PROBABILITY OF F INTEGRATION AND FREQUENCY OF STABLE Hfr DONORS IN F⁺ POPULATIONS OF *ESCHERICHIA COLI* K-12¹

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IN 1956, JACOB and WOLLMAN reported results obtained from fluctuation tests in which samples of individual F⁺ cultures of *Escherichia coli* K-12 that were descended from small inocula were separately mated with F⁻ bacteria. They observed a large variation in recombinant number from mating to mating and were able to isolate Hfr donors from those F⁺ cultures giving high recombinant yields. They, therefore, postulated that all recombinants arising in matings between F⁺ donors and F⁻ recipients were due to the presence of Hfr donors in the F⁺ population. However, data fluctuation tests of the sort conducted by JACOB and WOLLMAN (1956) cannot be used to obtain an accurate measure of the frequency of Hfr cells in populations of F⁺ cells or to determine the probability of F integration per bacterium per generation (CURTISS and RENSHAW 1969). We, therefore, decided to measure these parameters directly to partially test the hypothesis enunciated by JACOB and WOLLMAN (1956).

In 1962, CAMPBELL proposed that episomic elements were circular and were integrated into the circular bacterial chromosome by a single reciprocal crossover event. The CAMPBELL model for F integration is in accord with the recent finding that F is circular (FREIFELDER 1968) and with genetic data which suggest that F is linearly inserted into the Hfr chromosome (CURTISS 1964a; PITTARD 1965).

Two specific aspects of the above proposed model for F integration will affect our ability to accurately measure the parameters associated with F integration. First, the union between F and the chromosome could be either stable or unstable. Unstable unions would produce Hfr donors of the moment which could probably contribute to the total recombinant yield of F⁺ populations. However, these temporary Hfr cells would not give rise to a clone of descendants, which is a necessary requirement for any method designed to measure the frequency of Hfr cells and rates of F integration in F⁺ populations. Second, integration of F into the chromosome could be either lethal or nonlethal, depending on whether or not integration of F inactivated genes coding for vital functions. We have, therefore, been able to measure only the parameters associated with F integrations which are stable and nonlethal. A preliminary accounting of our results has been published (CURTISS and STALLIONS 1968).

MATERIALS AND METHODS

Bacteria: The strains of *E. coli* K-12 used in this study are listed in Table 1. The Hfr derivatives of the Type I F⁺ strain χ 209, which were isolated during this study, are listed in other

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TABLE 1
Bacterial strains

Strain number	Mating type	Relevant genotype*	Derivation
$\chi 99$	F ⁻	<i>thr⁻ ara⁻ leu⁻ lacY⁻ T6^s gal⁻ str^r xyl⁻ mtl⁻ thi⁻</i>	$\chi 12\dagger$
$\chi 137$	F ⁻	<i>thr⁻ ara⁻ leu⁻ proA-B⁻ lacY⁻ T6^r str^r thi⁻</i>	$\chi 134\dagger$
$\chi 204$	F ⁻	<i>ara⁻ leu⁻ lacY⁻ T6^r purE⁻ gal⁻ trp⁻ aroD⁻ str^r xyl⁻ mtl⁻ thi⁻</i>	$\chi 148\dagger$
$\chi 209$	F ⁺	prototroph T6 ^s str ^s	$\chi 42\dagger, \$$
$\chi 278$	F ⁻	<i>thr⁻ leu⁻ proB⁻ lacY⁻ T6^r str^r thi⁻</i>	C600 ⁺ ,
$\chi 462$	F ⁻	<i>ara⁻ leu⁻ proA⁻ lacZ⁻ T6^r purE⁻ trp⁻ lys⁻ str^r xyl⁻ mtl⁻ metE⁻ thi⁻</i>	$\chi 454\ \$
$\chi 474$	F ⁻	<i>ara⁻ leu⁻ proB⁻ lacZ⁻ T6^r purE⁻ trp⁻ lys⁻ str^r xyl⁻ mtl⁻ metE⁻ thi⁻</i>	$\chi 454\ \$
$\chi 478$	F ⁻	<i>ara⁻ leu⁻ lacZ⁻ proC⁻ T6^r purE⁻ trp⁻ lys⁻ str^r xyl⁻ mtl⁻ metE⁻ thi⁻</i>	$\chi 454\ \$
$\chi 680$	F ⁻	<i>thr⁻ leu⁻ proA⁻ lacY⁻ T6^r str^r thi⁻</i>	C600 ⁺ ,
$\chi 760$	F ⁻	<i>ara⁻ leu⁻ lacY⁻ proC⁻ T6^r purE⁻ gal⁻ trp⁻ his⁻ argG⁻ str^r xyl⁻ mtl⁻ ile⁻ met⁻ thi⁻</i>	$\chi 697\ \$
$\chi 775$	F ⁻	<i>T6^r purE⁻ pyr⁻ trp⁻ his⁻ str^r xyl⁻</i>	$\chi 540\ \$
$\chi 821$	F ⁻	<i>thr⁻ proA⁻ T6^r purE⁻ pyr⁻ his⁻ str^r xyl⁻</i>	$\chi 723\ \$

