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The formation of Hfr strains in *Escherichia coli* K12

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1. INTRODUCTION

The work described in this paper was undertaken to establish whether in a typical F^+ strain of *E. coli* K12 stable Hfr strains arise with their origins (that is, the points at which the sex factor is integrated) distributed randomly around the chromosome, or whether there are specific regions at which this integration may occur. A single F^+ strain was used as a source of independently and spontaneously arising Hfr strains. The analysis of these supports the view that there exist specific regions for sex factor integration. Further experiments bearing on the mechanism of formation of Hfr strains and on the nature of the fertility of F^+ cultures are also described.

2. MATERIALS AND METHODS

(i) *Bacterial strains*

The following strains of *E. coli* K12 were used:

W1655 F^+ *met*$^-$ *str*^{s} *T6*^{s} *lac*$^+$ *gal*$^+$ (λ)$^-$ λ^r (Lederberg & Lederberg, 1953).

W677 F^- *thr*$^-$ *leu*$^-$ B_1^- *pro*$^-$ *str*^{r} *azi*^{s}. A *str*^{r} derivative, kindly provided by Dr R. C. Clowes, of a *pro*$^-$ derivative of strain W677 (Hayes, 1953).

W677 F^- *thr*$^-$ *leu*$^+$ B_1^- *pro*$^-$ *str*^{r} *azi*^{s}. The *leu*$^+$ marker from strain W1655 was introduced into the previous strain, using phage P1*kc* (Lennox, 1955) as the transducing agent.

W945 F^- *thr*$^-$ *leu*$^-$ B_1^- *str*^{r} *T6*^{r} *lac*$^-$ *gal*$^-$ (Cavalli-Sforza & Jinks, 1956).

Hfr P4X *met*$^-$ *str*^{s} *lac*$^+$ *gal*$^+$ (Adelberg & Burns, 1960).

J62 F^- *pro*$^-$ *try*$^-$ *his*$^-$ *str*^{r} *lac*$^-$ (Clowes & Rowley, 1954).

1177 F^- *ade*$^-$ B_1^- *str*^{r} *T6*^{r} *lac*$^-$ (a *T6*^{r} derivative of a strain provided by Dr P. G. de Haan).

The origins and directions of transfer of the Hfr strains mentioned in the text, as well as the symbols for the genetic markers, are given in Fig. 1.

(ii) *Media*

Nutrient broth: 2·5% Oxoid Nutrient Broth No. 2.

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Minimal media; as described by Lederberg & Tatum (1947) but without asparagine.

Nutrient and minimal agar were made by solidifying the appropriate liquid media with 1·25% and 1·5% Davis New Zealand Agar respectively.

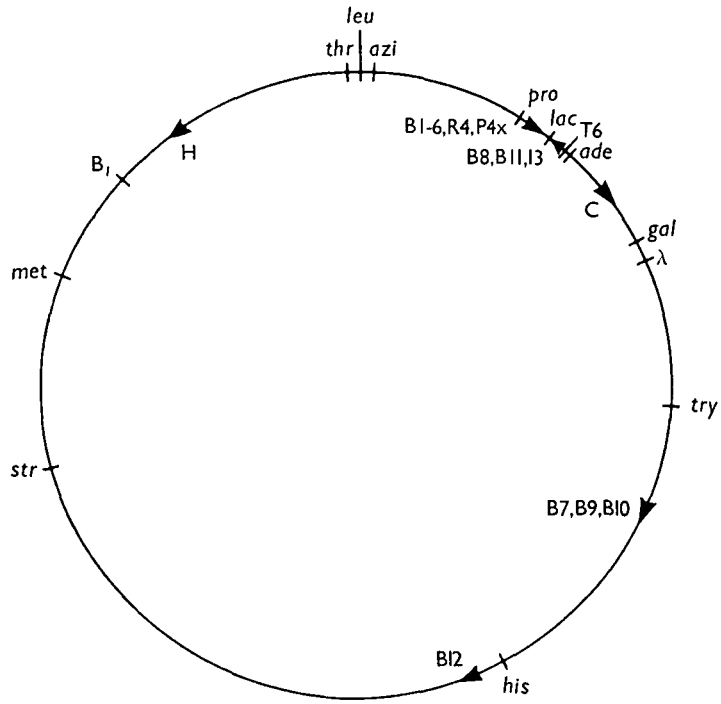

Fig. 1. The genetic map of *Escherichia coli* K12, showing the location of markers mentioned in this paper. The arrows represent the origins and directions of transfer of Hfr strains mentioned in the text. Markers are shown outside the circle and Hfr strain symbols inside. The abbreviations used are the following.

