GENE TRANSFER BY F′ STRAINS OF *ESCHERICHIA COLI* K-12. III. AN ANALYSIS OF THE RECOMBINATION EVENTS OCCURRING IN THE F′ MALE AND IN THE ZYGOTES

JAMES PITTARD[1] AND EDWARD A. ADELBERG

Department of Microbiology, Yale University, New Haven, Connecticut

Received February 18, 1964

IN previous communications (PITTARD, LOUTIT and ADELBERG 1963; PITTARD and ADELBERG 1963), it was shown that when certain F′ strains transfer chromosomal genes to a recipient, the first appearance of recombinants for any given marker occurs 9 minutes later than when the donor is the parent Hfr from which the F′ male was derived. One of the strains exhibiting this 9 minute delay was shown to possess an F-merogenote (F_{14}) which takes 9 minutes to be transferred. The F-merogenote F_{14} carries a number of genetic loci concerned with the biosynthesis of methionine (*met*), arginine (*arg*), and isoleucine-valine (*ilv*).[2] The order of transfer of these genes is *met*, *arg*, *ilv*, *F*. The transfer of F_{14} by this strain occurs at a frequency approaching 50 percent whereas the highest frequency for a chromosomal gene is 5 percent or less. When an F′ male with the genotype met^+, arg^+, ilv^+, F/met^+, arg^-, ilv^- transfers the chromosomal gene xyl^+ to a xyl^-, ilv^-, arg^-, met^- female, the xyl^+ recombinants have a fixed probability of integrating met^+, arg^+, or ilv^+, no matter at which time samples are removed from the mating mixture. The wild-type allele met^+ is inherited at a frequency of 70 percent, arg^+ at 30 percent, and ilv^+ at about 20 percent. It can be further demonstrated that this gradient of recovery of F-merogenote markers in the xyl^+ recombinants is not due to any post-zygotic elimination occurring in zygotes but is caused by an interaction between F-merogenote and chromosome before transfer to the recipient.

SCAIFE and GROSS (1963), working independently with an F′ strain carrying the F-merogenote, *F-lac*, proposed that chromosome transfer by F′ strains occurs as the result of a crossover between donor chromosome and F-merogenote. According to this model, the low recovery of arg^+ and ilv^+ in the xyl^+ recombinants occurs because in a large percent of the cases the crossover between F-merogenote and donor chromosome causes the chromosomal genes arg^- and ilv^- to be transferred instead of the wild-type alleles arg^+ and ilv^+ carried by the F-merogenote. When the male donor carries ilv^+ on both the F-merogenote and the chromosome, the percent of xyl^+ recombinants that inherit ilv^+ increases from 20 percent to 80 to 90 percent as would be predicted by the crossing-over model (PITTARD and ADELBERG 1963). In Figure 1 is presented a detailed comparison of the recovery

[1] Present address: Department of Bacteriology, University of Melbourne, Victoria, Australia.

[2] For an explanation of symbols see Table 1.

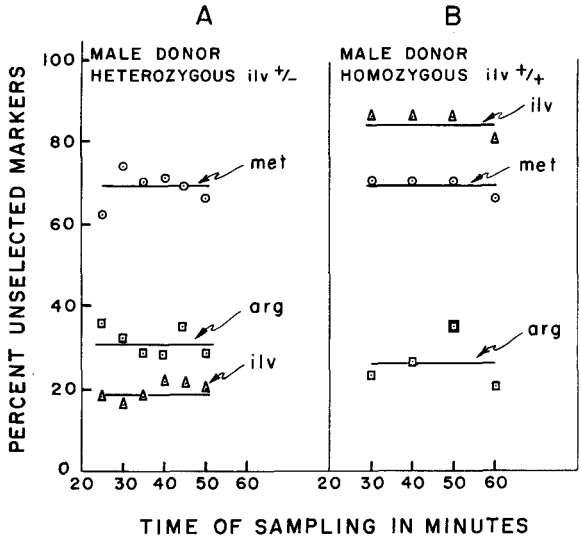

FIGURE 1.—A comparison of the frequency with which markers *met*+ *arg*+ and *ilv*+ are found in *xyl*+ recombinants when the F′ male donor is (A) heterozygous +/— for *ilv*, and (B) homozygous +/+ for *ilv*.

of prototrophic markers in the *xyl*+ recombinants when the F′ donor is (A) heterozygous *ilv*+*F*/*ilv*⁻ or (B) homozygous *ilv*+*F*/*ilv*+.

Having established the applicability of the crossing-over model to this F′ system we now wish to present an analysis of the various events that contribute to the genotype of recombinants having received chromosomal markers from the F′ male. Three distinct events will be considered: (1) The first event occurs in the male F′ donor and consists of a crossover between the F-merogenote and the homologous chromosomal region. We shall call this the donor crossover. As a result of this primary crossover event a new arrangement of genes is formed in the male and is subsequently transferred to the female. Evidence will be presented to support the idea that the donor crossover has a constant probability of occurring per unit length anywhere along the F-merogenote. (2) Once the recombinant chromosome resulting from the donor crossover is transferred to the female, further recombination events can occur between the transferred element and the corresponding genetic region of the recipient's chromosome. In the experiments to be reported, selection has been made for the male chromosomal marker *xyl*+, which is transferred approximately 18 minutes after the leading *met*+ marker. Thus, there is ample opportunity for recombination events to occur between *xyl*+ and *met*+. (3) Finally, when chromosomal genes have been transferred in the manner outlined above, it is found that some zygotes have simultaneously received an entire F-merogenote.

