F+ STRAINS OF *ESCHERICHIA COLI* K-12 DEFECTIVE IN Hfr FORMATION[1]

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IN *Escherichia coli* K-12, there are two basic mating types (HAYES 1953a; CAVALLI, LEDERBERG and LEDERBERG 1953). Donor bacteria, called F+, are characterized by the presence of a fertility factor (F) which exists autonomously in the cytoplasm. Recipient bacteria, lacking this fertility factor, are designated F-. Conjugation between these two mating types yields a low frequency of recombination for chromosomal markers (*ca.* 10^{-5}) but a high frequency of F transfer (*ca.* 0.5) to F- recipient cells.

Donor strains in which the F factor has integrated into the chromosome have been isolated from F+ strains. These strains are very efficient donors of genetic material (CAVALLI 1950; HAYES 1953b), transferring their chromosomes in a linear, sequential manner to F- recipient strains (WOLLMAN, JACOB and HAYES 1956). Called Hfr donors (for high-frequency recombination), they give recombinant frequencies of about 50 to 100% for markers transferred soon after commencement of mating. Essentially all recombinants in an Hfr × F- cross are F-, the chromosomally integrated fertility factor being transferred as the last Hfr marker. Genetic (JACOB and WOLLMAN 1958) and radioautographic (CAIRNS 1963) experiments provided evidence that the chromosome of *E. coli* is circular.

In 1956, JACOB and WOLLMAN used the fluctuation test of LURIA and DELBRÜCK (1943) to show that Hfr donors arise spontaneously from F+ strains by a mutation-like event. From their results they inferred that all recombinants in an F+ × F- cross were due to the presence of Hfr mutants in the F+ population. LEDERBERG (1958), REEVES (1960), HAYES (1960), CURTISS (1962, 1964) and BRODA (1967) presented evidence indicating that Hfr donors, having F stably integrated into or attached to the chromosome, could not account for all recombinants formed in F+ × F- matings. CURTISS (1962, 1964), also employing the fluctuation test, observed that in some F+ strains stable integration of F to yield Hfr "mutants" either did not occur, or occurred at an undetectable frequency, or occurred but was lethal. This led to the classification of F+ donor strains according to their ability to give rise to stable Hfr donors. *Type I* F+ donors have this ability, and *Type II* F+ donors do not (CURTISS and RENSHAW 1965).

The present study was undertaken to confirm the existence of Type I and Type II F+ donors and to determine the basis of the difference between them. The data confirm previous observations (CURTISS 1962, 1964) that some F+ strains give

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rise to stable Hfr mutants and others do not, but the data do not provide a definitive reason for the inability of Type II F+ strains to yield Hfr donors at detectable frequencies.

MATERIALS AND METHODS

Bacteria: The principal strains of *E. coli* K-12 used in this study are listed in Table 1; other derivatives of these strains are described in several other tables. In regard to the derivations of the K-12 sublines used, χ15 comes from W1485 (LEDERBERG and LEDERBERG 1953), χ42 from K-12–112 (WOLLMAN 1953), χ33 from 58–161 (LEDERBERG 1947), and both C600 (APPLEYARD 1954) and W945 (CAVALLI-SFORZA and JINKS 1956) were derived from Y10 (LEDERBERG 1947).

TABLE 1

Escherichia coli *K-12 strains*

Strain number	Mating type	Relevant genotype*	Derivation†
χ15	F+	prototroph *T6^s* λ$^-$ *str^s cyc^s*	W1485
χ33	F+	*T6^s* λ+ *str^s met$^-$ cyc^s*	58–161
χ42	F+	*T6^s* λ+ *his$^-$ str^s cyc^s*	K-12–112
χ45	F−	*T6^s* λ+ *his$^-$ str^s cyc^s*	χ42
χ59	F−	*thr$^-$ leu$^-$ lacY$^-$ T6^s* λ$^-$ *str^s cyc^s thi$^-$*	C600
χ76	F−	*thr$^-$ leu$^-$ proA-B$^-$ lacY$^-$ T6^s* λ$^-$ *str^s cyc^s thi$^-$*	χ59
χ89	F−	*thr$^-$ ara$^-$ leu$^-$ lacY$^-$ T6^s* λ$^-$ *str^s cyc^s thi$^-$*	W945
χ97	F+	*T6^s* λ+ *str^s cyc^s thi$^-$*	W945
χ101	F+	*T6^s* λ$^-$ *str^s cyc^s thi$^-$*	χ97
χ137	F−	*thr$^-$ ara$^-$ leu$^-$ proA-B$^-$ lacY$^-$ T6^s* λ$^-$ *str^r cyc^s thi$^-$*	χ76
χ148	F−	*ara$^-$ leu$^-$ lacY$^-$ T6^r purE$^-$* λ$^-$ *trp$^-$ str^r cyc^s thi$^-$*	χ89
χ160	F−	*ara$^-$ leu$^-$ lacY$^-$ T6^r purE$^-$* λ$^-$ *trp$^-$ his$^-$ str^r cyc^s thi$^-$*	χ148
χ209	F+	prototroph *T6^s* λ$^-$ *str^s cyc^s*	χ42
χ277	F−	*thr$^-$ leu$^-$ lacY$^-$ proC$^-$ T6^r* λ$^-$ *str^r cyc^s thi$^-$*	χ59
χ314	F′	*lac$^-$ T6^s* λ$^-$ *str^s cyc^s thi$^-$*/F-*lac*+	AB785
χ462	F−	*ara$^-$ leu$^-$ proA$^-$ lacZ$^-$ T6^r purE$^-$* λ$^-$ *trp$^-$ lys$^-$ str^r metE$^-$ cyc^s thi$^-$*	χ148
χ478	F−	*ara$^-$ leu$^-$ lacZ-proC$^-$ T6^r purE$^-$* λ$^-$ *trp$^-$ lys$^-$ str^r metE$^-$ cyc^s thi$^-$*	χ148
χ503	Hfr	prototroph *T6^s* λ$^-$ *str^s cyc^s*	χ15
χ637	F−	prototroph *lacY$^-$ T6^s* λ$^-$ *str^s cyc^r*	χ15
χ760	F−	*ara$^-$ leu$^-$ lacY$^-$ proC$^-$ T6^r purE$^-$* λ$^-$ *trp$^-$ his$^-$ argG$^-$ str^r ile$^-$ met$^-$ cyc^s thi$^-$*	χ160
χ830	F−	*thr$^-$ T6^r* λ$^-$ *trp$^-$ str^r cyc^s*	χ209
χ850	F−	prototroph *lac$^-$ T6^s* λ$^-$ *str^s cyc^r*	χ209
χ851	F−	*proC$^-$ T6^r* λ$^-$ *str^r cyc^r*	χ15
χ852	F−	*lac$^-$ T6^s* λ$^-$ *str^s cyc^r thi$^-$*	χ101
χ853	F−	*proC$^-$ T6^r* λ$^-$ *str^r cyc^s thi$^-$*	χ101
χ863	F′	*lac$^-$ T6^s* λ *str^s cyc^r thi$^-$*/F-*lac*+	χ852×χ314
χ864	F′	prototroph *lac$^-$ T6^s* λ$^-$ *str^s cyc^r*/F-*lac*+	χ850×χ314
χ865	F′	prototroph *lacY$^-$ T6^s* λ$^-$ *str^s cyc^r*/F-*lac*+	χ637×χ314

* The nomenclature used conforms with the proposals of DEMEREC *et al.* (1966) except for the modifications noted by CURTISS (1968). The genetic markers are arranged in the order in which they occur on the bacterial chromosome (see Figure 1). Only those markers relevant to this study are listed and certain mutations conferring resistance to phages and azide and inability to ferment carbohydrates have been omitted.