ade adenine, B_1 vitamin B_1, *his* histidine, *leu* leucine, *met* methionine, *pro* proline, *thr* threonine, *try* tryptophan, λ = prophage λ. *azi*, *str*, *T6*; resistance/sensitivity to azide, streptomycin, and phage *T6* respectively. *gal*, *lac*; fermentation of galactose, lactose.

The origins of the following Hfr strains are given: C (Cavalli, 1951); H (Hayes, 1953); P4X (Adelberg & Burns, 1960); R4 (Reeves, 1959); 13 (Hirota & Sneath, 1961); B1–12 (see text).

Buffer: An aqueous solution (pH approx. 7·2) with 0·7% anhydrous $NaHPO_4$, 0·4% NaCl, 0·3% KH_2PO_4 and 0·02% $MgSO_4.7H_2O$.

Aminoacids, vitamin B_1 and streptomycin were added to give final concentrations of 20, 10 and 250 μg./ml. respectively.

(iii) *Culture methods*

Strains were maintained at 4°C. on nutrient agar slopes. All experiments were performed at 37°C. Stationary-phase cultures were obtained by inoculating into

nutrient broth and incubating without aeration. After overnight growth such cultures contain about 10^9 cells per ml.

(iv) *Interrupted mating experiments*

In interrupted mating experiments a hundred-fold dilution of an overnight culture of the donor strain was made into fresh broth. This culture was incubated for $1\frac{3}{4}$ hours (giving about 2×10^7 cells per ml.) on a 33 r.p.m. rotor, at 37°C., before mixing with an equal volume of an overnight culture of the recipient strain. At appropriate times samples were withdrawn, diluted ten-fold into buffer, violently agitated for 1 min. on a Microid flask shaker running at full speed, and further diluted five-fold. Samples (0·2 ml.) were plated onto appropriate selective media.

(v) *Technique for the isolation of Hfr strains*

An overnight culture of the F^+ strain W1655 *met*$^-$ *str*^{s}, derived from a single colony, was diluted into fresh broth to give a suspension with about 100 cells per ml. Forty-four 1 ml. aliquots were dispensed into tubes, which were then incubated at 37°C. for $5\frac{1}{2}$ hours, to give populations of about 5×10^6 cells per ml. Samples (0·15 ml.) were withdrawn and mated for 1 hour at 37°C. with 0·1 ml. of a culture of an F^- strain (about 2×10^7 cells) suspended in buffer. A drop of this mixture was then plated onto selective medium. The remainder of the F^+ cultures were stored at 4°C. until it was known which of them would be used for the next cycle of the enrichment procedure.

The cultures which gave rise to many recombinant colonies were inferred to contain a large clone of Hfr cells with an origin relatively close to the selected markers. Such cultures were only enriched if they gave more than four times the average number of recombinants; they were then diluted into fresh broth, so that there was about one Hfr cell per 10 ml. The number of recombinants obtained in the above cross was used as the approximate, empirical, dilution factor. One millilitre aliquots were then dispensed into a fresh series of tubes and incubated for $4\frac{1}{2}$ hours before mixing 0·15 ml. samples with the female strain, and plating as before for recombinants. In the isolation of the different Hfr strains, one to three cycles of dilution, incubation and mating were required before the cultures were sufficiently enriched to allow plating on nutrient agar for the picking of Hfr colonies. This process was repeated to obtain each Hfr strain isolated.