MATERIALS AND METHODS

Organisms: The strains used in this work are described in Table 1. They are all derivatives of *Escherichia coli* K-12.

TABLE 1

*List of strains**

Strain no.	Auxotrophic characters										Energy source utilization			Response to T6	Sex
	ile	ilv	arg	met	thi	thr	leu	pro	his	try	xyl	mal	tpa		
AB1255	1†	+	1	1	2	+	+	+	1	+	4	1	+	R	♀
AB1450	+	16	1	1	2	+	+	+	1	+	4	1	+	R	♀
AB1203	+	7	3	+	1	+	+	+	+	+	5	?	+	R	♀
AB2151	+	132	+	+	?	+	6	+	+	+	+	+	2	S	♂ Hfr
AB1516	+/+	+/7	+/3	+/+	1	+	+	2	4	+	+	+	+	S	♂ F_{14}
AB1528	+/+	+/7	3	+	1	+	+	2	4	+	+	+	+	S	♂ F_{16}
AB2144	+/+	7/7	3	+	1	+	+	2	4	+	+	+	+	S	♂ F_{16}
AB2148	+	+/12	+	+	?	+	+	2	4	3	+	1	+	R	♂ F_{16}

* The following abbreviations are used: ile, isoleucine; ilv, isoleucine and valine; arg, arginine; met, methionine; thi, thiamine; thr, threonine; leu, leucine, pro, proline; his, histidine; try, tryptophan; xyl, xylose; mal, maltose; tpa, tryptophanase; T6, Bacteriophage T6; R, resistant; S, sensitive; +/—, heterozygous; ?, not tested.

† Numbers refer to allele numbers that have been alloted to mutant strains in these laboratories. See PITTARD, LOUTIT and ADELBERG (1963).

Media and culture methods: The media and culture methods were those described by ADELBERG and BURNS (1960).

Mating conditions: The conditions under which recombination experiments were carried out were the same as those described by ADELBERG and BURNS (1960) with the following exceptions. Male cells were grown to exponential phase in glucose minimal medium supplemented with the required growth factors. Mating cells were always mixed in the ratio of at least ten females to one male to exclude the possibility of two males mating with the same female. Conjugation was interrupted by subjecting 1 ml samples of mating mixture to violent agitation with a Vortex Junior mixer. Unless otherwise stated the male cells were then always killed with bacteriophage T6.

Scoring unselected markers: At least 80 recombinants were tested at each time interval by transferring colonies to master plates of the selective medium and then replicating the new growth onto appropriate media.

RESULTS

Recombination events occurring in the F′ male: A schematic representation of the alleles carried on the F-merogenote F_{14} and on the homologous chromosomal region of the male AB1516 is shown in Figure 2. The relative position of the chromosomal gene *xyl* is also shown. As can be seen from this figure, the *arg-1* allele (the structural gene for the enzyme concerned with the last reaction in arginine biosynthesis), has undergone a transposition on F_{14} (PITTARD, LOUTIT and ADELBERG 1963). Although *arg-1* is situated very close to *arg-3* on the chromosome, these two loci are separated by 5 minutes on F_{14}. The *arg-3* allele is the structural gene for the enzyme concerned with the third step in argininé biosynthesis, the conversion of acetylornithine to ornithine (T. RAMAKRISHNAN, personal communication). The *arg-3* locus occurs in the same relative position on both F_{14} and on the chromosome. In Figure 2 we have represented the transposition of *arg-1* by two symbols: *arg-1* for the location of the structural gene, and *arg-1(DEL)* for the deletion region on the F-merogenote. When F_{14} pairs with a normal chromosome this deletion will be directly opposite to the *arg-1* locus of the latter. Furthermore, in such pairings the transposed *arg-1* locus of

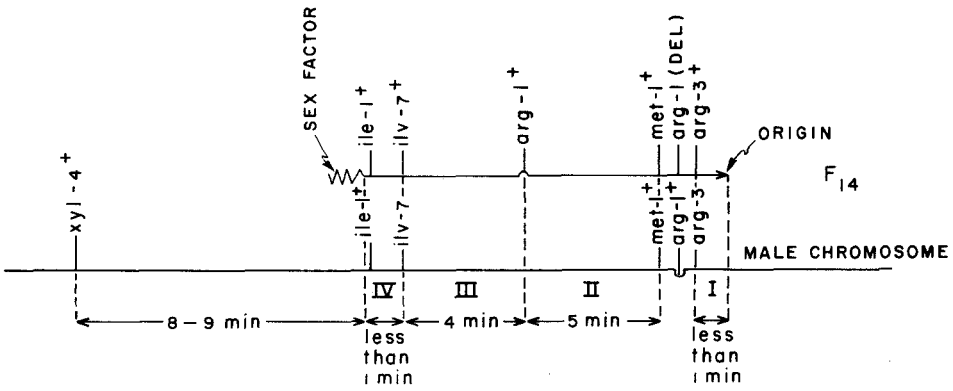

FIGURE 2.—A schematic representation of the genes carried on the F-merogenote F_{14} and on part of the chromosome of F′ strain AB1516.