† χ33, χ42 and χ314 were descended from single-colony isolates of strains received from N. SCHWARTZ. Certain other properties of these strains and further details on their derivation can be found in other papers published from this laboratory.

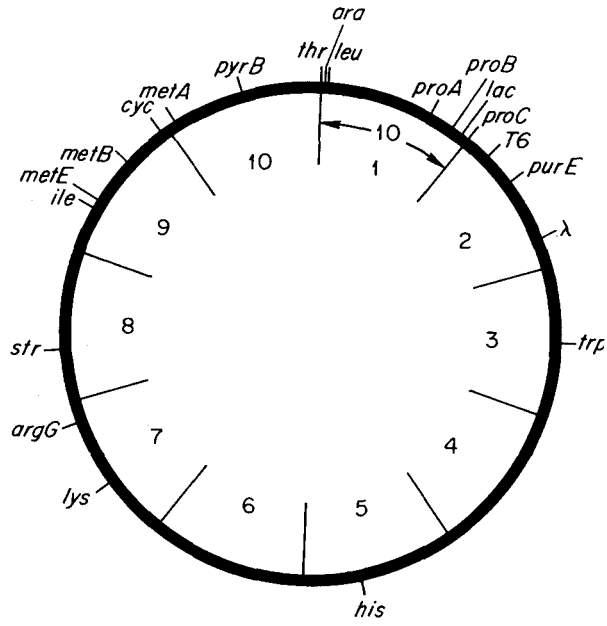

FIGURE 1.—Chromosome map of *Escherichia coli* K-12 showing position of markers employed in this study. The chromosome is divided into 10 sections each containing 10 units; each unit is roughly equivalent to one min of transfer time.

χ101 was obtained from W945 by sequential spontaneous selection of revertants for seven loci, by transduction to ability to utilize mannitol with P1*kc* grown on χ15, by introduction of F from χ42 and then by curing of λ prophage (CURTISS 1962). Figure 1 shows the position on the K-12 circular chromosome of the genetic markers used in this study.

Media: The following synthetic media were used: ML (minimal liquid) and MA (minimal agar) (CURTISS 1965); 3M (minimal mating medium) containing the same concentration of salts as ML but with different proportions of phosphates to give a pH of 6.3 (CURTISS, CHARAMELLA, STALLIONS and MAYS 1968); and ML-low phosphate, containing one-third as much phosphate as ML. Glucose, arabinose and lactose were used at 0.5% final concentrations. Nutritional supplements were purchased from California Biochemical Corp. and were used at the concentrations given by CURTISS *et al.* (1968). D-cycloserine (Eli Lilly) and streptomycin sulfate (Squibb) were used at 5 and 200 μg/ml final concentration, respectively.

The complex media used were: L broth (LENNOX 1955), Difco Penassay broth, beef-peptone broth (HIROTA 1960) containing 0.4% NaCl (pH 7.6), Difco Penassay agar containing 0.8% NaCl, and EMB agar (CURTISS 1965). BSG (buffered saline with gelatin, CURTISS 1965) was frequently used as a diluent.

Isolation of auxotrophic mutants: Auxotrophic mutants were induced by an ultraviolet (UV) dose of 600 ergs/mm^2 and isolated by a modification of the penicillin enrichment cycling procedure as described by CURTISS, CHARAMELLA, BERG, and HARRIS (1965).

Curing of F: The fertility factor F was removed from F+ and F′ strains by growing strains to be cured in beef-peptone broth containing 20 to 50 μg acridine orange per ml as described by HIROTA (1960). The acridine orange was purchased from MATHESON, COLEMAN and BELL. The cultures containing acridine orange were incubated in the dark, usually as 5 ml standing cultures in 16 × 150 mm tubes.

Curing of prophage λ: Cultures of the strains to be cured were suspended in BSG and irradiated with UV doses of 600, 1000 and 1400 ergs/mm^2. They were then diluted to give about 100 to

400 surviving colonies per Penassay agar plate previously spread with antiserum to λ. After growth, the colonies were replica plated to EMB agar containing 0.1% glucose (ZINDER 1958) spread with wild-type λ phage. Lysogenic colonies, immune to λ, were white to pink. Nonlysogenic colonies (i.e., colonies containing cured cells) were red, because of acid production during lysis of some of the sensitive cells. The colonies containing nonlysogenic cells were picked from the Penassay agar plates and retested for lysogenicity by streaking against λ on EMB-0.1% glucose and for inability to produce λ after UV induction.

Transduction: The methods for preparing transducing lysates of P1*kc* and P1L4, a mutant of P1*kc* which gives higher frequencies of transduction (BERG and CARO 1967), were described by CURTISS *et al.* (1965). The recipients to be transduced were grown in L broth containing 2.5×10^{-3}M $CaCl_2$ and, after 20 min for P1 adsorption, various dilutions were plated on selective media.

Fluctuation tests for F+→ Hfr mutation: For most experiments of this type, the JACOB and WOLLMAN (1956) application of the LURIA and DELBRÜCK (1943) fluctuation test was employed. In this method the F+ culture was diluted to contain approximately 100 cells in a volume of 1 ml or less, and replicate cultures of this volume were distributed in appropriate sterile culture tubes (either Wasserman tubes or 0.4-ml centrifuge tubes) and incubated in a 37°C water bath. When these cultures reached between 2 and 5×10^8 cells/ml, suitable amounts (either 10, 20 or 100 μl) were mixed at 1 min intervals with 0.4 ml of an F⁻ culture in sterile Wasserman tubes at 37°C. After a suitable mating period (usually 20, 40, 60 or 80 min), the F+ donor was lysed by addition of 0.05 ml of UV-killed T6 to give a final titer of 2×10^{10}/ml. After 10 to 15 min at 37°C, 0.05 ml of antiserum to T6 was added to neutralize unadsorbed T6. Generally, selection for two or more recombinant classes was carried out by plating 0.1 ml amounts of the interrupted mating mixture on each type of selective medium. Reversion controls for the F+ and F⁻ parents were routinely performed, and in many experiments each F+ culture was also assayed for viable titer. In these experiments, a variety of growth conditions were used. When the F+ and F⁻ cultures were grown in ML, the F⁻ parent was resuspended, before the matings commenced, in 3M supplemented to satisfy the requirements of both parents.