* The nomenclature used conforms with the proposals of DEMEREC *et al.* (1966) except for the modifications noted by CURTISS (1968). The markers are listed in the order in which they occur on the bacterial chromosome. All strains are nonlysogenic for λ and can ferment and utilize arabinose, lactose, galactose, maltose, xylose, and mannitol unless otherwise indicated. Certain mutations conferring resistance to phages, cycloserine, and azide have not been listed.

† CURTISS (1965).

‡ CURTISS (1964b).

§ CURTISS and RENSHAW (1969).

|| CURTISS *et al.* (1968).

¶ BERG and CURTISS (1967).

tables and figures. $\chi 209$ is a prototrophic, nonlysogenic derivative of WOLLMAN's (1953) strain K-12-112, which we received from N. SCHWARTZ in 1960 as a histidine-requiring, λ lysogen ($\chi 42$, see CURTISS and RENSHAW 1969). (The cysteine requirement of K-12-112 reported by WOLLMAN (1953) had been lost prior to our receipt of this strain.)

Media: The liquid (ML) and solid (MA) minimal media were described by CURTISS (1965) and supplemented with growth requirements, energy sources, and antibiotics at levels given by CURTISS, CHARAMELLA, STALLIONS, and MAYS (1968). Difco Penassay broth and agar and L broth (LENNOX 1955) were used as complex media.

Replica plating: New velveteen, cut into 6 $\times$ 6 inch squares, was purchased for replica plating (LEDERBERG and LEDERBERG 1952). An aluminum alloy core block with a brass O ring, to hold the sterile velveteen squares, was used. The entire assembly weighs 600 g, a sufficient weight to allow replica plating from upturned Petri plates with no requirement for any external pressure. When done in this way, the results of replica plating are very uniform.

Mating procedures: A detailed description of the mating methods has been given (CURTISS *et al.* 1968). All liquid matings performed in this study employed cells grown and mated in L broth. The donor:recipient cell ratios were 1:2 in F⁺ $\times$ F⁻ matings and 1:10 in Hfr $\times$ F⁻ matings. Interruptions of mating were accomplished with UV-irradiated T6 and agitation on a vortex mixer. Four to six plates, selective for each class of recombinant, were used to obtain significant numbers of recombinants in F⁺ $\times$ F⁻ matings.

Characterization of isolated Hfr strains: After purification and reisolation, all Hfr strains were tested for stability of F integration; for mutation to auxotrophy; for ability to ferment glucose, arabinose, lactose, galactose, xylose, mannitol, and maltose; for changes in sensitivity to the seven T phages, P1 κ c, and λ ; and for changes in sensitivity to streptomycin. Only one strain, Hfr OR77, had a genotypic change that was probably associated with F integration (see later).

A presumptive origin, direction, and gradient of marker transfer was established for each Hfr

by using the cross-streak method (BERG and CURTISS 1967) and a variety of multiply mutant F^- strains (Table 1). This was confirmed by performing a 40- or 60 min mating with an appropriate F^- to establish a more accurate gradient of marker transfer. Recombinants for the presumed first and last markers were tested for inheritance of donor ability by streaking cultures of recombinants across a donor specific phage on EMB agar containing 0.1% glucose. In certain instances, the assignment of a definite F integration location required doing interrupted matings, recombinant analyses for unselected markers, and/or comparative matings between F^- strains and Hfr donors with known origins and directions of chromosome transfer.