F_{14} will be directly opposite a chromosomal region lacking a corresponding base-pair sequence. The pairing anomalies which must result are shown diagrammatically in Figure 2. The positions of *arg-1* and *arg-3* relative to each other on the chromosome are arbitrary, as are the relative positions of *ilv-7* and *ile-1*. Map distances are given in minutes and represent the time required for a particular length of genetic material to be transferred during conjugation.

The distance between *Origin* and *arg-3* cannot be directly measured, but is estimated to be less than one minute. This figure is arrived at as follows. F_{14} contains a total of 9 minutes of chromosomal DNA as measured by the delay in transfer of chromosomal markers such as *xyl* and *mal*. The distance between F_{14} markers *arg-3* and *ilv-7* is also 9 minutes. Thus there can be very little DNA between *arg-3* and *Origin*.

In Figure 3 are represented four different products of donor crossovers occurring in regions I, II, III and IV respectively of F′ strain AB1516. By recovery of the different alleles contributed by each of these products to the zygotes, one can determine the frequencies with which crossing-over events occur in each of the four regions. If crossing over occurs with equal probability per unit length along F_{14}, one would expect less than 10 percent of the crossovers to occur in regions I and IV, since together these regions only make up 10 percent or less of the entire length of F_{14}. Similarly one would expect approximately 50 percent of the crossovers to occur in region II and 40 percent to occur in region III.

In the experiment to be reported, the F′ male strain AB1516 was mated with the *xyl⁻ met-1 arg-1 ile-1* female strain AB1255. Samples were withdrawn from the mating mixture at 5 min intervals and subjected to mechanical agitation to separate mating couples and to bacteriophage T6 to kill the male parent. These samples were then plated on minimal xylose medium to recover the *xyl*+ recombinants. At each time 80 *xyl*+ recombinants were tested for their retention of the female markers *met-1 arg-1* and *ile-1* and for their inheritance of markers from either the F-merogenote or chromosome of the male. All recombinants that were *arg⁻* were tested for their ability to grow on ornithine. Strains carrying *arg-3* can grow on this intermediate but strains carrying *arg-1* or *arg-1(DEL)* cannot. Furthermore, recombinant strains that could not grow in the absence of isoleucine and valine were tested for their ability to grow on isoleucine alone.

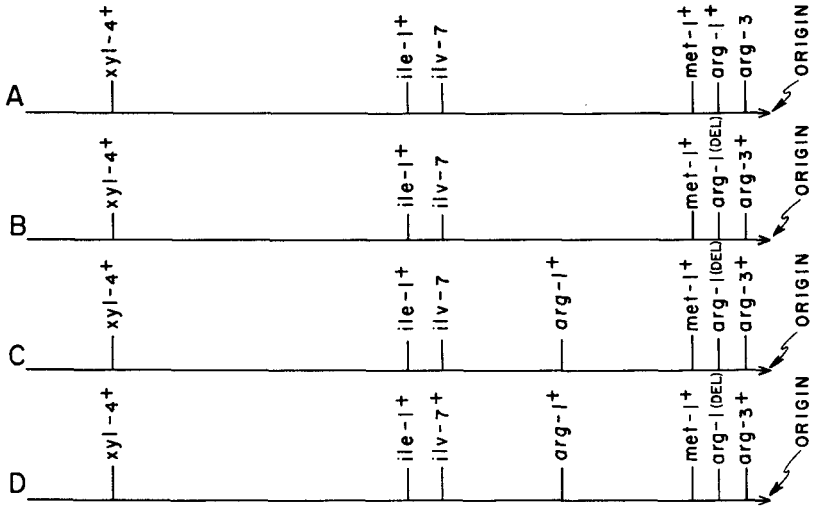

FIGURE 3.—A schematic representation of the genes that would be transferred to a female as a result of different primary crossing-over events occurring in the F′ male AB1516. (A) Crossing-over event in region I. (B) Crossing-over event in region II. (C) Crossing-over event in region III. (D) Crossing-over event in region IV. See Figure 2 for definition of the four regions.

Those recombinants which have retained the *ile-1* allele can grow on isoleucine alone, whereas recombinants inheriting the *ilv-7* allele are able to grow only in the presence of both isoleucine and valine.

The results of these experiments are shown in Figure 4. The frequency of donor crossovers in region I can be estimated from the recovery of the donor marker *arg-3* in the *xyl*$^+$ recombinants. Figure 4a shows this recovery to be less than 4 percent, in agreement with the estimated size of region I of less than 1 min. Similarly, an upper limit for the frequency of donor crossovers in region IV can be estimated from the recovery of the donor marker *ilv*$^+$ in the *xyl*$^+$ recombinants. Figure 4b shows the average recovery to be about 7 percent; however we will show later that one third of these recombinants owe their isoleucine-valine independence to the simultaneous transfer of an independent F_{14} element, so that the corrected figure for region IV is nearer to 5 percent. This figure is still only maximum since a genotype conferring isoleucine-valine independence can also arise by crossovers in the zygotes without previous donor crossovers in region IV.