Control fluctuation tests with replicate samples taken from the same large F+ culture were performed for most F+ strains and mating conditions employed. No significant fluctuations in the number of recombinants issuing from each individual mating were observed in these experiments.

For large-scale searches for Hfr derivatives, the less quantitative but more rapid and efficient fluctuation test procedure devised by BERG and CURTISS (1967) was used. In this method, F+ cultures descended from small inocula were analyzed by cross-streaking loopfuls of each F+ culture on appropriate selective media.

Mating procedure: F+ and F′ parents were grown in L broth to log phase as standing cultures at 37°C. F⁻ parents were grown to log phase in L broth with aeration at 37°C. Matings were of 10 ml volume in stationary 125 ml micro-Fernbach flasks at 37°C. The donor:recipient cell ratio was varied according to the experiment. Initial cell densities in the mating mixtures were usually below 5×10^8 cells/ml to avoid problems of oxygen depletion toward the end of the mating, after further growth of the parents had occurred. Matings were introduced by adding 0.8 ml of the mating mixture (or multiples thereof) to 0.1 ml of purified UV-killed T6 at 2×10^{11}/ml (or appropriate multiples thereof). After 15 min at 37°C, 0.1 ml of T6 antiserum (or appropriate multiples thereof) was added, and after 10 to 15 min for neutralization of unadsorbed T6 at 37°C, recombinant selections were made by spreading 0.05-ml amounts of the interrupted mating mixture to dryness on appropriate media. If dilutions were necessary, BSG containing 10% L broth was used. Recombinant frequencies were calculated by dividing the recombinant titers by the titer of the donor parent at the commencement of mating.

Mating type determination: The mating types of cells were determined by transferring tops of colonies into 2 ml of Penassay broth and, after growth for 5 to 6 hours, streaking these cultures against the donor specific RNA phage MS-2 on EMB-0.1% glucose as described by BERG and CURTISS (1967). The validity of this procedure was verified by cross-streaking the cultures to be tested, which were usually streptomycin-resistant, against various polyauxotrophic

cycloserine-resistant F⁻ strains on appropriate selective media. Cultures which were sensitive to MS-2 invariably produced 5 to 10 recombinants beyond the juncture of the cross streak, whereas cultures resistant to MS-2 never produced any recombinants.

RESULTS

Terminology: The isolation of Hfr donors from F+ cultures can be accomplished only if F has become stably associated with the bacterial chromosome at a site which does not cause loss of some irreparable function. We thus define *stable Hfr* donors as having arisen in this manner. *Unstable Hfr* donors are defined as those in which the association between F and the chromosome is transient. Therefore, unstable Hfr donors cannot be isolated by conventional techniques nor can Hfr donors in which F has inactivated a gene for an irreparable function.

Fluctuation tests for F+→ Hfr mutation with Type I F+ donors: Fluctuation tests with Type I F+ donors gave large fluctuations in the number of recombinants arising from matings between F⁻ bacteria and samples of individual F+ cultures descended from small inocula (Table 2). Most F+ cultures gave rise to few if any recombinants, although several cultures gave high numbers of recombinants. Note that F+ culture 18 (Table 2) gave only *leu*+ recombinants, suggesting the presence of a clone of similar Hfr cells. From this culture it was possible to isolate, by replica plating, a "P4X"-type Hfr (Origin *proB proA leu . . . lac* F). From F+

TABLE 2

*Fluctuation test for F+ → Hfr mutation with a Type I F+ donor**

Donor: χ42 / Recipient: χ148	*F+* / *F⁻*	*leu+* / *leu⁻*	*purE+* / *purE⁻*	*trp+* / *trp⁻*	*T6ˢ* / *T6ʳ*	*strˢ* / *strʳ*
	Number of recombinants					
Culture†	*leu+ strʳ*		*purE+ strʳ*		*trp+ strʳ*	
1–7	0		0		0	
8 & 9	1		0		0	
10	0		1		0	
11	0		0		2	
12	2		0		0	
13	2		1		0	
14	1		4		0	
15	3		0		2	
16	13		4		14	
17	19		15		16	
18	51		0		0	
19	80		75		87	
20	171		67		74	
Mean	17.2		8.4		9.8	
Variance	1647		444		580	
χ^2	1915		1058		1184	
P	<0.001		<0.001		<0.001	

* Donor and recipient cultures were grown in appropriately supplemented minimal medium and were mated for 80 min.

† Ranked in order of increasing recombinant yield.

culture 20, a "Hayes"-type Hfr was obtained (O *thr leu proA . . . pyrB* F). Analyses of the mating types of recombinants arising from matings involving F+ cultures, such as cultures 18–20 in Table 2, revealed that 80 to 98% of the recombinants remained F-. Thus most if not all of these recombinants were due to Hfr cells in the F+ cultures.

Fluctuation tests for F+→ Hfr mutation with Type II F+ donors: No significant fluctuation in the number of recombinants issuing from matings with individual cultures of Type II F+ donors was observed (Table 3). The mean number of recombinants per mating was almost equal to the variance, and the calculated χ^2 values indicated that the observed variation in recombinant number from mating to mating indicated a Poisson distribution.

Fluctuation tests for F+→ Hfr mutation in donor strains with Type I chromosomes and F from Type II F+ donors: Even though the fertility factor F in the Type II F+ donors χ59F+ and χ101 (Table 3) was originally donated by the Type I F+ donor χ42, it was thought possible that the sojourn in χ59+ and χ101 could have altered F. Thus, F was returned from several Type II F+ donors

TABLE 3

*Fluctuation tests for F+ → Hfr mutation with Type II F+ donor strains**

Donor: χ59F+	F+	*trp+*	*his+*	*str^s^*	χ101	F+	*leu+*	*proA-B+*	*str^s^*
Recipient: χ160	F-	*trp-*	*his-*	*str^r^*	χ137	F-	*leu-*	*proA-B+*	*str^r^*
		Number of recombinants					Number of recombinants		
Culture		*trp+ str^r^*		*his+ str^r^*			*leu+ str^r^*		*proA-B+ str^r^*
1		1		0			1		3
2		2		3			2		3
3		3		2			2		3
4		3		3			1		4
5		4		3			2		4
6		2		5			4		2
7		1		6			0		6
8		5		4			2		6
9		4		5			2		7
10		5		5			1		8
11		6		6			4		6
12		6		7			3		7
13		4		9			6		5
14		4		9			6		5
15		3		10			1		9
16		5		10			2		9
17		10		5			5		7
18		8		8			6		8
19		6		10			4		10
20		9		8			4		14
Mean		4.6		6.2			2.9		6.3
Variance		5.9		8.0			3.1		8.0
χ^2		25.4		25.8			21.4		25.4
P		0.2		0.2			0.3		0.15

* See Table 2 footnotes. χ59 F+ was obtained by infecting χ59 with F from χ42.