RESULTS

Experimental design for determining the frequency of stable Hfr cells and the probability of F integration in populations of F^+ cells: A culture of the Type I F^+ strain $\chi 209$ growing in Penassay broth was diluted to obtain about 100 colonies per Penassay agar plate. These plates were spread with the diluted culture and then incubated for 16 hrs at 37°C. Care was taken not to distribute cells within 1 cm of the edge of the Petri plates. These $\chi 209$ colonies were replica plated onto minimal agar plates, selective for $thr^+ leu^+ str^r$ recombinants, which had been prespread with 0.05 ml of a culture of the F^- strain $\chi 99$. The $\chi 99$ culture was at a cell density of about 5×10^8 /ml and was growing in Penassay broth. The minimal agar plates were prespread with the F^- culture within 5 min of the replica plating and were placed at 37°C within 5 min after replica plating. After two days' incubation, the minimal agar plates were examined and matched with the appropriate Penassay agar plates. All $\chi 209$ colonies which gave rise to five or more $thr^+ leu^+ str^r$ recombinants on the minimal agar plates were carefully resuspended in 2 ml of Penassay broth. These suspensions were then appropriately diluted to obtain about 100 colonies on each of 4 to 8 Penassay agar plates. After 16 hours incubation, these plates were replica plated to minimal agar prespread with $\chi 99$ as described above. After two days the minimal agar plates were matched with the Penassay agar plates and the frequency of Hfr cells in the original $\chi 209$ colony was calculated. (A colony of Hfr cells gave a confluent patch of recombinant growth on the minimal agar plate.)

The above procedure was repeated three times using separate cultures of $\chi 209$ which, however, were inoculated from the same slant. This method has allowed an unbiased isolation of stable Hfr donors having any origin and direction of chromosome transfer, provided that at least 5% of the cells in the $\chi 209$ colony were Hfr donors.

Frequency of stable Hfr cells in populations of F^+ cells: Table 2 presents the data obtained from the three $\chi 209$ cultures analyzed. Of the 79 $\chi 209$ colonies tested for the presence of stable Hfr cells, there were 18 which contained pure clones of Hfr cells and 16 which contained mixed clones. Eleven of the 18 pure clones contained Hfr donors of the same type (line 5, Table 2). This suggests that this Hfr type was present in the $\chi 209$ slant used to inoculate the three cultures, although Hfr donors of this same type were also found in 4 of the 16 mixed clones (see later). The frequencies of pure Hfr clones for the three $\chi 209$ cultures analyzed were similar (line 4, Table 2). It should be noted that six years had elapsed since the last single colony reisolation of the $\chi 209$ strain used in these

TABLE 2
Frequency of $\chi 209$ clones containing detectable numbers of Hfr cells*

	$\chi 209$ culture A	$\chi 209$ culture B	$\chi 209$ culture C	All cultures
1. Total number of $\chi 209$ colonies replica plated	22,060	28,460	57,010	107,530
2. Number of $\chi 209$ colonies analyzed for presence of Hfr cells†	22	41	16	79
3. Number of $\chi 209$ colonies containing 95 to 100% Hfr cells‡	3	5	10	18
4. Frequency of pure Hfr clones	1.4×10^{-4}	1.8×10^{-4}	1.8×10^{-4}	1.7×10^{-4}
5. Number of pure Hfr clones with given chromosome-transfer gradient	3 O-argG xyl...lys F 1 O-argG xyl...lys F 1 O-lys his...argG F 1 O-purE T6...gal F 1 O-proB proA...lac F 1 O-his aroD...lys F	1 O-argG xyl...lys F 1 O-lys his...argG F 1 O-purE T6...gal F 1 O-proB proA...lac F 1 O-his aroD...lys F	7 O-argG xyl...lys F 2 O-lys his...argG F 1 O-purE T6...gal F	
6. Number of $\chi 209$ colonies containing Hfr and F+ cells	6	6	4	16
7. Frequency of impure Hfr clones	2.7×10^{-4}	2.1×10^{-4}	7.0×10^{-5}	1.5×10^{-4}

* See text for description of methods used.

† The mean number of colonies (cells) tested from each original $\chi 209$ colony to be analyzed for the presence of Hfr cells was 490 (range of 237 to 1,212).

‡ While most of these clones contained 100% Hfr cells, some contained several percent F+ cells. We assumed that these infrequent F+ cells were due either to reversions of Hfr cells to F+ cells and/or to contaminating F+ cells picked up from colonies adjacent to the resuspended colony.

