GENETIC VARIATION IN THE SEX FACTOR OF *ESCHERICHIA COLI*[1]

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The mating type of *Escherichia coli* is determined by the presence or absence of the sex factor (F). Cells which lack F are genetic recipients or females, and are designated F^-; cells which possess F may be genetic donors, or males (Lederberg *et al.*, 1952). Two types of males have been described: F^+, in which the sex factor behaves as though it were a population of autonomous, extrachromosomal particles, transmissible by cell-to-cell contact; and Hfr, in which the sex factor is firmly bound to the chromosome and is not transmissible except as a chromosomal locus (Jacob and Wollman, 1957). The name "episome" has been proposed for genetic determinants of this type, which have alternative states of existence as cytoplasmic particles or as attached chromosomal elements (Jacob and Wollman, 1958). The genetic material of a temperate phage is another example of an episome.

Male cells of *E. coli* appear to transfer their chromosome to females only when F is in the attached state. Thus, all Hfr cells transmit chromosome, but in a population of F^+ cells, only those few in which F is attached act as donors. Such donor cells probably include both stable Hfr mutants as well as cells in which the attachment is unstable and transitory. The chromosome of the F^+ cell forms a closed circle of genetic markers (figure 1); the attachment of F causes a break in the chromosome such that F remains attached to one broken end, whereas the other end becomes the leading point or "origin" in chromosome transfer. Thus, in chromosome transfer by an Hfr, attached sex factor is always the last marker to be transferred (Jacob and Wollman, 1957). Since conjugation may be interrupted at any time by spontaneous separation of the mating couple, partial transfer of the chromosome is the general rule. As a consequence, the farther away a marker is from origin, the less chance it has of being transferred, and transfer of attached sex factor in Hfr $\times$ F^- crosses is a very rare event. The rare recombinant which receives attached F becomes a donor, usually Hfr.

The stable attachment of F to the chromosome has as a second consequence the disappearance of the autonomous population of F particles. This effect is analogous to the repression of vegetative multiplication of temperate phage by chromosomally attached prophage. Since Hfr cells lose their autonomous F population, and transfer attached F as a very rare event during conjugation, the progeny of Hfr $\times$ F^- crosses are usually F^-.

The stability of attachment of F in Hfr cells is a relative matter; most Hfr strains are capable of reversion, at varying rates, to the F^+ state. This phenomenon serves to confirm the identity of attached and autonomous F.

We propose that the sex factor of strain K-12 be designated as wild-type F. *Wild-type F is characterized by having a low affinity for the chromosome, with no preferential site of attachment; and by the fact that when attachment occurs, extrachromosomal multiplication of F ceases.* In the present paper, a genetic variant of F is described, and evidence is presented for its origin by recombination of attached F and chromosome. The existence of sex factors with recognizably different properties requires a system for their designation. We will accordingly refer to the wild-type F of strain K-12 as F^1, and the variant F to be described below as F^2.

A preliminary report of this work has appeared elsewhere (Adelberg and Burns, 1959).

MATERIALS AND METHODS

Media. Liquid cultures were prepared in the broth described by Luria and Burrous (1957) supplemented with 0.1 per cent glucose. Nutrient agar was prepared by solidification of the same medium (without glucose) by addition of 2 per

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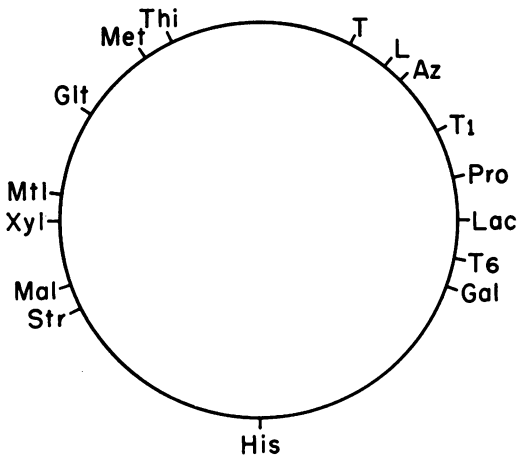