Thus, the data of Figure 4a and 4b show that donor crossovers in regions I and IV occur collectively with a mean frequency of less than 10 percent as predicted from the hypothesis of random crossing over within the F_{14} region. Let us turn now to regions II and III which, together with region I, make up over 90 percent of F_{14}. Donor crossovers in these regions should account for over 90 percent of all *xyl*$^+$ recombinants, and will contribute the marker *ilv-7* to the zygotes. Integration of *ilv-7*, however, will only occur in approximately two thirds of the zygotes, as shown by the fact that one third of all *xyl*$^+$ recombinants retain the female markers *met-1* and *ile-1* (Figure 4c). We can therefore predict that about

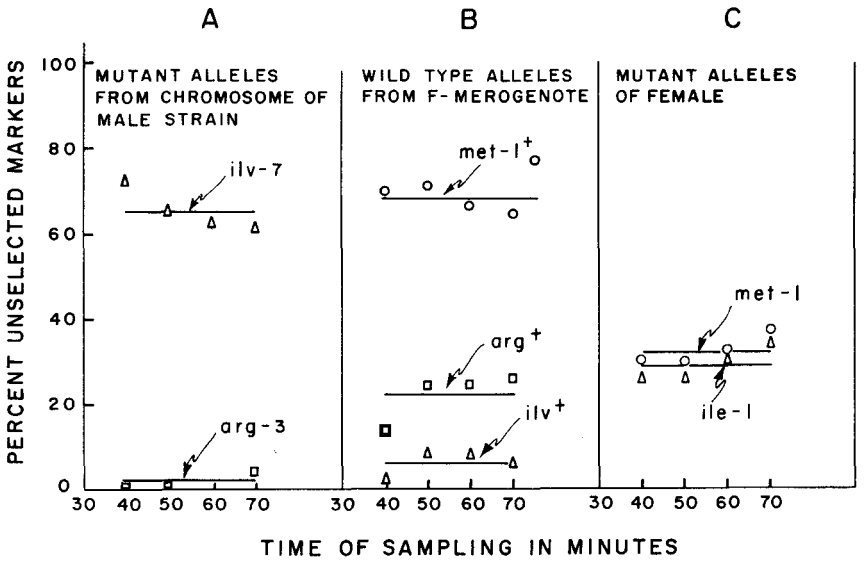

FIGURE 4.—The percent of *xyl*+ recombinants obtained at different times during mating which have (A) inherited the mutant alleles *ilv-7* and *arg-3* from the chromosome of male strain AB1516; (B) inherited the wild-type alleles *ilv*+ *arg*+ and *met*+ from the F-merogenote of the male strain; and (C) retained the mutant alleles *met-1* and *ile-1* of the female strain AB1255.

60 percent of all *xyl*+ recombinants will inherit the *ilv-7* marker from the F′ donor, and this is seen to be the case in Figure 4a.

We can also make a testable prediction about the frequency of crossing over in regions III and IV collectively. These regions total approximately 4 min, or about 45 percent of F_{14}. Donor crossovers in these regions will contribute the markers *arg-1*+ and *arg-3*+ to the zygotes; again correcting for the integration effect, we can predict that approximately two thirds of 45 percent, or 30 percent, of the *xyl*+ recombinants should be *arg-1*+ *arg-3*+. Figure 4b shows that the actual percentage averages 22 percent, in fair agreement with prediction.

Recombination events in the zygote: crossing over in terminal regions: When we examine recombination events occurring in the zygotes, we find that a disproportionately high frequency of crossing over occurs in the very small region immediately adjacent to the leading end or *Origin* of the transferred DNA. Zygotes receiving the *xyl*+ gene from the male all receive *met-1*+ as a proximal marker, since the male AB1516 is homozygous *met-1*+ on the chromosome and on F_{14}. In order to give rise to *xyl*+ recombinants, these zygotes must undergo at least two crossover events. One of these crossovers must occur on the side of *xyl* distal from *Origin*, and the other must occur somewhere between *xyl* and *Origin*. Let us consider in detail the occurrence of the latter crossover. If we assume an equal probability of crossing over per unit length for the region *xyl-met-Origin*, we should expect approximately 94 percent of the crossovers to occur between *xyl* and *met* (17 min) and approximately 6 percent to occur between *met* and *Origin* (1 min). As a consequence of this, the expected ratio of *xyl*+

met-1 to *xyl*$^+$ *met-1*$^+$ would be approximately 18 to 1. However, as can be seen from Figure 4, the observed ratio is 3 to 7: i.e., twice as many crossovers occur in the small region *met-Origin* as occur in the larger region *xyl-met*. This forces us to conclude that the general rule of equal probability of crossover per unit length cannot be applied to the small region immediately adjacent to the leading end of the transferred DNA. In the present case the probability of crossover per unit length is 36 times greater in this region than it is in those regions not immediately adjacent to an end. This conclusion is further supported by the observation that in a cross between Hfr strain AB2151 (*xyl*$^+$ *tpa-2*) and the F$^-$ strain AB1203 (*xyl-5 tpa*$^+$), 70 percent of the *xyl*$^+$ recombinants inherit the proximal male marker *tpa-2*. In this case, the distance between *Origin* and *tpa* is less than 1 min whereas the distance between *tpa* and *xyl* is approximately 8 min. Nevertheless, 70 percent of the crossovers occur in the small region between *tpa* and *Origin*. DE HAAN and GROSS (1962) report, similarly, that they find a high probability of crossing over at the distal end of a transferred fragment.