TABLE 3

*Generation times and stabilities of F integration for Hfr strains isolated**

Strain number	Hfr number	Origin, direction and gradient of chromosome transfer	Generation time† (min)	Stability of F integration			
				% Hfr	Gener-ations‡	% Hfr	Gener-ations‡
χ209	F+		17.1 ± 0.4	..	..	..	..
χ884	OR64	O- <i>thr ara leu proA</i> ... <i>met</i> F	17.3	3§	>80	98	~45
χ896	OR76	O- <i>thr ara leu proA</i> ... <i>met</i> F	15.9	96	~65	100	~45
χ876	OR56	O- <i>proC T6 purE gal</i> ... <i>lac</i> F	17.6	98	~65	100	~45
χ874	OR54	O- <i>purE gal pyr trp</i> ... <i>T6</i> F	18.8	20§	~65	76§	~45
χ869	OR49	O- <i>argG xyl ile metE</i> ... <i>lys</i> F	18.1	100	>80	...	..
χ870	OR50	O- <i>argG xyl ile metE</i> ... <i>lys</i> F	18.9	100	>80	...	..
χ437	OR8	O- <i>ile metE met thr</i> ... <i>xyl</i> F	19.4	23§	~65	86§	~45
χ875	OR55	O- <i>proB proA leu ara</i> ... <i>lac</i> F	17.8	83	>80	100	~45
χ886	OR66	O- <i>proB proA leu ara</i> ... <i>lac</i> F	16.5	100	>80	...	..
χ892	OR72	O- <i>proB proA leu ara</i> ... <i>lac</i> F	16.9	100	>80	...	..
χ436	OR7	O- <i>T6 proC lac proB</i> ... <i>purE</i> F	18.8	2§	~65	97	~45
χ879	OR59	O- <i>purE T6 proC lac</i> ... <i>gal</i> F	18.3	100	>80	...	..
χ894	OR74	O- <i>purE T6 proC lac</i> ... <i>gal</i> F	16.3	100	>80	...	..
χ901	OR81	O- <i>trp pyr gal purE</i> ... <i>aroD</i> F	16.8	98	~65	3§	~45
χ877	OR57	O- <i>his aroD trp pyr</i> ... <i>lys</i> F	16.8	100	~65	...	..
χ895	OR75	O- <i>his aroD trp pyr</i> ... <i>lys</i> F	17.3	83	>80	100	~45
χ897¶	OR77	O- <i>his aroD trp pyr</i> ... <i>lys</i> F	20.1	0	~65	98	~45
χ898	OR78	O- <i>lys his aroD trp</i> ... <i>argG</i> F	14.6	100	>80	...	..

* The strains were grown in Penassay broth with aeration at 37°C. Plating on Penassay agar to determine viable titers was done at 30 min intervals.

† The generation time for χ209 is an average of four determinations. All other values represent single determinations for populations containing the maximum percentage of Hfr cells attainable.

‡ The approximate number of generation cycles intervening between plating a single cell and testing the population for the frequency of Hfr cells was calculated by determining the mean number of cells per colony on Penassay agar, the mean number of cells obtained by picking colonies with a sterile needle, the mean number of generation cycles of growth occurring during transfers from one slant to another, and the mean number of generation cycles of growth occurring during all liquid culturing manipulations. The Hfr strains that had grown for more than 80 generations had not been reisolated since they were isolated and placed on slants in November and December 1967.

§ The majority of non-Hfr donors obtained from χ884, χ874, χ437, χ436, and χ901 were F2 donors (ADELBERG and BURNS 1960) giving elevated frequencies of recombinants for proximal markers. Most of these recombinants became donors. All other non-Hfr segregants from these five Hfr strains and from the other strains tested behaved like F+ donors.

|| In the first experiment on the stability of F integration with χ901, 98% of the isolates tested behaved like fair to good Hfr donors with regard to the frequency of recombinants, but about 80% of the recombinants tested became donors. A very high frequency Hfr donor was thus reisolated from the χ901 stock that had been frozen since isolation. This isolate was used in the second experiment, but only 3% of the colonies tested after growth of this isolate were good Hfr donors whose recombinants remained F-. The remaining 97% of the colonies were high frequency F2 donors whose recombinants became donors.

¶ χ897 has a requirement for diaminopimelic acid and homoserine. Thus, this strain grows very slowly in Penassay broth unless the medium is supplemented with these two amino acids. In the first experiment with χ897, the culture was grown in unsupplemented Penassay broth after having been grown and subcultured on unsupplemented Penassay agar slants. All 100 isolates tested were F+ donors and were prototrophic. The generation time of 20.1 min is for a culture growing in Penassay broth supplemented with LL+ meso α,ε- diaminopimelic acid (20μg/ml) and L-homoserine (20μg/ml). The inoculum for this culture was obtained from our χ897 stock which had been frozen since isolation. In extensive tests with χ897, all F+ segregants were prototrophs and all prototrophic revertants isolated and tested were F+.

studies. Thus the mean frequency of stable Hfr cells present in the $\chi 209$ population (1.7×10^{-4}) should represent a close approximation to the equilibrium frequency which is a function of the probabilities of F integration in F^+ cells and of F excision in Hfr cells and of the relative growth rates of F^+ and Hfr cells.