(vi) *Rate of reversion from the Hfr to the F$^+$ state*

Overnight broth cultures of each of these strains were diluted $\times 2 \cdot 10^{-10}$ into broth and dispensed as 1 ml. aliquots so that the initial number of cells in any one tube is unlikely to be more than one. After overnight incubation those tubes which yielded

growth were checked for fertility in crosses with appropriate recipient strains and dilutions were plated for the recovery of isolated colonies. One hundred colonies derived from each Hfr strain were resuspended in 1 ml. of broth, and 0·5 ml. portions were used for scoring fertility of the Hfr or the F^+ type by mixing with an equal volume of a culture of an F^- strain and after 3 hours' incubation plating standard loopfuls (*ca.* 0·02 ml.) of the mixture for recombinant colonies on selective media. This technique gives better discrimination than the use of replica plating. In general each clone gave a clear Hfr or F^+ level of fertility. The few clones which were doubtful, and all apparent F^+ clones, were retested to ensure that low fertility was not an artefact.

3. RESULTS

(i) *Isolation and characterization of Hfr strains*

Hfr strains were isolated by a modification of the method of Jacob & Wollman (1956). These authors obtained F^+ cultures containing a high proportion of Hfr cells by the Luria and Delbrück fluctuation method (1943), and isolated Hfr colonies from such cultures by replica plating. In the present study, the method (see Materials and Methods, Section (v)) differs in three respects. First, no ultraviolet irradiation was used. Second, cells were kept in broth at all stages of the isolation procedure, so that mutations to auxotrophy which might accompany the transition from the F^+ to the Hfr state would not necessarily be lethal. Third, Hfr strains were isolated from the original Hfr-rich F^+ cultures by Cavalli and Lederberg's sib-selection enrichment technique (1955) instead of by replica plating. This involves estimating how many Hfr cells there are in a culture so that when aliquots from a dilution of this culture are dispensed into a series of tubes only a few of them will receive an Hfr cell. In this minority of cultures the Hfr population will therefore be enriched with respect to the background of F^+ cells because the ratio of F^+ cells to Hfr cells in these few tubes will be smaller than in the original undiluted culture. This method has been used independently by Mäkelä (1963) for the isolation of Hfr strains in *Salmonella abony*. Each Hfr strain isolated was derived from a culture started from a single F^+ colony; there was therefore no possibility of the repeated isolation of the same Hfr clone.

The strain used in this study was W1655 F^+, a methionineless, non-lysogenic derivative of the original Lederberg (1947) strain 58-161. In matings it gives approximately equal numbers of recombinants for markers distributed around the chromosome, so that there is no special affinity of the sex factor for any particular region of the chromosome like that described by Richter (1961). In the first series of isolations, selection was for Hfr strains which transfer thr^+leu^+ early; the recipient strain used was W945 $thr^-leu^-B_1^-str^r$. Six independently arising Hfr strains were obtained; these were characterized by 1 hour crosses and by interrupted matings (see Methods), in which it was found that each of these strains (B1–6) began to transfer the pro^+ and thr^+leu^+ genes at about $5\frac{1}{2}$ and 13 min. mating respectively.

None of them transferred the *lac*$^+$ locus with high efficiency. It was therefore concluded that the origins of all these strains are in the small region between *pro* and *lac*. This result strongly suggests that there are specific regions where the sex factor can integrate stably to give Hfr strains.

To determine whether another class of Hfr strains exists in which the sex factor is also integrated between the *pro* and *lac* loci, but which transfer the chromosome in the opposite direction, a second series of isolations was undertaken. The recipient strain used was J62 (*pro*$^-$*try*$^-$*his*$^-$*str*^{r}), and the isolation of Hfr strains transferring the *try*$^+$ gene (which is about the same distance as the *thr leu* loci from the *pro-lac* region) was attempted. Six further Hfr strains (B7–12) were isolated; they fell into three groups. Whereas strain B12 transferred *his*$^+$ very early, the origins of strains B8 and B11 fell between the *T*6 and *lac* loci; these two strains, which have very similar transfer kinetics, transfer *ade*$^+$ early and *lac*$^+$ late, and were found to be very similar to Hfr strain 13 (Hirota & Sneath, 1961). Hfr strains B7, 9 and 10 form the third class. All three transfer *try*$^+$ after 7–10 min. and *his*$^+$ late. However, since Hfr strains with the sex factor integrated between *pro* and *lac*, transferring *lac* first, were not found, it was tentatively concluded that in this region at least, the sex factor can only integrate to give Hfr strains transferring the chromosome with one polarity.