We have, however, already reported the failure to observe any similar regions of high-frequency crossing over in the case of recombination in the donor; i.e., donor crossover frequencies per unit length for the terminal regions: *arg-3-Origin* and *ile-1–sex-factor* are the same as for other regions. This apparent contradiction is most easily explained if we assume that, in the male donor, F_{14} exists as a closed ("circular") linkage group without ends, and that F_{14} pairs and recombines with the chromosome while in this form. This assumption is consistent with the model for episome structure proposed by CAMPBELL (1962).

Although in Figure 2 we have for simplicity depicted F_{14} as a linear structure paired with chromosome, we believe that it exists as a closed linkage group in the F′ male. The distribution of donor crossovers shows that F_{14} is able to pair with the homologous chromosomal region anywhere along the entire length of the F-merogenote, but it seems likely that at any given time only a very small region of F_{14} will be synapsed with its homologous chromosomal region.

The closed structure proposed for the F-merogenote would, according to this model, be converted to an open structure for transfer by the same mechanism that opens the circular chromosome of conjugating males (JACOB and BRENNER (1963); BOUCK and ADELBERG (1963).

Recombination events in the zygote: Crossing over in nonterminal regions: In considering this group of recombination events, we shall first consider those cases where the transferred gene arrangements have arisen as a result of (a) a donor crossover in region II and (b) a donor crossover in region III. The genes present in the zygotes in each case are shown in Figure 5 and Figure 6. Since, among the *xyl*$^+$ recombinants, the female *ile-1* allele is retained in approximately one third of the cases, we shall use this figure as a measure of the frequency of crossing over between *xyl* and *ilv*, *ile* (region A in Figure 6). Since the distances *xyl–ilv*, and *ilv–met* are approximately the same, we can expect a frequency of one third for recombination events occurring between *ilv* and *met*. This in turn leads to the prediction that the recombination frequency in either region B or region C of Figure 6 should be approximately one in six. On the basis of these figures, we

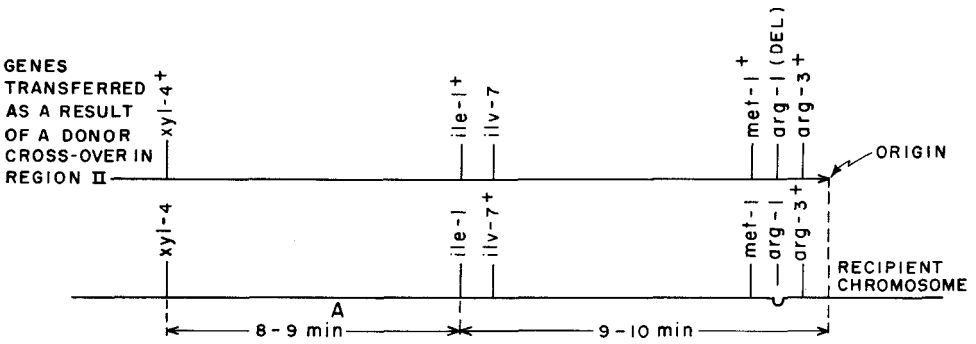

FIGURE 5.—Genetic constitution of zygotes formed when the transferred genetic material from the male has arisen as a result of primary crossing over in region II of Figure 2.

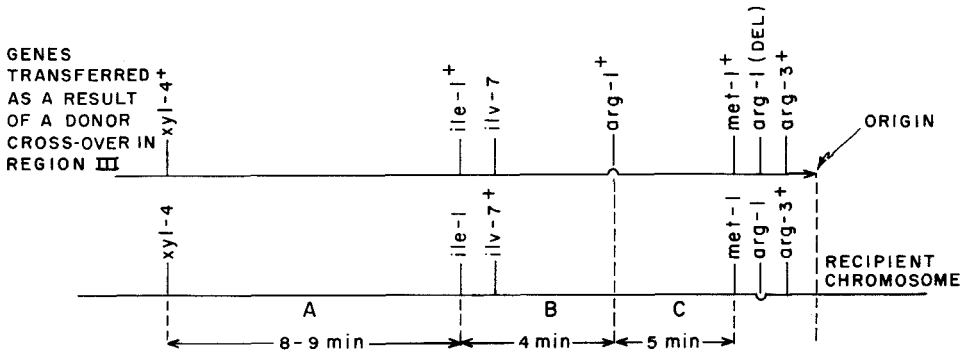

FIGURE 6.—Genetic constitution of zygotes formed when the transferred genetic material from the male has arisen as a result of primary crossing over in region III of Figure 2.

can predict the frequency of different genotypes among the *xyl*$^+$ recombinants as follows.

In the experiment summarized in Figure 4, 300 *xyl*$^+$ recombinants were checked for the inheritance of the *met*, *arg*, *ile*, and *ilv* markers. Assuming that 50 percent of these *xyl*$^+$ recombinants arose from zygotes represented in Figure 5 and that 40 percent arose from zygotes represented in Figure 6, we can make the following calculations. Fifty percent of 300 *xyl*$^+$ recombinants, (150), should have arisen from zygotes represented in Figure 5. In approximately one third of these 150 zygotes, (50), a crossover should occur between *xyl* and *ile* to give *xyl*$^+$ *ile-1* recombinants. In one third of these 50, (17), a second crossover should occur between *ile* and *met* to give *xyl*$^+$ *ile-1 met-1*$^+$ *arg-1(DEL)* recombinants. Of the 100 zygotes that did not undergo recombination events between *xyl* and *ile*, approximately one third, (33), should have crossed over between *ile* and *met* to give *xyl*$^+$ *ilv-7*, *arg-1*, *met-1* recombinants. Therefore, from the 150 *xyl*$^+$ recombinants arising from zygotes with the genotype expressed in Figure 5 the distribution of recombinant genotypes listed in column B of Table 2 should be found.