Generation times and stabilities of F integration for Hfr strains: Table 3 presents the generation times for 18 Hfr strains and for the F^+ strain $\chi 209$ growing in Penassay broth with aeration at 37°C . Under these conditions most of the Hfr strains grew at rates that were similar to the rate of the parental F^+ strain. The generation time of $\chi 897$ could not be determined under these growth conditions, since this Hfr strain was found to have an obligate requirement for diaminopimelic acid and L-homoserine. Based on results with this Hfr (see Table 3 footnote), we conclude that F integration has inactivated a gene to cause the requirement for diaminopimelic acid and homoserine.

The data on stabilities of F integration for the Hfr strains tested (Table 3) reveal that independently isolated Hfr donors having the same gradient of marker transfer have similar stabilities of F integration; an observation previously noted by BRODA (1967). Hfr OR54, Hfr OR8, and Hfr OR81 were very unstable; in fact, we never obtained a culture of Hfr OR81 that contained more than a few percent Hfr cells.

FIGURE 1.—Percentage of Hfr cells in clones containing both F^+ and Hfr cells and the chromosome-transfer gradients of the Hfr donors in each clone (see text).

TABLE 4

*Probability of stable F integration per bacterium per generation**

	Number and designation of clones	Generation in which Hfr cell arose	Total number of cells present when Hfr cell arose†
~50% Hfr cells	1 (A-21)	1	215,060
~25% Hfr cells	3 (B-10, C-7, C-5)	2	430,120
~12.5% Hfr cells	3 (B-23, B-21, A-17)	3	860,240
Total	7		1,515,420

Calculation:

Probability of stable F integration per bacterium per generation

$$\frac{7}{1.5 \times 10^6} \times \log_e 2 = 3 \times 10^{-6}$$

* The data analyzed are presented in Figure 1 and data on the generation times and stabilities of F integration for the Hfr strains used for this calculation are presented in Table 3.

† Based on number of cells plated in 0 generation (Table 2).

Probability of stable F integration per bacterium per generation: The frequency of mixed clones containing various percentages of stable Hfr cells can be used to estimate the probability of stable F integration per bacterium per generation. Figure 1 presents the data used for the calculation given in Table 4. The accuracy of this calculation depends on the precision of the resuspension of the original $\times 209$ colonies, on the relative growth rates of Hfr and F^+ cells within the colony, and on the stabilities of F integration in the Hfr cells. The mixed clones used for this calculation contained five Hfr donors (OR66, OR64, OR56, OR72, and OR81) having generation times similar to the generation time of the F^+ strain $\times 209$ and two Hfr strains (OR54 and OR77) growing more slowly than the F^+ parent (Table 3). Four of the seven Hfr donors (OR66, OR64, OR56, and OR72) are stable with regard to F integration, whereas the remaining three (OR54, OR77, and OR81) revert to F^+ and/or F_2 donor-types at a measurable rate (Table 3). It is apparent from the data in Table 3 that it is quite possible that the cell giving rise to the mixed clone containing Hfr OR54 was an Hfr OR54 cell and that during the 30 generations of growth required to form a colony, a sufficient number of more rapidly growing F^+ segregants arose to account for the finding that only 43% of the cells in the final colony were Hfr cells (Figure 1). If the clone containing Hfr OR54 is considered to represent a pure clone of Hfr cells, then the calculated frequency of Hfr cells in the F^+ population (Table 2) would have to be increased, whereas the calculated probability of stable F integration per bacterium per generation (Table 4) when recomputed and rounded off would not change. All other possible reassignments of the generation in which the first Hfr cell arose by correcting for growth rates and stabilities of F integration would not alter the calculated probability of stable F integration per bacterium per generation. We, therefore, believe that the three types of errors associated with the calculation given in Table 4 would tend to be insignificant and random.