(ii) *To distinguish between apparently similar Hfr strains*

(*a*) *Transduction*

An obvious approach was to compare the linkage between the sex factor and the closest known chromosomal markers, using transduction with phage P1*kc* (Lennox, 1955). In such an experiment, using Lennox's methods, Hfr strain B1 and a *pro*$^-$*lac*$^-$ strain were used as the donor strain and the recipient strain respectively. The recipient strain was a *pro*$^-$ derivative of strain W677 *thr*$^-$*leu*$^-$*B*$_1^-$ made *leu*$^+$ so that a marker transferred early by Hfr strain B1 was available for the testing of *lac*$^+$ transductants for the Hfr property. Although both *pro*$^+$ and *lac*$^+$ transductants were obtained, of several hundred recombinants tested, none had inherited either the unselected marker (that is, *lac*$^+$, *pro*$^+$ respectively) or the sex factor.

Since DeWitt & Adelberg (1962) had reported joint transduction of the *lac*$^+$ character and the sex factor in an experiment in which Hfr strain P4X was used as the donor and a recipient strain analogous to strain W677 *pro*$^-$ as the recipient, Hfr strain P4X was then used in a control experiment. Again, none of the *lac*$^+$ transductants obtained carried the sex factor. Because of the absence of any positive result, this approach was not pursued.

(*b*) *The rate of reversion from the Hfr to the F*$^+$ *state*

It was thought that each species of Hfr strain might have a characteristic instability. The proportion of F$^+$ cells among the descendants, in a known number of generations, of single cells of the different Hfr strains was therefore determined and compared (see Methods).

It was found that with none of the Hfr strains tested (B1–11) had the proportion of F^+ bacteria became greater than 2% after the thirty generations of growth from one cell to about 10^9 cells. Therefore, to obtain greater resolution, clones which had been serially subcultured through a total of about eighty generations were examined. It was important to show that the results obtained by this method with a given Hfr strain are reproducible. All determinations were therefore made on two or three parallel cultures, each starting from a different single cell of the Hfr strain.

Table 1. *The formation of F^+ derivatives during subculture of Hfr strains B1–11*

Hfr strain	Approximate number of generations of growth	Number of colonies tested	Number of revertant clones
B1	80	200	3
B2	80	100	0
B3	80	100	0
B4	80	100	0
B5	80	100	0
B6	80	100	0
B7	30	100	0
	80	100	91
B9	30	100	2
	80	100	96
B10	30	100	0
	80	100	99
B8	80	100	1
B11	80	100	3

Overnight broth cultures of Hfr strains B1–11 were diluted $2 \cdot 10^{-10}$ into broth and dispensed as 1 ml. aliquots. After overnight incubation, dilutions were made, first 2×10^6 into broth for a second cycle of overnight growth, and, second, for plating on nutrient agar for the recovery of 30-generation colonies. After three cycles of dilution and overnight incubation, dilutions and platings were made for the recovery of 80-generation clones. Colonies were tested for the Hfr character by suspending in broth, incubating and crossing with appropriate F^- strains (W677 *thr⁻leu⁻B₁⁻pro⁻str^r* for strains B1–6, and J62 *pro⁻try⁻his⁻str^r* for strains B7–11).

Standard loopfuls (*ca.* 0·02 ml.) of the mating mixtures were plated on to selective media for the recovery of *thr⁺leu⁺* (strains B1–6) and *try⁺* (B7–11) recombinants. In each case the results given are the sum of two or three parallel experiments.

It was confirmed that within an Hfr strain the data from parallel experiments are very similar. For example, in the experiment given in Table 1 the three Hfr B7 clones subcultured in parallel gave rise to 32/34, 30/33 and 29/33 revertants.