TABLE 4

*Fluctuation test for F+ → Hfr mutation in a donor with a Type I chromosome and a Type II F**

Donor: χ45F+ Recipient: χ148	*F+* *F-*	*leu+* *leu-*	*purE+* *purE-*	*trp+* *trp-*	*str^s* *str^r*
	Number of recombinants				
Culture	*leu+ str^r*		*purE+ str^r*		*trp+ str^r*
1–5	0		0		0
6 & 7	1		0		0
8	0		0		1
9	0		1		1
10	1		1		0
11	2		0		0
12	0		2		0
13	1		0		2
14	3		0		0
15	1		5		0
16	4		0		10
17	7		7		3
18	132		0		2
19	124		93		148
20	227		185		324
Mean	25.2		14.7		24.6
Variance	3586		1878		5747
χ^2	2846		2555		4673
P	<0.001		<0.001		<0.001

* See Table 2 footnotes.

to χ45, an F- derivative of χ42 obtained by acridine-orange treatment. In all cases, significant fluctuations in the number of recombinants in matings with individual F+ cultures were observed, as is shown for one such fluctuation test in Table 4. The similarity of the results presented in Table 4 to those obtained with Type I F+ donors (Table 2) indicated that the difference between Type I and Type II F+ donors is not due to differences in F.

Summary of fluctuation tests for F+→ Hfr mutation: Table 5 lists, for both Type I and Type II F+ donors, the number of tests in which significant and non-significant fluctuations in the number of recombinants were obtained. Most (31 out of 38) tests with Type I F+ donors gave significant fluctuations. About 5% of the individual F+ cultures gave elevated recombinant frequencies, and about 40% of these (2% of total Type I F+ cultures) had a sufficient number of Hfr cells (0.1 to 1.0%) such that stable Hfr derivatives could be isolated by replica plating from plates with well isolated colonies. Percentages of recombination, based on the titer of stable Hfr cells in some of these cultures (as determined by replica plating), varied from 0.2 to 10% with a mean of 2%. Thus, on the average, one recombinant was formed for every 50 Hfr cells present in the F+ culture. Based on this value and the mean recombinant frequency per culture in fluctuation tests with Type I F+ donors, it was calculated that the frequency of stable Hfr cells would have to be about 1×10^{-3} if all recombinants were due to stable

TABLE 5

*Summary of results of fluctuation tests for F+ → Hfr mutations**

F+ donor type	F+ strains tested†	Number of tests in which fluctuations in the number of recombinants were: Significant	Not significant
Type I	K-12–112 derivatives (χ42, χ45F+, χ209, χ395)	17	7
	58–161 derivatives (χ33, χ402)	3	0
	W1485 derivatives (χ15, χ593)	11	0
		—	—
		31	7
Type II	W945 derivatives (χ89F+, χ97, χ101, χ206F+, χ206Ex6, χ397, χ399, χ401, χ519)	5‡	18
	C600 derivatives (χ59F+, χ76F+, χ98Ex11, χ603)	0	13
		—	—
		5	31

* The above fluctuation tests were done under a variety of conditions. The F+ cultures were grown in minimal medium with low phosphate and high phosphate, with 0.5% glucose and 0.1% glucose, and with optimal and suboptimal levels of required supplements; in Penassay broth; and in L broth. Generation times in these media varied from 40 min to 3 hr. Matings were done either in minimal mating medium, Penassay broth or L broth and varied in duration from 20 to 80 min. The number of F+ cultures used for each test varied from 20 to 300. Each culture was descended from approximately 100 to 200 cells. A variety of F− recipient strains was used.

† Derivation of strains not listed in Table 1 was as follows: χ45F+ (4 strains) by introduction of F from χ101 (2 strains) or from χ98Ex11 (2 strains) into χ45; χ395 from χ42 by λ curing; χ402 from χ33 by λ curing; χ593 from χ15 by introduction of *proB-lac* deletion and *thy*− mutations; χ89F+ by introduction of F from χ42 into χ89; χ206F+ by introduction of F from χ42 into χ206, which was derived from χ89 by selecting a *proA-B*− mutation and reversion to *thr*+ (see CURTISS 1964); χ519 by introduction of F from χ15 into χ478; χ59F+ and χ76F+ by introduction of F from χ42 into χ59 and χ76, respectively; and χ603 by introduction of F from χ593 into χ277. χ206Ex6 and χ98Ex11 are partially diploid strains described by CURTISS (1964); χ397, χ399 and χ401 were isolated from χ101 and contain *his*−, *met*− and *ade*− mutations, respectively.

‡ Even though significant fluctuations in the number of recombinants from matings of individual F+ cultures were observed in these five experiments, we were unable to isolate any stable Hfr mutants.

Hfr cells. All seven of the nonsignificant fluctuations in number of recombinants with the Type I F+ donors derived from K-12–112 were obtained in tests with only 20 to 25 individual cultures—a reasonable result in view of the low frequency (5%) of individual Type I F+ cultures giving high recombinant yields.

Most (31 out of 36) tests with Type II F+ donors did not give significant fluctuations in the number of recombinants arising in matings with individual F+ cultures (Table 5). However, five tests with the W945-derived Type II F+ donors did give significant fluctuations in the number of recombinants. Repeated attempts to isolate stable Hfr derivatives from any of the Type II F+ cultures which gave high recombinant yields (no more than 4 to 5 times the average yield for all cultures) were unsuccessful. All five significant fluctuations in the number of recombinants were obtained in large tests with 40, 200 or 300 individual F+ cultures. Three of these "significant" fluctuations were obtained in tests in which the

F+ cultures were growing in "poor" media, which resulted in very long generation times (2 to 3 hr) and a greater variation in viable-cell titer between the F+ cultures than was observed when the F+ cells were growing in "rich" medium. In the test with 40 F+ cultures growing in ML plus 0.1% glucose, viable-cell titers were determined for all cultures. Thus, when the variance was calculated using recombinant frequency for each mating, the value of P was less significant (0.02) than when calculated on recombinant number per plate for each mating ($P = < 0.001$). Although the variances in the other four tests could not be calculated in this way, it is possible that the significant fluctuations in the number of recombinants were fortuitously due to variations in viable-cell titers of the individual F+ cultures. Alternatively, unstable Hfr derivatives could have been present as clones in some individual F+ cultures at the time of mating and then reverted to F+ before we could isolate them.