A more important source of error concerns the ability of the method to detect,

in mixed clones, all stable Hfr donors, especially those which transfer the *thr*⁺ *leu*⁺ alleles as distal markers. The *thr*⁺ and *leu*⁺ markers are transferred in the first quarter of the donor chromosome by 7 Hfr strains, in the second quarter by 3 Hfr strains, in the third quarter by 4 Hfr strains, and in the distal quarter by 2 Hfr strains (Figure 1). Such a bias is not evident, however, if one ignores those mixed clones which contain less than 5% Hfr cells (Figure 1). Therefore, the calculation in Table 4 is based on an analysis of mixed clones that contain more than 5% Hfr cells. This computation reveals that the probability of stable F integration per bacterium per generation is approximately 3×10^{-6} .

Frequency of recombinants in F⁺ × F⁻ matings which are accounted for by stable Hfr cells in the F⁺ population: To determine the frequency of recombinants, issuing from F⁺ × F⁻ matings, that are accounted for by stable Hfr cells in the F⁺ population, we performed reconstruction experiments in which an equal mixture of the 12 Hfr types isolated from χ 209 (Table 5) was mated with a poly-auxotrophic F⁻ strain. Two of these Hfr strains were isolated in fluctuation tests performed by CURTISS and RENSHAW (1969) and ten were isolated in the present series of experiments. The data from two matings of this type, and from matings between the F⁺ strain χ 209 and the same F⁻ strain, are presented in Table 6. These data, along with the value for the frequency of stable Hfr cells in populations of χ 209 (Table 2), have been used to compute that 15% of the recombinants in matings between χ 209 and an F⁻ strain are due to the presence of stable Hfr donors in the χ 209 population (Table 7). If we allow a contribution from

TABLE 5

*Types of chromosome-transfer gradients obtained for Hfr derivatives of the E. coli K-12 subline K-12-112**

Strain number	Hfr number	Origin, direction, and gradient of chromosome transfer	Number isolated
Clockwise direction of transfer			
χ 896	OR76	O- <i>thr ara leu proA</i> ... <i>met</i> F	5
χ 876	OR56	O- <i>proC T6 purE gal</i> ... <i>lac</i> F	1
χ 874	OR54	O- <i>purE gal pyr trp</i> ... <i>T6</i> F	1
χ 869	OR49	O- <i>argG xyl ile metE</i> ... <i>lys</i> F	15
χ 437	OR8	O- <i>ile metE met thr</i> ... <i>xyl</i> F	1
Counterclock direction of transfer			
χ 875	OR55	O- <i>proB proA leu ara</i> ... <i>lac</i> F	5
χ 436	OR7	O- <i>T6 proC lac proB</i> ... <i>purE</i> F	1
χ 879	OR59	O- <i>purE T6 proC lac</i> ... <i>gal</i> F	5
χ 901	OR81	O- <i>trp pyr gal purE</i> ... <i>aroD</i> F	1
χ 877	OR57	O- <i>his aroD trp pyr</i> ... <i>lys</i> F	2
χ 897†	OR77	O- <i>his aroD trp pyr</i> ... <i>lys</i> F	1
χ 898	OR78	O- <i>lys his aroD trp</i> ... <i>argG</i> F	3
Total			41

* Thirty-four of the Hfr strains were isolated during the investigations reported in this manuscript and seven were isolated from fluctuation tests conducted by CURTISS and RENSHAW (1969).

† This Hfr requires diaminopimelic acid and homoserine for growth.

TABLE 6
*Recombinant frequencies obtained in matings with an F⁺ strain and with a mixture of twelve Hfr strains isolated from it**