Figure 1. Genetic map of *Escherichia coli*, showing relative location of markers referred to in this paper (see table 1 for abbreviations used). (After Jacob and Wollman, 1957).

cent agar. The defined medium used for selection of various recombinants was a half-strength preparation of medium 56 described by Monod *et al.* (1951). Sugars were added as carbon-source at a final concentration of 0.2 per cent. To score for fermentation markers, sugars were added to nutrient agar at a final concentration of 1.0 per cent, with bromothymol blue at 0.0012 per cent final concentration as an indicator of acid production. Amino acids, when used as supplements, were added at predetermined optimal concentrations ranging from 5×10^{-4} M to 2×10^{-3} M. Streptomycin was used at a final concentration of 200 μg per ml.

For good growth of recombinant clones, it was found essential to autoclave the agar, the mineral base, and the sugar separately.

Culture methods. Stock cultures were maintained on slants of nutrient agar. Cultures to be used in an experiment were grown overnight in

TABLE 1

Summary of strains

Strain No.	Auxotrophic Characters						Energy-Source Utilization					Response to Phages		Response to Antibacterial Agents			Sex
	TL	*pro*	*his*	*glt*	*met*	*thi*	*lac*	*gal*	*mal*	*xyl*	*mtl*	T1	T6	*Az*	*str*	*TA*	
AB-1	+	+	−	+	+	−	−	−	−	−	−		s	s	r	r	♂
AB-9	+	+	−	−	+	−	−	−	−	−	−		r	s	r		♂
AB-13	+	+	−	+	+	−	−	−	−	−	−		r	s	s		♂
AB-57	+	−	−	+	−	−	−	−	−	−	−	s	s	s	r	s	♀
AB-89	+	+	−	+	−	−	+						s	s	r	s	♀
AB-91	+	+	+	+	−	+	+	+	+	+	+	s	s	s	s	s	♀
AB-113	−	+	−	+	+	−	−	−	−	−	−		r		r	s	♀
AB-122																	
AB-124	+	+	+	+	+	+	−	+	+	+	+	s	s	s	s	s	♂
AB-126																	
AB-127																	
AB-128	+	+	+	+	+	+	+	+	+	+	+	s	s	s	s	s	♂
AB-129																	
AB-151	−	−	−	+	+	−	−	−	−	−	−	r	r	r	r	s	♀
AT-12	−	+	+	+	+	−	−	+	+	+	+	s	s	s	r	s	♂
K12	+	+	+	+	+	+	+	+	+	+	+	s	s	s	s	s	♂
P4x																	
P4x-1	+	+	+	+	−	+	+	+	+	+	+	s	s	s	s	s	♂
P697	−	+	−	+	+	−	−	−	−	−	−	r	s	s	s	s	♀
PA-214S^R/T6	−	+	−	−	+	−	−	−	−	−	−	r	r	s	r	s	♀
TA-15S^R	−	+	−	+	+	−	−	−	−	−	−	r	s	s	r	r	♀
W208S^R	−	+	+	+	+	−	−	+	+	+	+	s	s	s	r	s	♀
W2961	−	−	+	+	+	−	−	−	+	−	−	s	s	s	r	s	♀

The following abbreviations are used: *TL*, threonine and leucine; *pro*, proline; *his*, histidine; *glt*, glutamic acid; *met*, methionine; *thi*, thiamine; *lac*, lactose; *gal*, galactose; *mal*, maltose; *xyl*, xylose; *mtl*, mannitol; *Az*, azide; *str*, streptomycin; *TA*, thienylalanine; s, sensitive; and r, resistant.

broth with shaking. They were then diluted approximately 1:100 in fresh broth and reincubated with shaking at 37 C until they were in exponential phase. Erlenmeyer flasks fitted with Klett-tube side arms were employed, so that exponential growth could be verified by periodic readings in the Klett colorimeter.