TABLE 2

A comparison of the frequencies with which different genotypes were predicted to be found in the xyl$^+$ *recombinants and the actual numbers found in the recombination experiment depicted in Figure 4*

Genotypes of *xyl*$^+$ recombinants	Numbers of each genotype expected to be formed from zygotes having received the gene arrangements depicted in Figure 3				Total	Numbers of each genotype found in the experiment reported in Figure 4
	A	B	C	D		
ile-1 arg-1 met-1	3	33	26	3	65	54
ile-1 arg-1 met-1+	..	17	7	1	25	22
ile-1 arg+ *met-1*+	..	..	7	1	8	7
ile-1 arg+ *met-1*	..	..	..	..	0	3
ile-1 arg-3 met-1+	2	..	..	..	2	1
ilv-7 arg+ *met-1*	..	..	14	..	14	0
ilv-7 arg-1 met-1	3	33	14	..	50	49
ilv-7 arg-1 met-1+	..	67	..	..	67	94
ilv-7 arg+ *met-1*+	..	..	52	..	52	54
ilv-7 arg-3 met-1+	7	..	..	..	7	2
ilv+ *arg-1 met-1*	..	..	..	2	2	5
ilv+ *arg*+ *met-1*	..	..	..	2	2	1
ilv+ *met-1*+	..	..	..	6	6	8*

* Fourteen *ilv*$^+$ *met-1*$^+$ recombinants were found, but six of these were shown to have been formed as a result of independent transfer of F_{14}. See text.

† The column headings (A,B,C,D) refer to the four gene arrangements depicted in Figure 3. A, results from a donor crossover in Region I; B, from a donor crossover in Region II; C, from a donor crossover in Region III; D, from a donor crossover in Region IV.

If we now consider the 120 *xyl*$^+$ recombinants which are predicted to have arisen from zygotes represented in Figure 6 we come to the following conclusions. In one third of these 120 zygotes, (40), a crossover should occur between *xyl* and *ile* to give *xyl*$^+$ *ile-1* recombinants; the remainder, (80), will have the genotype *xyl*$^+$ *ilv-7*. In one sixth of the 40 zygotes, (7), a second crossover should occur in region B of Figure 6 to give *xyl*$^+$ *ile-1 arg-1*$^+$ *met-1*$^+$ recombinants; in another one sixth of the zygotes the second crossover will occur in region C of Figure 6 to give *xyl*$^+$ *ile-1 arg-1(DEL) met-1*$^+$ recombinants. The remaining 26 zygotes should produce recombinants of the genotype *xyl*$^+$ *ile-1 arg-1 met-1*.

Returning now to the 80 zygotes in which no crossover should have occurred between *xyl* and *ile*, similar calculations lead to the predicted distribution of recombinant genotypes shown in column C of Table 2. Finally, if we assume that the gene sequences depicted in Figure 3a and 3d are each transferred to the female in 5 percent of the cases, we can similarly calculate the number of different genotypes arising from the resulting zygotes. These are shown in columns A and D of Table 2.

The observed numbers of recombinants having each predicted genotype are given in the last column of Table 2. It can be seen that, in general, there is close agreement between the numbers that were predicted and the numbers that were actually found. The major discrepancy involves the *ilv-7 arg-1*$^+$ *met-1* class. No

recombinants with this genotype were found although 14 had been predicted. There is no explanation for this at the moment.

The frequency of independent transfer of F-merogenote to zygotes which receive chromosomal markers from the same F′ male: When an F′ male is mated with a suitable recipient the number of zygotes which receive the F-merogenote alone generally outnumber those receiving chromosome by at least ten to one. Since mating conditions were such that triparental matings between two males and one female were excluded, we wished to see whether any of the zygotes which received chromosomal markers simultaneously received an entire copy of F_{14} from the male donor. In order to answer this question, direct selection was made for recombinants which had received both *xyl*$^+$ and *ilv-7*$^+$ *ile-1*$^+$. Eighty-five xylose-positive, isoleucine-valine-independent recombinants were tested by the replica plating method for the ability to transfer F_{14} to a suitable recipient; at the same time they were analysed to see if they had received *met* and *arg* as unselected markers. Twenty-seven of these behaved as high frequency donors of F_{14}. All of these 27 were, in addition, *met*$^+$ and *arg*$^+$. Ten of these males were purified and grown under conditions favouring the loss of the F-merogenote F_{14}. Five of these segregated unambiguous *xyl*$^+$ *arg-1 met-1* females. Of the five, three proved to have integrated into their chromosome the *ilv-7* marker of the donor chromosome, indicating that they had arisen from zygotes which had received an intact F_{14} element as well as a gene arrangement arising by a crossover between a second F_{14} and the donor chromosome. The other two had retained their *ile-1* allele under similar circumstances. In this experiment approximately 3 percent of the *xyl*$^+$ recombinants therefore had simultaneously received chromosomal markers including the F_{14} region and a second copy of the F-merogenote F_{14}.