The results are presented in this table. Hfr strains with similar origins tended to give similar numbers of F^+ cells, but Hfr strains with different points of origin yielded very diverse numbers of F^+ cells. Thus Hfr B8 and B11 resembled each other, as did strains B7, 9 and 10. An exception was strain B1. Two out of three subcultures of this strain gave rise to F^+ revertants. It therefore appears to differ from strains B2–6, if these are considered together. No revertants were found among any subcultures of these strains, so that they are indistinguishable within the limits of the experiment.

A very interesting point emerges from a comparison of the thirty- and eighty-generation samples from strains B7, 9 and 10. It appears from these data that during growth of these strains the proportion of F^+ cells increases exponentially. The simplest explanation, assuming a constant rate for the Hfr→F^+ transition, is that the Hfr cells grow more slowly than the F^+ cells. To account for the observed rate of increase of the F^+ population in these cultures the Hfr cells would have to grow at about 0·76 of the rate (1·0) for F^+ cells. The growth rates of strains Hfr B10 and W1655 F^+ in broth at 37°C., measured by dilution and plating for viable colony counts, were compared; the experimental result obtained was 0·79. We may therefore conclude that there is a difference in growth rate, sufficient to explain this effect, between Hfr strains of the B7 type and the F^+ strain from which they arose, and that the latter grows at a similar rate to the F^+ revertants. However, slower growth rate is not a general property of Hfr strains, since strains W1655 F^+ and Hfr B1 grow at the same rate.

(c) *The transfer race technique*

The object of this refinement of the interrupted mating experiment (suggested by Dr Julian Gross) is to exclude some of the factors which cause fluctuations between the results of different experiments. Comparisons between pairs of Hfr strains are made by using them in a mixed mating with the same F^- strain, so that all sampling errors and environmental effects cancel out. The contribution which each of the two donor strains makes to the recombinant population can be assessed by labelling one of them with a non-selective marker which is inherited with a high and constant frequency by the selected recombinants. In the present study the entry times of thr^+leu^+ for Hfr strains B1 and B3 were compared, since although they were indistinguishable in simple interrupted mating experiments, in the reversion experiment (see above) only strain B1 gave F^+ derivatives at a measurable rate. Azide resistance (azi^r) was used as the marker labelling one of the Hfr strains; since both strains transfer their *azi* allele just before thr^+leu^+, all the selected recombinants will have received the *azi* locus, irrespective of the time at which mating is interrupted. If one of the strains transfers the thr^+leu^+ loci earlier than the other strain does, in mixed matings the 'winning' strain will contribute a higher proportion of the first-formed recombinants; these can be recognized by the *azi* allele which they carry.

Azide-resistant derivatives of strains B1 and B3 were isolated and the following pairs of strains were together mated with the female strain W677 $thr^-leu^-B_1^-pro^-str^r azi^s$

$$\text{B3 } azi^s + \text{B3 } azi^r$$

$$\text{B3 } azi^s + \text{B1 } azi^r$$

The former cross (Table 2) showed that the *azi* character itself did not affect the kinetics of mating. The result of the second cross shows that within the limits of the

experiment, Hfr strains B1 and B3 are indistinguishable with respect to the time of transfer of the *thr*$^+$ and *leu*$^+$ loci.

Table 2. *The transfer-race experiment*

Hfr strains in cross	Time at which mating is interrupted	Total number of *thr*$^+$*leu*$^+$ recombinants obtained per 0·2 ml. sample	Number of recombinants tested	*azi*^{r}	
				No.	%
B3 *azi*^{s} + B3 *azi*^{r}	12	96	108	71	66
B3 *azi*^{s} + B3 *azi*^{r}	25	712	108	66	61
B3 *azi*^{s} + B1 *azi*^{r}	12	150	72	47	65
B3 *azi*^{s} + B1 *azi*^{r}	25	> 1000	72	47	65