Recombinant class selected	Recombinant frequencies in experiment 1 for:						Recombinant frequencies in experiment 2 for:					
	F ⁺ donor at:			12 Hfr donors at:			F ⁺ donor at:			12 Hfr donors at:		
	40 min	60 min		40 min	60 min		40 min	60 min		40 min	60 min	
<i>Leu</i> ⁺ <i>str</i> ^r	9.8 × 10 ⁻⁶	3.1 × 10 ⁻⁵		6.8 × 10 ⁻³	2.7 × 10 ⁻²		2.5 × 10 ⁻⁵	4.9 × 10 ⁻⁵		1.7 × 10 ⁻²	3.3 × 10 ⁻²	
<i>proC</i> ⁺ <i>str</i> ^r	1.1 × 10 ⁻⁵	2.5 × 10 ⁻⁵		1.0 × 10 ⁻²	2.3 × 10 ⁻²		2.8 × 10 ⁻⁵	3.9 × 10 ⁻⁵		2.3 × 10 ⁻²	3.1 × 10 ⁻²	
<i>purE</i> ⁺ <i>str</i> ^r	1.9 × 10 ⁻⁵	3.8 × 10 ⁻⁵		1.9 × 10 ⁻²	3.4 × 10 ⁻²		3.7 × 10 ⁻⁵	5.7 × 10 ⁻⁵		2.0 × 10 ⁻²	4.5 × 10 ⁻²	
<i>trp</i> ⁺ <i>str</i> ^r							1.7 × 10 ⁻⁵	3.7 × 10 ⁻⁵		3.7 × 10 ⁻³	1.0 × 10 ⁻²	
<i>his</i> ⁺ <i>str</i> ^r	5.6 × 10 ⁻⁶	1.9 × 10 ⁻⁵		1.4 × 10 ⁻²	3.0 × 10 ⁻²		1.4 × 10 ⁻⁵	2.5 × 10 ⁻⁵		1.9 × 10 ⁻²	4.1 × 10 ⁻²	
<i>argG</i> ⁺ <i>str</i> ^r	9.2 × 10 ⁻⁶	1.7 × 10 ⁻⁵		1.1 × 10 ⁻²	2.2 × 10 ⁻²		5.6 × 10 ⁻⁶	6.3 × 10 ⁻⁶		8.2 × 10 ⁻³	1.1 × 10 ⁻²	
<i>ile</i> ⁺ <i>str</i> ^r	3.4 × 10 ⁻⁶	1.1 × 10 ⁻⁵		1.2 × 10 ⁻⁴	2.1 × 10 ⁻³		4.5 × 10 ⁻⁶	7.3 × 10 ⁻⁶		1.2 × 10 ⁻³	4.1 × 10 ⁻³	
<i>met</i> ⁺ <i>str</i> ^r	2.3 × 10 ⁻⁶	1.3 × 10 ⁻⁵		1.1 × 10 ⁻⁴	2.2 × 10 ⁻³		3.2 × 10 ⁻⁶	1.1 × 10 ⁻⁵		6.8 × 10 ⁻⁴	2.8 × 10 ⁻³	
Average	8.6 × 10 ⁻⁶	2.2 × 10 ⁻⁵		8.8 × 10 ⁻³	2.0 × 10 ⁻²		1.7 × 10 ⁻⁵	2.9 × 10 ⁻⁵		1.4 × 10 ⁻²	2.2 × 10 ⁻²	

* Bacteria were grown and mated in L broth as indicated in the MATERIALS AND METHODS. χ^{760} was used as the F⁻ recipient. An equal mixture of the twelve Hfr cultures was made prior to inoculation of the nonaerated Hfr culture. Prior to both experiments, the Hfr strains were checked for purity of their Hfr behavior and reisolates were used if necessary.

TABLE 7

Calculation of the frequency of recombinants, from $F^+ \times F^-$ matings, due to stable Hfr donors in the F^+ population

	Experiment 1		Experiment 2	
	40 min	60 min	40 min	60 min
1. Frequency of stable Hfr cells in F^+ population*	1.7×10^{-4}	1.7×10^{-4}	1.7×10^{-4}	1.7×10^{-4}
2. Mean recombinant frequency per Hfr cell†	8.8×10^{-3}	2.0×10^{-2}	1.4×10^{-2}	2.2×10^{-2}
3. Recombinant frequency due to stable Hfr cells in F^+ population (1×2)	1.5×10^{-6}	3.4×10^{-6}	2.4×10^{-6}	3.7×10^{-6}
4. Mean recombinant frequency per cell in F^+ population†	8.6×10^{-6}	2.2×10^{-5}	1.7×10^{-5}	2.9×10^{-5}
5. Frequency of recombinants, from $F^+ \times F^-$ mating, due to stable Hfr donors in F^+ population ($3 \div 4$)	1.7×10^{-1}	1.5×10^{-1}	1.4×10^{-1}	1.3×10^{-1}

* From Table 2.

† From Table 6.

those cells in which F has just integrated into the chromosome, this value of 15% would have to be increased by only another 0.25%. This last value was calculated by multiplying the probability of F integration per bacterium per generation (3×10^{-6} ; Table 4) by the mean recombinant frequency per Hfr cell (line 2, Table 7) and dividing this product by the mean recombinant frequency per cell in the F^+ population (line 4, Table 7).