From the results obtained, it is evident that Type I F+ donors readily give rise to stable Hfr donors and Type II F+ donors do not. It is obvious from the variety of growth and mating conditions used (Table 5) that this difference is not under any environmental control. It should be noted, however, that the mean frequency of recombinants per F+ culture was always much higher in fluctuation tests with F+ cultures grown in broth than in tests with F+ cultures grown in synthetic media. It was therefore necessary to mix smaller samples of the broth grown F+ cultures (usually 10 or 20 μl) with the 0.4 ml F- cultures than used in the tests with F+ cultures grown in minimal media (100 μl) in order to obtain a low number of recombinants from the majority of the individual F+ × F- matings. Even so, the data from fluctuation tests with broth grown Type I F+ cultures could be interpreted by assuming that there existed two types of F+ donor behavior in the population: one which gave rise to a Poisson distribution in the number of recombinants per individual mating and one which gave rise to the large fluctuations in recombinant number due to the mutation-like integration of F to give rise to a clone of Hfr donor cells. Such superimposed distributions in recombinant number were also noted occasionally in those fluctuation tests with Type I F+ donors grown in minimal medium in which high mean yields of recombinants per mating were obtained. In addition to ruling out environmental factors as being responsible for the ability or inability of F+ strains to give rise to stable Hfr donors, it is also concluded that this difference is not due to the presence or absence of λ prophage. [The Type I F+ strains χ42, χ45F+ and χ33 (all lysogenic) and χ209, χ395, χ402, χ15 and χ593 (all nonlysogenic) were equally capable of giving Hfr mutants, whereas no Hfr derivatives could be obtained from the Type II F+ strains χ97 (lysogenic) and χ101 (nonlysogenic).] Also, as previously concluded, this difference is not due to differences in the fertility factor.

Recombinant formation in F- derivatives of Type I and Type II F+ donors: The inability of Type II F+ donors to give rise to stable Hfr donors could possibly be due to a defect which affected genetic recombination so as to prevent stable integration of F into the chromosome from being completed. To test this, F- derivatives of both Type I and Type II F+ donors, obtained by acridine-orange treatment, were mated with Hfr and F+ donors (Table 6). The *proC*+ *str*^{r} and *trp*+

TABLE 6

*Recombinant yields in matings of Hfr and F+ donors with F⁻ strains derived from Type I and Type II F+ strains**

F⁻ recipient and its relevant genotype and derivation		Recombinant frequencies in matings with F+ donor		Recombinant frequencies in matings with Hfr donor	
		proC⁺ str^r	*trp⁺ str^r*	*proC⁺ str^r*	*trp⁺ str^r*
χ830 F⁻ *thr⁻ T6^r trp⁻ str^r*	Type I	.....	5.9×10^{-6}	.....	9.1×10^{-2}
χ851 F⁻ *proC⁻ T6^r str^r*	Type I	1.5×10^{-5}	.....	9.3×10^{-1}	.....
χ277 F⁻ *thr⁻ leu⁻ proC⁻ T6^r str^r thi⁻*	Type II	7.3×10^{-6}	.....	5.8×10^{-1}	.....
χ853 F⁻ *proC⁻ T6^r str^r thi⁻*	Type II	1.0×10^{-5}	.....	6.4×10^{-1}	.....

* The matings were conducted as described in MATERIALS AND METHODS and were interrupted after 40 min. All recombinants were selected by plating on MA supplemented with threonine, leucine, thiamine, glucose and streptomycin. χ15(F+) and χ503 (Hfr OR21 O-*proC purE gal trp . . . lacF*) were the prototrophic *T6^s str^s* donor strains used and are isogenic with the F⁻ strain χ851, a fact which probably explains the higher recombinant frequencies in matings with this F⁻ strain. The donor:recipient cell ratios were about 1:2 in the matings with χ15 and about 1:10 in the matings with χ503. Type II strain χ277 has never possessed F but its F+ derivative χ603, fails to give rise to detectable frequencies of Hfr donors as revealed in fluctuation tests (Table 5).

str^r recombinant frequencies were reasonably similar in all the F+ × F⁻ matings. Similarly, the frequencies of *proC⁺ str^r* recombinants were nearly equal in matings with the Hfr parent, although the frequency of *trp⁺ str^r* recombinants is reduced in the mating with the F⁻ strain χ830, since Hfr OR21 does not begin to transfer the *trp⁺* allele until 20 min after commencement of mating. An analysis of recombinants indicated that there was no persisting heterozygosity at the *T6* locus. Since the *T6* locus is closely linked to the *proC* locus, it can be concluded that most if not all of the *proC⁺ str^r* recombinants were haploid. The results presented in Table 6 indicate that F⁻ derivatives of Type I and Type II F+ strains are equally capable of carrying out genetic recombination following conjugal transfer of genetic material.

Chromosome mobilization and transfer by F′ derivatives of Type I and Type II F+ strains: According to the model of episome integration into chromosomes proposed by CAMPBELL (1962), the formation of an Hfr donor would result from a reciprocal crossover between a circular F and the chromosome. Evidence in support of this model for Hfr formation has been presented and discussed by BRODA (1967) and CURTISS and STALLIONS (1969). F′ *lac⁺* factors were introduced into F⁻ derivatives of Type I and Type II F+ donors to test the ability of these strains to carry out reciprocal recombination. Chromosome mobilization and transfer by F′ donors requires a reciprocal recombination event between the chromosome and F′ factor (SCAIFE and GROSS 1963; SCAIFE 1967). The frequencies (Table 7) and kinetics (data not shown) of chromosome transfer relative to F′ *lac⁺* transfer were equivalent for all three strains tested. Based on the results presented in this and the preceding section, it is concluded that the inability of Type II F+ donors to give rise to stable Hfr donors is not due to any defect in the basic recombination mechanism(s).

TABLE 7

*F′ lac+ and chromosome transfer by F′ donors having chromosomes derived from Type I and Type II F+ strains**

F′ donor and its genotype and derivation		Frequency of recombinants inheriting donor:							
		lac+ allele		*proA*+ allele		*leu*+ allele		*metE*+ *allele*	
		Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
χ864 F′ *lac+/lac⁻ T6ˢ strˢ cycʳ*	Type I	2.5×10^{-1}	9.7×10^{-1}	.....	1.2×10^{-2}	2.7×10^{-3}	5.3×10^{-3}	4.3×10^{-6}	1.9×10^{-5}
χ865 F′ *lac+/lac⁻ T6ˢ strˢ cycʳ*	Type I	4.5×10^{-1}	1.0×10^{0}	.....	2.4×10^{-2}	6.5×10^{-3}	1.6×10^{-2}	2.0×10^{-5}	3.3×10^{-5}
χ863 F′ *lac+/lac⁻ T6ˢ strˢ cycʳ thi⁻*	Type II	2.9×10^{-1}	1.3×10^{0}	.....	1.9×10^{-2}	3.7×10^{-3}	8.9×10^{-3}	9.0×10^{-5}	8.8×10^{-5}

* The matings were conducted as described in MATERIALS AND METHODS and were interrupted after 40 min. χ462 (F⁻*lac⁻ proA⁻ leu⁻ metE⁻ T6ʳ strʳ*) was used as the recipient. The donor:recipient cell ratios were about 1:20.