Strains. The strains used in these experiments are described in table 1. They are all originally descended from strain K-12 of *E. coli*.

Mating conditions. Exponential growth cultures, as described above, were adjusted to a cell density of from 1 to 4 $\times$ 10^8 per ml and mixed in appropriate volume ratios. Generally, 0.5 ml of the minority parent was diluted into 4.5 ml of the majority parent in a 125-ml Erlenmeyer flask, which was incubated without shaking at 37 C. When larger volumes were required, an equivalent surface to volume ratio was maintained. Initial cell densities were adjusted to provide the majority parent in approximately 20-fold excess. Unless otherwise stated, mating mixtures were plated for recombinant selection at 60 min.

Interruption of mating. To follow the kinetics of chromosome or sex factor transfer, periodic samples of a mating mixture of cells were treated in the Waring Blendor (Wollman and Jacob, 1955) or with phage T6 (Hayes, 1957). In the latter case, T6-resistant strains of F^- bacteria were employed.

In several experiments, attempts were made to interrupt mating by placing the sample in a small test tube attached to an electric vibrator and shaking it rapidly for 90 sec at 0 C. The results indicated that complete separation of all mating couples was not achieved, and the method was abandoned.

Replica plating. When colonies appearing on the experimental plates were to be scored for fertility or for unselected markers, they were transferred to a "master" plate containing agar medium of the same composition. A large sample of each colony was inoculated in the form of a patch, and the master plate was incubated until heavy growth was obtained. It was then replicated onto various test plates as described by Lederberg and Lederberg (1952). Clones to be tested for fertility were replicated onto a plate in which about 2 $\times$ 10^9 F^- cells had been spread on the surface of a medium selective for recombinants. A control plate of similar medium without F^- cells was always included in the replication series, as well as a control of the same medium used for the master plate.

Purification of cultures. When new strains, obtained by selection of mutants or recombinants, were to be used in further experiments, they were purified by at least two successive single colony isolations, from plates streaked with dilute cell suspensions.

RESULTS

Discovery of an unusual type of genetic donor. Strain P4x is a typical Hfr strain. It transfers its chromosome in an oriented manner, such that its markers enter in the order O–*pro–TL–thi–met–mal–str–his–gal–lac*–SF, where O represents origin and SF the attached sex factor (see table 1 for other symbols). The recombinant progeny of P4x $\times$ F^- crosses are F^-, except when the lac^+ allele of P4x is selected; lac^+ recombinants are males, as a result of the close linkage of SF and *lac*.

When we first began work with this strain,[2] we were troubled by low frequencies of recombination. Suspecting that the strain had reverted to F^+, we isolated a large number of subclones and tested them for fertility by replica plating. About half of the clones tested as F^+; the others gave dense patches of recombinants on the F^- plate. One each of the "Hfr" and F^+ clones were purified and kept for further study, the presumed Hfr strain being numbered P4x-1. We assumed at the time that we had reisolated strain P4x, and were therefore surprised to find that recombinants receiving the markers TL^+ from P4x-1 all tested as genetic donors by the replica plating method.

A number of the male recombinants were purified and crossed in broth with a $TL^-str\text{-}r$ F^- strain in broth for 100 min. They were found to give $TL^+str\text{-}r$ recombinants at the same rate as strain P4x-1; that is, from 1 to 3 per cent of the donor cell input. A "second generation" recombinant was isolated and found to be again a genetic donor, as was a randomly chosen "third generation" recombinant, strain AB-13 (table 2). It thus appeared that a sex factor was being transferred through repeated crosses, and that recombinants receiving it became donors with a relatively high frequency of recombination.

Since strain P4x-1 differed from strain P4x

[2] Kindly furnished by Dr. Francois Jacob of the Pasteur Institute, Paris.