DISCUSSION

From the experimental data reported in this communication, we calculated that the equilibrium frequency of stable Hfr cells in Type I F^+ populations was 1.7×10^{-4} , that the probability of stable F integration per bacterium per generation was 3×10^{-6} , and that 15 to 16% of the recombinants arising in $F^+ \times F^-$ matings were due to stable Hfr donors in the F^+ population. Since these values were calculated for one F^+ strain growing in rich medium, they may not be representative of values which would be obtained with other F^+ strains and/or for different environmental conditions. However, CURTISS and RENSHAW (1969) were unable to notice significant differences in results from fluctuation tests designed to demonstrate indirectly the rates of F integration for Type I F^+ derivatives of the K-12-112, W1485, and 58-161 sublines grown under a variety of environmental conditions. We, therefore, believe that the values calculated from the data presented in this report are reasonable approximations of the values which would be obtained in more extensive studies with other F^+ strains and/or growth conditions.

BRODA (1967) has also shown, by using a different method, that only about 20% of the recombinants issuing from $F^+ \times F^-$ matings are due to stable Hfr donors in the F^+ population. It is, therefore, evident that 80% of the recombinants

ants formed in $F^+ \times F^-$ matings must be due to lethal and/or unstable integrations of F into the chromosome or to some means of chromosome transfer which does not require F integration.

Of the 131 Hfr strains known to us, 82 of which were isolated in our laboratory, 4 have mutations in genes coding for reparable or nonessential functions which resulted as a consequence of F integration. Hfr 4 (RICHTER 1961) and Hfr P10(J4) (SCHWARTZ 1967) have lesions in the *malB* locus, Hfr OR1 (CURTISS 1964a) has a lesion in the *lac* operon, and Hfr OR77 (this report) has a lesion in a gene involved in the biosynthesis of diaminopimelic acid and homoserine. It therefore seems unlikely that 80 to 85% of all F integrations would inactivate genes coding for vital functions. In addition, it might be expected that regions of homology between F and genes coding for vital functions would have been selected against during the course of bacterial evolution. We, therefore, do not believe that integrations of F, which result in lethality due to inactivation of genes coding for vital functions, account for an appreciable number of recombinants formed in $F^+ \times F^-$ matings.

It is possible, however, that integration of F into the chromosome does not always occur according to the model proposed by CAMPBELL (1962). If the reciprocal crossover event were incomplete, then it is possible that insertion of F into the chromosome might result in the formation of a linear, nonviable chromosome. Such a possibility has been proposed as an explanation for the inability to recover stable Hfr donors from Type II F^+ strains (CURTISS and RENSHAW 1969).

The data presented in this communication are, therefore, compatible with the JACOB-WOLLMAN (1956) postulate that all recombinants arising from $F^+ \times F^-$ matings are due to Hfr donors in the F^+ population, provided that 80 to 85% of these Hfr cells arise as a consequence of lethal and/or unstable associations between F and the chromosome. Alternatively, F^+ donors may possess a mechanism for chromosome transfer which does not depend on integration of F into the chromosome.

SUMMARY

Integration of the fertility factor F into the chromosome of *Escherichia coli* K-12 results in the formation of an Hfr donor. By replica plating over 10^5 F^+ colonies to selective media spread with an F^- culture, we have determined the probability of stable F integration/bacterium/generation and the frequency of stable Hfr donors in populations of the prototrophic Type I F^+ strain $\times 209$ at genetic equilibrium. (Stable F integrations were operationally defined as those which give rise to isolable clones of stable Hfr donors.) This method allowed detection and isolation of Hfr donors transferring the selected marker on the distal 20% of their chromosomes and present at frequencies from 1 to 100% in F^+ colonies. The frequency of pure clones of stable Hfr donors was 1.7×10^{-4} and the probability of stable F integration was 3×10^{-6} /bacterium/generation. Thirty four Hfr strains were obtained representing 10 unique marker-transfer gradients. Matings between a multiply marked F^- strain and an equal mixture of 12 Hfr strains having unique marker-transfer gradients (2 of these Hfr types

were isolated from $\chi 209$ by CURTISS and RENSHAW (1969)) were conducted to determine the mean recombination frequency per Hfr cell for any chromosomal marker. Matings between this F^- strain and $\chi 209$ were also performed to determine the mean recombination frequency per cell. By using these mean recombination frequencies and the equilibrium frequency of stable Hfr cells in the $\chi 209$ population, we calculated that 15% of the recombinants formed in $F^+ \times F^-$ matings are due to stable Hfr donors in the F^+ population. Another 0.25% of the recombinants formed in $F^+ \times F^-$ matings are accounted for by stable integrations of F which occur just before or during mating. Therefore, 80 to 85% of the recombinants formed in $F^+ \times F^-$ matings must be due either to unstable and/or lethal integrations of F into the chromosome or to a mechanism of chromosome transfer which does not require F integration into the donor chromosome.

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