Overnight broth cultures of the Hfr strains were diluted 10^{-2} into fresh broth. After 1¾ hours' incubation at 37°C. on a rotor the two Hfr cultures were mixed, and a 5 ml. volume of the mixture was added to an equal volume of an F$^-$ strain (W677 *thr*$^-$*leu*$^-$*B*$_1^-$*pro*$^-$*str*^{r}*azi*^{s}). At appropriate times samples were withdrawn, diluted tenfold into buffer, violently agitated for 1 min. and further diluted fivefold. Samples (0·2 ml.) were plated on minimal medium supplemented with glucose, proline, vitamin B$_1$ and streptomycin, for *thr*$^+$*leu*$^+$ recombinants. Recombinant colonies were resuspended in buffer and streaked on plates of the selective medium supplemented with M/1600 (final concentration) sodium azide. Under these conditions an unambiguous resistance/sensitivity result was obtained for the *azi* character.

(iii) *The analysis of recombinants formed in F*$^+$ *crosses*

In the present study selection at each stage in the isolation procedure of the tube giving rise to most recombinants, resulted in the enrichment and isolation of Hfr strains, so that we may conclude that these strains are indeed derived from the observed fertile clones. However, there is no proof that such clones are the sole basis for the fertility of F$^+$ cultures. We may ask whether Hfr strains such as those isolated (B1–12) in the present study are sufficient to explain the fertility of strain W1655 F$^+$.

Strains B1–12 divide into three groups with respect to the transfer of the *pro lac try* region of the chromosome. While strains B1–6 transfer *pro*$^+$ but not *lac*$^+$ or *try*$^+$ with high frequency, and strains B8 and B11 transfer only *try*$^+$, strains B7, 9 and 10 and also strain B12 transfer all three loci with high frequency. Thus whereas *pro*$^+$ and *try*$^+$ are transferred by two of the three groups of strains, *lac*$^+$ is only transferred by the B10 group. Therefore, if F$^+$ fertility is due to Hfr clones (and assuming that strains B1–12 are a representative sample of these), we should expect that in F$^+$ × F$^-$ crosses *lac*$^+$ will be transferred less frequently than either *pro*$^+$ or *try*$^+$ and also that the proportion of the *lac*$^+$ recombinants which are *pro*$^+$ will be greater than the proportion of *pro*$^+$ recombinants which are *lac*$^+$.

Strain W1655 F$^+$ (from which strains B1–12 had been isolated) was mated with strain J62 F$^-$ *pro*$^-$*try*$^-$*his*$^-$*lac*$^-$*str*^{r} in a 1-hour cross. Dilutions were plated onto selective media for *pro*$^+$ recombinants on the one hand, and *lac*$^+$ recombinants on the other. It was not possible to draw conclusions from the analysis of the *lac*$^+$

recombinants, since it was found that all these, both in this cross and the control Hfr cross (see below), were *pro*$^+$ and *try*$^+$. This phenomenon, also observed by Professor B. A. D. Stocker (personal communication), has been termed by him 'inadvertent selection'. However, the frequency of inheritance of the *lac*$^+$ character can be examined among the *pro*$^+$ recombinants, which were also tested for the *try* character.

Table 3. *Linkage between* pro, lac *and* try *among* pro$^+$ *recombinants in crosses of strains Hfr B*10* *and W*1655 *F*$^+$ *against strain J*62 pro$^-$try$^-$his$^-$lac$^-$str^r

1. Approximate number of recombinant per ml. of mating mixture

Cross	Recombinant class	
	pro$^+$	*try*$^+$
Hfr B10 × F$^-$	$9{\cdot}4 \times 10^6$	$3{\cdot}4 \times 10^7$
F$^+$ × F$^-$	$1{\cdot}8 \times 10^4$	$2{\cdot}8 \times 10^4$

2. Analysis of *pro*$^+$ recombinants

Donor strain	Number of recombinants tested	Percentage of the different classes of recombinants			
		lac$^+$*try*$^+$	*lac*$^+$*try*$^-$	*lac*$^-$*try*$^+$	*lac*$^-$*try*$^-$
Hfr B10	160	53	28	9	10
F$^+$	232	11	53	1	35

Logarithmically growing broth cultures were mated for 1 hour before dilution and plating on minimal selective medium for *pro*$^+$ and *try*$^+$ recombinants. Purified *pro*$^+$ recombinant colonies were suspended in buffer and streaked onto selective media to score for the *try* and *lac* characters.