TABLE 2

Transfer of a sex factor through repeated sexual cycles

Generation	Cross			Recombination Frequency† (% of ♂ Input)	♂ Recombinant Isolated
	♂	♀	Selection*		
1	P4x-1	TA-15S^R	*TL*$^+$ [*str-r*]	1.0	AB-1
2	AB-1	PA-214S^R/T6	*TL*$^+$ [*T6-r*]	1.3	AB-9
3	AB-9	P697	*TL*$^+$ [*glt*$^+$]	1.7	AB-13
4	AB-13	PA-214S^R/T6	*TL*$^+$ [*str-r*]	1.1	—

* The first symbol stands for the marker selected from the donor strain; the symbol in brackets stands for the marker selected from the recipient strain. See table 1 for meaning of symbols.

† Mated 100 min with F$^-$ in 10- to 20-fold excess.

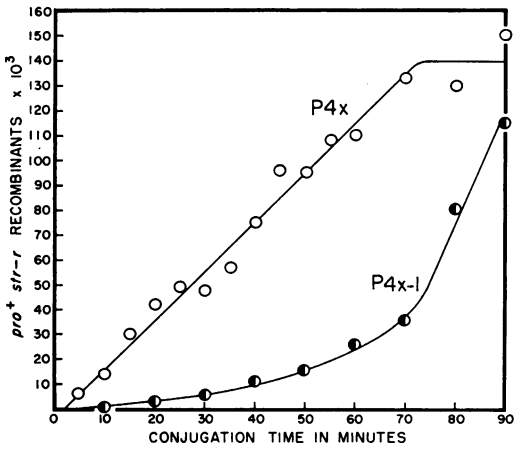

Figure 2. Kinetics of transfer of the *pro*$^+$ locus by strains P4x and P4x-1. The F$^-$ parent was AB-151 in both crosses. Conjugation interrupted by T6 in the P4x cross, and by Waring Blendor in the P4x-1 cross. Similar curves are obtained for P4x-1 regardless of the method used for interrupting conjugation.

with respect to sex factor transfer, the two strains were also compared for rate and orientation of chromosome transfer, to determine whether any other differences existed. Using the technique of interrupted conjugation, it was found that the two strains were identical with respect to the order and times of entry of markers transferred; e. g., both transferred *pro* at about 5 min and *TL* at about 20 min. In both strains, *lac* is transferred last. They differed, however, in their kinetics of zygote formation, P4x-1 showing a much lower initial rate (figure 2), and a consequently lower frequency of recombination as measured under our standard conditions. With a given F$^-$, and a conjugation time of 60 min, strain P4x forms *pro* recombinants at 4.8 per cent of the donor cell input, whereas P4x-1 gives 0.3 to 0.5 per cent.

Transmission of the sex factor of strain P4x-1. Since the original parent strain, P4x, carried the sex factor as an added chromosomal locus, our first hypothesis was that F had undergone a transposition to a new locus somewhere near the *TL* region. Because the sex factor cannot be used as a selective marker, attempts were made to map it by the kinetics of its appearance as an unselected marker in variously selected zygotes, using the Waring Blendor method for interrupting conjugations. However, when recombinants in *pro*$^+$ and *TL*$^+$ selections were isolated at different times and scored for the presence of F, the kinetics of sex factor transfer could not be reconciled with any postulated linkage. Regardless of the selection employed, approximately 60 per cent of the recombinants were males by 30 min, and 100 per cent were males by about 45 min (figure 3*A*). Furthermore, the sex factor did not segregate with either *TL* or *pro*, when the latter were themselves scored as unselected markers.