In a previous report (PITTARD and ADELBERG 1963), chromosome transfer by F′ strain AB1528, which carries the F-merogenote F_{16}, was also described. The F-merogenote F_{16} is very small, and carries only the genes concerned with the biosynthesis of isoleucine and valine. F_{16} requires less than 2 min for transfer in comparison with 9 min for F_{14}. When strain AB1528, which is heterozygous *F-ilv*$^+$*/ilv-7*, transfers the chromosomal marker *xyl*$^+$ to a *xyl*$^-$ *ilv-16* recipient, approximately 90 percent of the recovered *xyl*$^+$ recombinants also inherit *ilv*$^+$. This figure is much higher than would be expected from the crossing-over model, since at least 30 percent of the *xyl*$^+$ recombinants would be expected to retain their initial *ilv-16* allele; furthermore, as a result of crossing-over events in the male, the *ilv-7* allele should have been transferred to zygotes at a measurable frequency.

In order to test whether this high recovery of *ilv*$^+$ in the *xyl*$^+$ recombinants was due to a very high independent transfer of F_{16}, the experiment was repeated using F′ male strain AB2148 which was heterozygous *F-ilv*$^+$*/ilv-12*. The *ilv-12* mutation carried by this male affects the gene controlling the structure of the transaminase enzyme involved in isoleucine-valine biosynthesis. This gene is situated very close to sex-factor on F_{16}, and the majority of donor crossovers occurring in AB2148 should therefore cause *ilv-12* to be transferred. Strains

carrying the *ilv-12* allele are unable to grow on α-keto-β-methylvalerate and valine, and therefore can be readily distinguished from strains carrying the *ilv-16* allele of the recipient, which strains can grow on these supplements. Male strain AB2148 and female AB1450 were mated for 60 min, after which time samples were withdrawn, subjected to mechanical agitation to separate mating couples and plated on minimal xylose medium containing isoleucine and valine. When the xyl^+ recombinants were checked for the inheritance of ilv^+, approximately 40 percent were found to be prototrophic for *ilv*. Forty of the xyl^+ ilv^+ recombinants were tested for the presence of F_{16}; 38 of them were found to be F′ males transferring F_{16} at high frequency. Ten of these were purified and then treated with acridine orange to remove F_{16}. All ten yielded female segregants upon treatment. Five of the segregant strains could grow on α-keto-β-methylvalerate and valine and thus has retained the original *ilv-16* mutation of the recipient. Four strains were unable to grow on α-keto-β-methylvalerate and valine but could grow on isoleucine and valine. These had integrated *ilv-12* from the chromosome of strain AB2148. The tenth strain could grow in the absence of isoleucine and valine and had integrated ilv^+ from the male donor. It appears from this experiment that the small F-merogenote F_{16} can be transferred to approximately 40 percent of those zygotes which are receiving chromosome as compared with the frequency of 3 percent found for the larger F_{14}. Consequently the high frequency at which xyl^+ recombinant also inherit ilv^+ from F_{16} male donors is due in the majority of cases to the independent transfer of F_{16} to the zygotes.

In the above experiment only a nutritional contraselection was employed against the male donor. Although the male to female ratio in the mating mixture of 1:20 should have prevented any matings in which two males conjugated with the same female, it was still possible that more than one mating event may have occurred while the recombinants were growing on the selective media. To rule out this possibility one further experiment was carried out. A xyl^+, T6-sensitive, F_{16}-carrying male, AB2144, was constructed that was homozygous *F-ilv-7/ilv-7*. This strain was mated with the xyl^- *ile-1* T6-resistant female strain AB1255; mating was interrupted at 60 min by mechanical agitation in the Vortex mixer, and the male was killed by treatment with bacteriophage T6. Recombinants for the xyl^+ marker were selected as in the previous experiment. In this experiment 80 percent of the xyl^+ recombinants were found to be ilv^+. Since the male donor was homozygous *ilv-7 F/ilv-7*, all gene arrangements transferred from the male must have carried the *ilv-7* allele.

In such a cross, xyl^+ ilv^+ recombinants could arise in either of two ways. If independent transfer of F_{16} occurred, the recombinants would be heterozygous *ilv-7 ile-1*$^+$ *F/ilv-7*$^+$ *ile-1;* since *ilv-7* and *ile-1* complement each other, the resulting phenotype would be ilv^+. If, on the other hand, there had been no independent transfer of F_{16}, ilv^+ recombinants could only have been formed as a result of recombination events between the transferred *ilv-7 ile-1*$^+$ and the recipient's *ilv-7*$^+$ *ile-1*. Since these two genes are very close together, we would expect such an event to occur at a frequency of 1 percent or less. However, 80 percent of the

xyl$^+$ recombinants are found to be *ilv*$^+$. When the *xyl*$^+$ *ilv*$^+$ recombinants were examined for their ability to transfer F_{16}, 154 out of a total of 160 acted as high-frequency donors of F_{16}. Eight of these were purified and shown to yield female strains carrying the *ile-1* allele after treatment with acridine orange.