TABLE 8

*P1L4-mediated transduction of F+ and F⁻ strains with a Type II chromosome**

Transductant class selected	Number of transductant colonies			
	F⁻ recipient		F+ recipient	
	Actual	Relative	Actual	Relative
ara+	941	28.5	1183	27.4
leu+	982	29.8	1280	29.7
ara+ *leu*+	644	19.5	901	20.9
lac+	304	9.2	422	9.8
proC+	365	11.1	448	10.4
lac+ *proC*+	63	1.9	80	1.9
Total	3299	100.0	4314	100.1

* χ478 (F⁻) and χ519 (F+) were used as recipients at cell titers of 1.0×10^8/ml and 1.4×10^8/ml, respectively. P1L4 was added to a final titer of 4.0×10^7 pfu/ml.

P1-mediated transduction of F+ and F⁻ strains with a Type II chromosome: It has been shown that F from Type II F+ donors is capable of attaching to or integrating into the chromosome when returned to an F⁻ derivative of a Type I F+ donor (Table 4). Also, F⁻ derivatives of Type II F+ strains are capable of undergoing genetic recombination with material transferred by conjugation (Table 6) and with F′ factors (Table 7). The last result indicates that the presence of F in an F⁻ derivative of a Type II F+ donor does not interfere with the expression and/or function of the products of chromosomal genes necessary for reciprocal recombination. However, F attachment or integration may not involve the usual type of reciprocal exchange, and it is thus possible that the presence of F in Type II F+ donors interferes with some other recombination mechanism. A partial test of this notion was achieved by studying P1-mediated transduction of the F⁻ strain χ478 and its F+ derivative χ519. Note that χ519 was unable to yield stable Hfr derivatives in a fluctuation test employing the cross-streak method (see Table 5). As the data in Table 8 show, there was no significant difference between χ478 and χ519 in transduction frequencies and in linkage between the *ara* and *leu* loci and the *porC* and *lac* loci. Therefore, the presence of F in Type II F+ strains does not interfere with the expression and/or function of products of chromosomal genes necessary for the integration of genetic material introduced by the transducing phage P1.

Equality of recombinant frequencies obtained with Type I and Type II F+ donors: Since Type I F+ donors give rise to stable Hfr mutants which can account for some of the recombinants formed when these cultures are mated with F⁻ bacteria, it can be asked whether recombinant frequencies in matings with these donors are higher than in matings with Type II F+ donors. The mean recombinant frequencies calculated from fluctuation tests done under the same conditions were very similar for both Type I and Type II F+ donors. (The data shown in Tables 2, 3, and 4 do not convey this point, since these experiments were chosen for presentation because of the large number of individual F+ cultures giving

none or low numbers of recombinants.) A more quantitative comparison of ability to produce recombinants can be obtained from results of matings of Type I and Type II F$^+$ donors with the same F$^-$ culture. We have used χ209 and χ101 as Type I and Type II F$^+$ donors, respectively, for most of the experiments reported in a following communication (CURTISS and RENSHAW 1969). In these experiments, cultures of the two donors were mated with samples of the same F$^-$ culture. The mean recombinant frequency for 23 matings of 40 min duration for χ209 was 2.0×10^{-5} (range from 8.2×10^{-6} to 4.1×10^{-5}), and for χ101 it was 1.9×10^{-5} (range from 8.5×10^{-6} to 4.5×10^{-5}). Thus it is concluded that Type I and Type II F$^+$ donors are equally capable of recombinant production.

Acridine-orange curing of F and F′ factors in Type I and Type II strains: During the preparation of strains used to obtain the data in Tables 6 and 7, it was observed that it was much more difficult to cure F, by growth in the presence of acridine orange, from Type II F$^+$ strains than from Type I F$^+$ strains. A search through data collected from experiments making use of curing by acridine orange, obtained during the past 8 years, confirmed this observation. All Type II F$^+$ strains from the W945 and C600 K-12 sublines, regardless of whether they had received F from Type I F$^+$ donors of the 58–161, K-12–112 or W1485 subline, behaved in a similar manner: their growth was markedly inhibited by 40 μg acridine orange/ml and more severely inhibited by 50 μg acridine orange/ml, and the percentage of curing of F after 15 to 20 generations of growth in acridine orange at these two concentrations was usually between 10 and 40%. On the other hand, the growth of all Type I F$^+$ strains was inhibited much less by acridine orange, and more than 95% curing of F could be obtained by overnight growth in 30, 40, or 50 μg acridine orange/ml. Previous studies (CURTISS 1964) have shown that in the presence of 50 μg acridine orange/ml, the rate of F curing was 9%/generation at a generation time of 69 min for the Type I F$^+$ strain χ15 and 5%/generation at a generation time of 87 min for the Type II F$^+$ strain χ98Ex11. Table 9 presents results from a more recent experiment which demonstrates the effects just described of acridine orange on growth and F curing in Type I and Type II F$^+$ strains. The differential effect of acridine orange on growth of Type I *versus* Type II F$^+$ donors is not associated with properties of F, since the same relationship exists when comparing growth in acridine orange of F$^-$ derivatives of Type I and Type II F$^+$ strains.

A somewhat paradoxical situation was found when we examined the curing of F′ *lac*$^+$ factors in strains with chromosomes derived from Type I and Type II F$^+$ strains. The data in Table 10 reveal that any given concentration of acridine orange gives comparable frequencies of F′*lac*$^+$ curing regardless of the derivation of the chromosome. This inability to observe a difference in F′ *lac*$^+$ curing between Type I and Type II strains was also true for experiments with Type I and Type II donors possessing a different F′ *lac*$^+$ factor. Since acridine orange inhibits the growth of Type II F′ *lac*$^+$ donors much more than the growth of Type I F′ *lac*$^+$ donors (see footnote to Table 10) but has an equal effect on F′ *lac*$^+$ curing, it is reasonable to believe that there is no direct relationship between the effects of acridine orange on inhibiting growth and on F curing (Table 9).

TABLE 9

*Curing of F in Type I and Type II F+ strains by growth in the presence of acridine orange**

F+ strain and type	Acridine orange concentration (μg/ml)	Number of generations of growth	Hours of incubation	Percent F+
χ15 Type I	0	21.1	25.0	100
	30	19.7	25.0	5.4
	40	20.4	25.0	<0.5
	50	21.1	30.2	<0.5
χ209 Type I	0	21.8	25.0	100
	30	21.6	25.0	<0.5
	40	20.0	25.0	<0.5
	50	17.5	30.2	<0.5
χ101 Type II	0	19.8	25.0	100
	30	20.4	25.0	60
	40	20.6	30.2	3.6
	50	19.8	48.5	88†

* Each 20 ml culture was started with an inoculum of 10^2 to 10^3 cells/ml and was aerated at 37°C in the dark.