These results suggested that sex factor transfer was independent of chromosome transfer, like the transfer of F^1 in F$^+$ × F$^-$ crosses. This was confirmed by "conversion" experiments, in which *str-r* F$^-$ cells were exposed to an excess of P4x-1 cells for varying lengths of time, separated in the Waring Blendor, and reisolated on streptomycin agar. From 20 to 60 clones from each sample were transferred to master plates and replicated onto cells of an appropriate F$^-$ strain to score for donor ability. The results of several such experiments are plotted in figure 3*B*. The

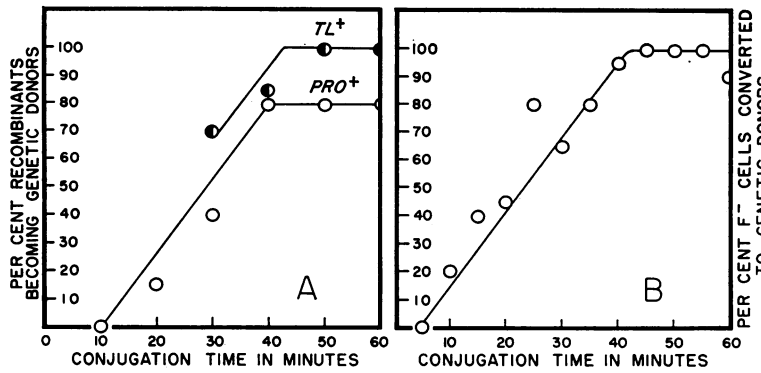

Figure 3. Kinetics of transfer of the sex factor of strain P4x-1. *A*. Sex factor scored as an unselected marker in recombinants receiving the loci *pro*$^+$ or *TL*$^+$ from P4x-1; conjugation interrupted by Waring Blendor. The F$^-$ parent, AB-151, was present in excess. *B*. Sex factor scored in clones of F$^-$ strain AB-57, reisolated on streptomycin agar after exposure to a 20-fold excess of P4x-1 cells. Conjugation interrupted by Waring Blendor.

TABLE 3

Fertility of F$^-$ strain AB-89 after conversion with F^1 and F^2

Donor Strain	Markers Selected from Donor	
	TL$^+$-*pro*$^+$	*TL*$^+$-*lac*$^+$
AB-89 (F^1)	<1	<1
AB-89 (F^2)	123	2

Values in table are recombinants per 10^5 donor cells. All data are from crosses with F$^-$ strain W2961, selecting for the markers shown from the donors and for *str-r* from the F$^-$. Crosses performed as described under Materials and Methods.

sex factor of P4x-1 is seen to enter the F$^-$ cells at 5 min, 100 per cent of the F$^-$ cells being converted to high frequency donors by about 45 min. On the other hand, fewer than 3 per cent of the reisolated F$^-$ cells proved to have received the *pro*$^+$ locus by the end of 60 min.

Thus, strain P4x-1 possesses a freely transmissible F agent, but one which is clearly different from the F agent (F^1) of strain K-12. The F of strain P4x-1 has been designated F^2; the differences between F^1 and F^2 are summarized in the following sections.

Chromosome transfer by strains carrying F^2. When a population of F$^-$ cells is converted to maleness by acquisition of F^1, it becomes a typical F$^+$ strain. As described earlier, only a very small minority of such cells act as chromosome donors at any one time. When F$^-$ cells are converted to maleness by acquisition of F^2, however, the majority of the population become active donors. An even more striking difference between F^1 and F^2 is the fact that cells converted to maleness with F^2 all transfer their chromosome *with the same orientation as was exhibited by Hfr strain P4x*, in which F^2 first appeared. An experiment which demonstrates this clearly is summarized in table 3. In this experiment, the same F$^-$ strain was converted with F^1 and F^2, respectively, and the two resulting donors compared for their frequency of transfer of the loci *pro* and *lac*. (Linked selections with *TL*$^+$ were employed, to eliminate reversions in the F$^-$ strain). It can be seen that strain AB-89 (F^1) transmits both *TL*$^+$-*pro*$^+$ and *TL*$^+$-*lac*$^+$ at very low frequency. AB-89 (F^2), on the other hand, transfers *TL*$^+$-*pro*$^+$, but not *TL*$^+$-*lac*$^+$, at relatively high frequency. The relative order of transfer O-*pro*-*TL*-*lac* has been verified in a number of strains carrying F^2; no exceptions have ever been observed. It can thus be inferred that F^2 has a high and specific affinity for a chromosomal site between *pro* and *lac*.

