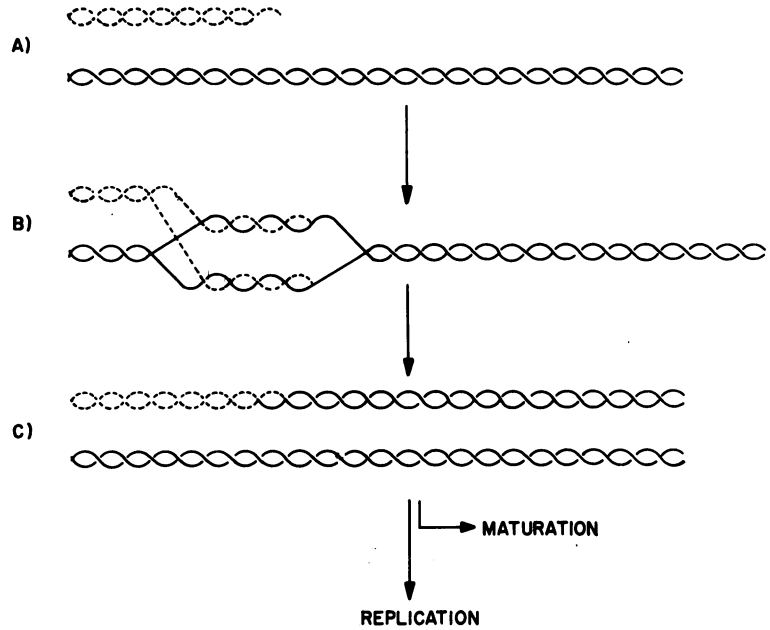

FIG. 8.—*Break and Copy.* (*A*) An intact molecule and a fragment from different parental lines. (*B*) Association by complementary base-pairing over a region of common nucleotide sequence. (*C*) The fragment is restored by copying the intact molecule. The recombinants found with 90% parental DNA would correspond to the restored fragment.

than to progeny with the genotype of the unlabeled parent. Although the P^{32} experiments give no information on the subunits of the DNA, they do give the integrated total amount of the original parental DNA transferred to the recombinants. This value also seems to show that distances along the genetic map are proportional to the amounts of DNA in the phage chromosomes.

Summary.—We have performed experiments designed to detect original parental DNA in recombinant phages. Density-gradient centrifugation was used to determine the distribution of labeled parental DNA among both parent and recombinant genotypes emerging from a two-factor cross between unlabeled phage λ and λ heavily labeled with the isotopes C^{13} and N^{15}. Our results demonstrate the presence of preferred quantities of original parental DNA in recombinant phages and support the following main conclusions:

(1) The DNA complement of λ is contained in a single semiconservatively replicating chromosome.

(2) Recombination occurs by chromosome breakage, although other mechanisms are not excluded.

(3) Both chromosomal subunits are broken during recombination.

(4) Distances along the genetic map are proportional to the amounts of DNA in the phage chromosome.

(5) The phage chromosome need not replicate in order to undergo recombination by breakage.

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