† The donor cells in this culture were thoroughly tested and were found to be F+ donors whose growth was still inhibited by 50 μg acridine orange/ml and which lost F at the same frequency during growth in acridine orange as did cells from the parent χ101 culture.

Effects of acridine orange on F transfer and recombinant production during conjugation: CUZIN and JACOB (1966) presented evidence indicating that acridine orange, when present during conjugation with Hfr and F′ *lac*+ donors, reversibly inhibited initiation of episome and chromosome transfer. We therefore undertook

TABLE 10

*Curing of F′ lac+ by growth in the presence of acridine orange in strains with chromosomes derived from Type I and Type II F+ strains**

F′ *lac*+ strain and type	Acridine orange concentration (μg/ml)	Percentage of cells harboring F′		
		Experiment 1	Experiment 2	Experiment 3
χ865 Type I	0	97.3	89.6	98.9
	20	...	...	32.2
	30	...	...	18.0
	40	4.7	...	16.0
	50	...	1.7	17.9
χ863 Type II	0	96.7	90.4	98.9
	20	...	...	45.0
	30	...	...	16.4
	40	11.1	...	16.3
	50	...	<0.2	11.6

* Experiments 1 and 2 were conducted as described in MATERIALS AND METHODS and experiment 3 as described in the footnote to Table 9. All cultures had undergone about 20 division cycles of growth at the time of plating. The growth of χ863 was significantly inhibited by 40 and 50 μg/ml of acridine orange, an effect similar to that noted for the Type II F+ strains χ101 (Table 9).

a study to investigate the effects of acridine orange on F and chromosome transfer by F+ donors in hopes of determining whether or not all chromosome transfer by F+ donors was initiated and controlled by F. The results presented in Table 11, obtained in experiments with Type I and Type II F+ donors, reveal that acridine orange inhibits recombinant production to a greater extent than it inhibits F transfer to recombinants. In the experiment with χ15 (Table 11), we examined F transfer to nonrecombinants and observed that F transfer occurred in the mating with acridine orange present (condition C) 85% as often as in the control mating (condition A). In any event, we have not observed a consistent difference between Type I and Type II F+ strains with regard to the effects of acridine orange on F transfer and recombinant production, and we certainly have not observed inhibitions like the 100- to 1000-fold inhibitions of F′ *lac*+ transfer and recombinant production noted by CUZIN and JACOB (1966) for matings conducted in the presence of acridine orange. With regard to this last point, it should be noted that acridine orange as purchased is not a pure chemical (ours contains 36% $ZnCl_2$ by weight), and therefore it might not be expected that similar results would be obtained with different sources and/or batches of the dye.

If all chromosome transfer by F+ donors requires some interaction between F and the chromosome, then the data in Table 11 suggest that either this interaction or the transfer of the chromosome formed by this interaction is inhibited more by acridine orange than is F transfer. Alternatively, acridine orange might have little or no effect on chromosome and F transfer but rather exert its effect in the F- recipient by inhibiting the integration of chromosomal material more than the survival and/or replication of the transferred F. This second explanation is favored by the observation that the inhibitions of F transfer and recombinant production are about equal in matings allowed to initiate transfer for 5 min in the absence of acridine orange (condition B, Table 11) and in matings with parents treated before and throughout mating with acridine orange (condition C, Table 11).

DISCUSSION

This study confirmed the existence of two types of F+ donors: Type I, which does give rise to stable Hfr derivatives, and Type II, which fails to give rise to detectable frequencies of stable Hfr derivatives. We have shown that the difference between these two F+ donor types is not due to differences in F, to the presence or absence of prophage λ, to the environmental growth conditions prior to and during mating, or to any *known* genetic marker. Furthermore, we have shown that F- derivatives of Type I and Type II F+ strains are equally capable of supporting recombination events that lead to haploid recombinant formation following conjugation and can also carry out reciprocal recombination to allow chromosome mobilization and transfer by F′ *lac*+ factors. Therefore, Type II strains are not "recombination deficient" in terms of the usual ways of measuring recombination proficiency. It was also shown that the presence of F in Type II strains did not inhibit or stimulate recombination events necessary for integration of genetic material introduced by the transducing phage P1. On the basis of

TABLE 11

*Frequency of recombinant formation and F transfer in matings with Type I and Type II F+ donors in the absence and presence of acridine orange**

Recombinant class selected	χ209 (Type I) × χ760			χ15 (Type I) × χ760			χ101 (Type II) × χ760		
	Condition A	Condition B	Condition C	Condition A	Condition B	Condition C	Condition A	Condition B	Condition C
leu+ *str*^{r}	4.5×10^{-5}	1.5×10^{-5}	1.1×10^{-5}	6.9×10^{-6}	6.8×10^{-6}	5.3×10^{-6}	1.2×10^{-5}	7.3×10^{-6}	1.4×10^{-5}
proC+ *str*^{r}	2.6×10^{-5}	8.9×10^{-6}	4.9×10^{-6}	7.6×10^{-6}	7.2×10^{-6}	4.6×10^{-6}	1.4×10^{-5}	7.7×10^{-6}	1.3×10^{-5}
purE+ *str*^{r}	8.6×10^{-5}	2.4×10^{-5}	1.8×10^{-5}	1.2×10^{-5}	5.8×10^{-6}	5.1×10^{-6}	2.0×10^{-5}	1.0×10^{-5}	1.4×10^{-5}
his+ *str*^{r}	1.1×10^{-5}	3.4×10^{-6}	1.3×10^{-6}	3.9×10^{-6}	5.0×10^{-6}	3.8×10^{-6}	2.0×10^{-5}	2.5×10^{-5}	2.4×10^{-5}
argG+ *str*^{r}	9.2×10^{-6}	4.1×10^{-6}	3.9×10^{-6}	5.5×10^{-6}	6.3×10^{-6}	3.7×10^{-6}	1.8×10^{-5}	1.1×10^{-5}	9.7×10^{-6}
Mean	3.5×10^{-5}	1.1×10^{-5}	7.7×10^{-6}	7.2×10^{-6}	6.2×10^{-6}	4.5×10^{-6}	1.7×10^{-5}	1.2×10^{-5}	1.5×10^{-5}
Relative	100	31	22	100	86	63	100	71	88
Frequency of F transfer to recombinants	9.9×10^{-1}	8.9×10^{-1}	6.9×10^{-1}	9.0×10^{-1}	.....	7.3×10^{-1}	8.5×10^{-1}	7.3×10^{-1}	7.1×10^{-1}
Relative	100	90	70	100	..	81	100	86	84