* Hfr strain B10 transfers its markers in the order *try*$^+$*lac*$^+$*pro*$^+$.

A control cross was made with Hfr strain B10, which transfers genes in the order 0 *try lac pro*. It was found that of the *pro*$^+$ recombinants 81% were *lac*$^+$ and 62% were *try*$^+$ (Table 3). In the F$^+$ cross 64% of the *pro*$^+$ recombinants were also *lac*$^+$. Since linkage between these genes (as determined in the Hfr cross) is 81%, in the F$^+$ cross, in about 79% (that is, $64 \times \frac{100}{81}$) of the *pro*$^+$ recombinants the *lac*$^+$ gene will have entered the recipient cell.* Since Hfr strains B1–6 do not transfer *lac*$^+$, according to these results only $100 - 79 = 21\%$ of the *pro*$^+$ colonies can possibly have come from this type of strain.

It might be argued that Hfr clones of the B10 type would account for the *pro*$^+$ recombinants which are *lac*$^+$. However, only 12% of the *pro*$^+$ recombinants from the F$^+$ cross are *try*$^+$. Since Hfr strain B10 showed 62% linkage between the *pro*$^+$ and *try*$^+$ markers, the maximum proportion of the *pro*$^+$ recombinants which could

* It is assumed that the sizes of the DNA fragments transferred in the F$^+$ and Hfr matings are similar. If this assumption is false, it follows that F$^+$ fertility cannot be due to Hfr clones.

have been derived from this type of donor is $12 \times \frac{100}{62} = 19\%$. We must therefore conclude that less than half of the *pro*$^+$ recombinants in F$^+$ crosses originated from Hfr cells of the B1 and B10 types.

4. DISCUSSION

When Hfr strains transferring the *thr*$^+$ and *leu*$^+$ loci as proximal markers were selected for, all six isolates (strain B1–6) were found to have their origins in a small chromosomal region between *pro* and *lac*, about 10 min. of transfer time away from the *thr leu* region. The length of this *pro lac* segment is about 2% of that of the whole chromosome (Jacob & Wollman, 1961). A total of at least twelve Hfr strains transferring *pro* early and *lac* late have now been isolated. As well as the six Hfr strains B1–6 and three more strains isolated by the present writer using a replica plating method, Hfr strains R2 and R4 (Reeves, 1959) and P4X (Adelberg & Burns, 1960) also have the sex factor integrated in this region. A firm conclusion is therefore that Hfr strains isolated by this method arise by non-random integration of the sex factor into the chromosome. This conclusion has also been drawn by Sanderson & Demerec (1965) (for Hfr strains in *Salmonella typhimurium*) and Matney *et al.* (1964).

In simple interrupted mating experiments the origins of Hfr strains B1–6 were indistinguishable. In more refined transfer experiments Hfr strains B1 and B3 could still not be distinguished, although these strains differed in that F$^+$ derivatives were found in sub-cultures of strain B1 but not in those of strain B3. Unsuccessful attempts were also made to compare the linkage of the sex factors of Hfr strains B1–6 to neighbouring chromosomal genes by transduction using phage P1.

The isolation of other Hfr strains (B7–12) is also described; the fact that five out of six fall into two groups is strong support for the proposition that there are specific small regions where the sex factor can integrate to give rise to stable Hfr strains. Hfr strains of the reciprocal type to B1–6 (that is, strains with the sex factor integrated in the same region but transferring the chromosome in the opposite direction) were looked for but not found; this is evidence that these integration sites have polarity.

The repeated isolation of similar types of Hfr strain, and the absence of the reciprocal type to strains B1–6 would both be expected if the formation of Hfr strains is dependent on pairing between homologous regions of the DNA of the sex factor and of the chromosome. Models postulating that the sex factor can integrate with equal ease at all points on the chromosome are excluded, as are those which propose that the sex factor associates with non-DNA regions of the chromosome, unless assumptions on the nature of this attachment are made to account for the observed polarity. These results are consistent with Campbell's model (1962) for insertion by recombination, although they do not exclude pairing without recombination as a mechanism for integration.