DISCUSSION

As a result of these studies it is apparent that the transfer of chromosomal markers by F_{14}-carrying strains can be quantitatively accounted for in terms of the crossing-over model proposed by SCAIFE and GROSS (1963). CUZIN and JACOB (1963) have selected relatively stable Hfr strains from the F′ strain carrying *F-lac*. These Hfr's no longer transfer *F-lac* as an independent element; rather they possess two integrated copies of the *lac* region. One of these is transferred as a proximal marker and the other as a terminal marker linked to sex-factor. There seems no doubt that these stable Hfr strains have arisen as a result of a reciprocal crossing-over event between F-merogenote and chromosome. We do not, however, believe that chromosome transfer by F′ strains occurs only from stable Hfr cells in the F′ population. During conjugation approximately 5 percent of the F′ cells transfer chromosome as a result of donor crossovers, but no stable Hfr clones were found among 300 clones isolated from an F′ male carrying F_{14}. Furthermore, the attempted selection of such strains by treating F′ strain AB1516 with acridine orange and selecting for *ilv*$^+$ *arg*$^+$ males only produced strains which transferred either *xyl*$^+$ or *ilv*$^+$ at barely detectable frequencies. We assume that the crossover event which normally occurs in the F′ male is reversible unless (a) it occurs during conjugation, in which case the product of the crossover is transferred, or (b) the crossover event is followed by a second heritable change which stabilizes the chromosome containing the duplicated region, so that the frequency of the reverse reaction (restoring the autonomous F-merogenote) is greatly decreased. The strains isolated by CUZIN and JACOB would presumably have undergone this second reaction.

In a number of experiments, we have observed that F′ males carrying the small F-merogenote F_{16} transfer chromosomal markers with the same or with a greater frequency than strains carrying the larger F-merogenote F_{14}. Experimental results reported in this paper indicate that there exists a constant probability for donor crossovers to occur per unit length of F_{14}. If we assume the same probability per unit length for F_{16}, it is apparent that equal rates of chromosome transfer will only occur if the effective pairing region between F_{14} and chromosome at any given time does not exceed the total length of F_{16}. This is in agreement with the model for recombination in *E. coli* proposed by MACCACARO and HAYES (1961) which states that regions of effective pairing between endogenote and exogenote are very small, any one region being less than 1 percent of the *E. coli* chromosome.

SUMMARY

When an F′ male carrying the F-merogenote F_{14} transfers chromosomal markers to a recipient, it does so as a result of a recombination event between the

F-merogenote and chromosome. This recombination event, termed "donor crossover", can occur with equal probability per unit length anywhere along the F-merogenote. In the zygotes, the genes transferred as a result of this donor crossover also undergo recombination events with the recipient chromosome. Except for the region immediately adjacent to the leading end of the transferred material, crossing over in the zygote also occurs with equal probability per unit length. In the very small region immediately adjacent to the leading end, the recombination frequency is elevated about 40-fold. The failure to observe a similar elevated frequency in this region in the F′ male suggests that, in the male, the F-merogenote exists as a closed ("circular") structure. Under conditions in which triparental matings cannot occur, 3 percent of the F′ males transfer both chromosome (integrated with F_{14}) and a second entire copy of F_{14} to the recipient. When F′ strains carrying the smaller F-merogenote F_{16} are used as donors, this independent transfer of F-merogenote to zygotes receiving chromosomal markers can reach 80 percent. Under these circumstances, chromosome transfer itself still results from recombination between chromosome and another copy of the F-merogenote.

LITERATURE CITED

ADELBERG, E. A., and S. BURNS, 1960 Genetic variation in the sex factor of *Escherichia coli*. J. Bacteriol. **79**: 321–330.

BOUCK, N., and E. A. ADELBERG, 1963 The relationship between DNA synthesis and conjugation in *Escherichia coli*. Biochem. Biophys. Res. Commun. **11**: 24–27.

CAMPBELL, A., 1962 Episomes. Advan. Genet. **11**: 101–145.

CUZIN, F., and F. JACOB, 1963 Intégration réversible de l'épisome sexuel F′ chez *Escherichia coli* K12. Compt. Rend. **257**: 795–797.

DEHAAN, P. G., and J. D. GROSS, 1962 Transfer delay and chromosome withdrawal during conjugation in *Escherichia coli*. Genet. Res. **3**: 251–272.

JACOB, F., and S. BRENNER, 1963 Sur la régulation de la synthèse du DNA chez les bactéries: l'hypothèse du réplicon. Compt. Rend. **256**: 298–300.

MACCACARO, G. A., and W. HAYES, 1961 Pairing interaction as a basis for negative interference. Genet. Res. **2**: 406–413.

PITTARD, J., and E. A. ADELBERG, 1963 Gene transfer by F′ strains of *Escherichia coli* K-12. II. Interaction between F-merogenote and chromosome during transfer. J. Bacteriol. **85**: 1402–1408.

PITTARD, J., J. S. LOUTIT, and E. A. ADELBERG, 1963 Gene transfer by F′ strains of *Escherichia coli* K-12. I. Delay in initiation of chromosome transfer. J. Bacteriol. **85**: 1394–1401.

SCAIFE, J., and J. D. GROSS, 1963 Thé mechanism of chromosome mobilisation by an F-prime factor in *Escherichia coli* K-12. Genet. Res. **4**: 328–331.