* The bacteria were grown in L broth and prior to mating were resuspended in L broth which had been adjusted to pH 7.6. Acridine orange was added to a final concentration of 30 μg/ml either 5 min after (Condition B) or 12 min before mating commenced (Condition C). Acridine orange at an appropriate final concentration was added to the UV-irradiated T6 suspension used to interrupt the control mating (Condition A) so that the same concentration of acridine orange would be present during T6 treatment, T6 antiserum treatment and plating for all three mating conditions. All manipulations were conducted in subdued light and the matings were interrupted after 60 min. The donor:recipient cell ratio was about 1:2.

all these observations, we infer that Type II strains either possess all of the active enzymes necessary to permit the integration of F into the chromosome, or that one or more unique enzymes coded for by chromosomal genes are necessary for F integration but not "normal recombination" and that one of these chromosomal genes is mutant in Type II strains. The former interpretation is favored by KAHN's (1968) observation that stable Hfr derivatives can be induced in F$^+$ derivatives of the Type II C600 subline of *E. coli* K-12 by treatment with N-methyl-N′-nitro-N-nitrosoguanidine. No Hfr strains were obtained without this mutagenic treatment. However, it is not clear whether the mutagenic treatment used to obtain Hfr donors caused alterations in F and/or the chromosome to permit stable F integration and/or expression of the Hfr phenotype.

That Type I and Type II F$^+$ donors are equally capable of chromosome transfer is deduced from the fact that the frequencies of recombinant formation obtained in mass matings with the same F$^-$ strain were nearly identical. The same was true of the mean recombinant frequencies from fluctuation tests done under identical conditions for both Type I and Type II F$^+$ donors. This observation suggests that the majority of recombinants in F$^+$ × F$^-$ matings must be due to a mechanism which does not require stable F integration into the chromosome, since F integration to yield stable Hfr donors does not occur at detectable frequencies in Type II F$^+$ strains. By comparing recombinant frequencies per Hfr cell in individual Type I F$^+$ cultures containing high frequencies of Hfr cells with the mean recombinant frequency for all F$^+$ cultures in fluctuation tests, it was calculated that the mean frequency of stable Hfr cells had to be about 1×10^{-3} to account for all recombinants formed in F$^+$ × F$^-$ matings. CURTISS and STALLIONS (1969) have shown by directly measuring the frequency of stable Hfr cells in a Type I F$^+$ population that about 15% of the recombinants formed in F$^+$ × F$^-$ matings are due to the presence of stable Hfr cells in the F$^+$ population. Therefore, it obviously would not be possible to measure a reduction of only 15% in recombinant yield by Type II F$^+$ donors which would have any significance, especially when it is realized that the Type I and Type II F$^+$ strains are only distantly related and are not isogenic.

In addition to the inability to isolate stable Hfr donors from Type II F$^+$ strains, we also noted that acridine orange is much less effective in curing F, but not F′ *lac*$^+$, from Type II strains than from Type I strains. It is difficult to assess the significance of these observations because the mechanism of episome curing by acridine orange is poorly understood. However, it seems reasonable to assume that episome curability might be affected by the structure and/or the location of episomes within the cell. The F and F′ *lac*$^+$ episomes usually exist in the bacterial cell as circular elements (FREIFELDER 1968) which are presumably attached to the bacterial membrane (JACOB, RYTER and CUZIN 1966) or to some other structure in a way that allows the parental strands of the episome and chromosome to be transmitted to daughter cells as a unit (CUZIN and JACOB 1967). If episomic elements were linear in Type II strains, due to the defectiveness of a bacterial enzyme(s) necessary for closure, then integration of such episomes into the chromosome would result in a linear "Hfr-type" chromosome which might not

be genetically viable. While such an occurrence could account for our inability to obtain stable Hfr donors from Type II F^+ strains, it does not at first appear to provide an explanation as to why F and F′ *lac*$^+$ episomes should be cured by acridine orange with different efficiencies in Type II strains. The frequency of chromosome mobilization by F′ *lac*$^+$ partially diploid strains indicates that the *lac* regions of the episome and chromosome must be synapsed about 10% of the time. It is unlikely that F is as frequently synapsed with the chromosome because there are no substantial segments of genetic homology between F and the chromosome (CURTISS and STALLIONS 1969). Therefore, it is possible that Type II strains possess membrane attachment sites with altered affinities for episomic elements which prevent efficient curing of F by acridine orange but have little or no effect on curing of F′ *lac*$^+$ episomes, because of the possible alternation of F′ *lac*$^+$ between a membrane site and a site for homologous pairing with the bacterial chromosome. Since the affinity between two structures can be affected by alterations in either structure, it is conceivable that the suggested linearity of episomes in Type II strains could therefore affect the efficiencies with which different episomes are cured by acridine orange.

In summarizing and extending our speculations as to why stable Hfr donors cannot be observed at detectable frequencies in Type II F^+ strains, we can consider three basic possibilities that are compatible with our results. First, stable F integration may not occur either because a bacterial enzyme(s) specifically necessary for F integration is absent or because the F attachment site on the membrane is altered, which in effect prevents pairing and/or exchange between F and the chromosome. Second, stable F integration may occur without causing the expression of the usual Hfr phenotype. Third, stable F integration resulting in chromosome lethality through the formation of a linear Hfr chromosome may occur because of the absence of a functional bacterial enzyme(s) necessary for complete integration of F by a reciprocal exchange into the chromosome or for the circularization of the F episome. If any one of the three explanations is correct, then Type II F^+ populations should be less fertile than Type I F^+ populations. However, if the third type of explanation is correct, then these lethal F integrations would permit the formation of "Hfr donors of the moment" which could contribute slightly to the total fertility of Type II F^+ populations.

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SUMMARY

The existence of F^+ donor strains which give rise to detectable frequencies of stable Hfr derivatives (Type I) and of F^+ donor strains which do not (Type II) has been confirmed. The basis for the difference between these two F^+ donor types is still not known. It is not due to differences in F, to the presence or absence of prophage λ, to the environmental growth conditions prior to or during mating,

or to any *known* genetic marker. F- derivatives of Type I and Type II F+ strains are capable of supporting recombination events leading to haploid recombinant formation following conjugation and can also carry out reciprocal recombination to allow chromosome mobilization and transfer by F′ factors. The presence of F in Type II strains does not affect recombination events necessary for integration of genetic material introduced by the transducing phage P1. It was therefore concluded that Type II strains are not deficient in recombination proficiency as customarily measured. Since they give nearly identical recombinant frequencies when mated with the same F- parent, it may be deduced that the two F+ donor types are similarly capable of chromosome transfer. Acridine orange was observed to be less effective in curing F, but not F′ *lac*+, from Type II strains than from Type I strains. The presence of acridine orange during matings of either F+ type with an F- strain reduced F transfer by 10 to 20% and recombinant production by 20 to 80%.

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