In experiments with Hfr strains B1–11 cultures were grown for a similar number of generations from a single fertile cell. Striking differences were observed in the

frequencies of F^+ cells in the sub-cultures of the different Hfr strains, although in general the strains within a group gave comparable results; thus strains B7, B9 and B10 gave values respectively of 91%, 96% and 99% F^+. An exception was strain B1, which gave revertants and appeared to differ significantly in this respect from strains B2–6 which yielded none.

However, further investigation showed that the high proportion of F^+ cells in the cultures of Hfr strains B7, 9 and 10 was in each case mainly due to the fact that these Hfr strains grew much more slowly than the F^+ cells arising from them rather than to an inherently high degree of instability. This result suggests that the growth rate of an Hfr strain is a function of the region at which the sex factor is integrated.

Jacob and Wollman's observation (1956) that persistent fertile clones arise in F^+ populations has been confirmed in the present study by the isolation of Hfr strains B1–12 following enrichment from F^+ cultures chosen for their fertility. However, the examination of recombinants obtained in an $F^+ \times F^-$ cross, using the F^+ strain from which the Hfr strains were isolated, gave very different frequencies for the inheritance of unselected markers from those predicted on the assumption that F^+ fertility is due solely to Hfr strains of the types isolated in the present study. Analysis showed that Hfr clones of the B1 and B10 types together cannot contribute more than half the *pro*$^+$ recombinants obtained in the F^+ cross. It is therefore inferred that the types of Hfr strain actually isolated can only contribute a component to F^+ fertility. However, we must treat this conclusion with some caution, because if a large number of Hfr clones arose similar in transfer properties to the Hfr Cavalli strain (that is, transferring both *lac*$^+$ and *pro*$^+$) this type of result would be obtained.

A Campbell-type model for the formation of Hfr strains by recombination allows us to propose an explanation for this result, as well as for the observation that Hfr strains B7, 9 and 10 grow more slowly than their F^+ revertants and the strain from which they arise. If recombination between sex factor and chromosome occurs at non-allelic regions of limited homology distributed randomly around the chromosome, insertion within a gene or operon could lead to impairment of function and the consequent slow growth rate or even death of the cell. Such clones with a potentially lethal integration of the sex factor might persist for a number of generations, contributing to the fertility of F^+ cultures, but would be impossible to isolate. Since the procedure employed in the isolation of strains B1–12 involved competitive growth of the Hfr strains with strain W1655 F^+ (although growth was limited as far as possible) we should not expect to isolate very defective Hfr strains. However, one class of Hfr types which might still have been isolated was not observed; although at all stages in their isolation strains B1–12 were kept in broth, none had any nutritional requirements additional to those of the ancestral strain. Also, like strain W1655 F^+, they were able to utilize lactose.

Another possible explanation for the experimental result that strains B7, 9 and 10 grow more slowly than strain W1655 F^+ is that growth rate is determined by the length of the chromosome, the DNA of which is replicated at a constant rate, and

that Hfr strains, with the sex factor inserted into the chromosome, grow more slowly than the F+ strains because they have a longer chromosome. However this is excluded on two grounds. In the first place, it predicts that all Hfr strains should grow more slowly than F+ ancestors; this was not observed with strain B1. Secondly, the predicted slowing in growth rate would be of the order of 1–2% (the size of the sex factor relative to that of the chromosome, a difference which is undetectable by the methods used in these experiments). The observed slowing in growth rate for strains B7, 9 and 10 was about 21%.

SUMMARY

The isolation and characterization of an isogenic series of twelve Hfr strains is described. From their points of origin it was concluded that the sex factor integrates at a limited number of sites on the bacterial chromosome. Although the nutritional requirements of these Hfr strains were similar to those of the parent F+ strain, it was observed that one group had a markedly slower growth rate. The relevance of these observations to theories on the nature of F+ fertility and on the formation of Hfr strains is discussed.

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