Maintenance of the autonomous state by F^2. Cells of *E. coli* which carry F^2 transfer their chromosome at relatively high frequency. This property reflects, in typical Hfr strains, the attachment of F to the chromosome. By analogy, and from the specificity of chromosome orientation during transfer, it may be inferred that F^2 also attaches to the chromosome.

In typical Hfr strains, the attached sex factor

actively represses autonomous multiplication of F. F^2, on the other hand, always maintains itself as a freely transmissible population of particles, in spite of its apparent attachment between *pro* and *lac*. It thus appears to be nonrepressive.

One possible explanation for the apparent loss of repression is that F^2 forms an unstable attachment to the chromosome, so that at any given moment some cells in the population have an attached sex factor, while others do not. A dynamic state would then exist, with no particle remaining attached long enough to effect repression. This model predicts that at the moment a population of cells carrying F^2 is mixed with F^- cells, only part of the male population will have the sex factor attached and will be able to initiate chromosome transfer. The rest of the population will gradually become donors, however, as F^2 chances to attach to its specific site in each cell. This model is supported by the observed kinetics of zygote formation in P4x-1 × F^- crosses: an increase in slope is always observed, in contrast with the linear kinetics observed in P4x × F^- crosses (figure 2). To explain these kinetics by the proposed model, it is necessary to postulate that attachment of F is irreversible in conjugating cells.

Origin of F^2. Any theory of the origin of the observed sex factor variation must ultimately account for both properties in which F^2 differs from F^1: its high and specific affinity for a chromosomal site, and its maintenance in the autonomous state. At first glance, the simplest theory would be that F mutated. However, it must be remembered that F^2 has acquired an affinity for the precise site at which the sex factor had been located in Hfr strain P4x, making necessary the postulation of directed mutation. To avoid this, it could be proposed that the original random mutation by which Hfr strain P4x arose from an F^+ cell represented the acquisition of specific affinity by an extrachromosomal F particle, with consequent attachment to the specific site. However, this would require that we postulate a second, later mutation to nonrepressiveness, since attached F is repressive in strain P4x but appears not to be in P4x-1.

TABLE 4

Fertility of F^- strain AB-91 after conversion with F^1 and F^2

Donor Strain	Markers Selected from Donor	
	TL⁺-pro⁺	*TL⁺-lac⁺*
AB-91 (F^1)	684	6
AB-91 (F^2)	2080	66

Values in table are recombinants per 10^5 donor cells. All conditions as described in table 3.

TABLE 5

Linkage of sfa locus to lac in strain AB-91 (F^-)

Recombinant Strain	Parent from which *lac* Allele Inherited	Recombination Frequency after Conversion to ♂ with F^1*
AB-122	AT-12	438
AB-124	AT-12	9
AB-126	AT-12	3
AB-127	AB-91	111
AB-128	AB-91	375
AB-129	AB-91	624

* Each strain crossed with $W208S^R$ (F^-), selecting *TL⁺* from the donor and *str-r* from the F^-. Values are recombinants per 10^5 donor cells.

Strains listed in first column were isolated from a cross of the Hfr strain AT-12 × AB-91 (F^-). See text for details.

We are indebted to Dr. Elie Wollman for first suggesting to us another hypothesis, which has in its favor its excellent simplicity as well as the further unity which it brings to the episome concept. According to this hypothesis, Hfr strain P4x arose by chance attachment of F^1 to the chromosome between *pro* and *lac*; and *F^2 arose by genetic exchange between F^1 and the chromosome.* This would be analogous to the acquisition of the *gal* locus by λ-prophage (Morse *et al.*, 1956). In the case of F^2, the acquisition by the episome of some chromosomal material from its attachment site would give it a large region of structural homology with that site, and explain its "memory" for its previous point of stable attachment.

The second consequence of the genetic change, the apparent loss of repression, is more difficult to explain, especially since the mechanism of repression is entirely unknown. It may, however, only reflect instability of attachment rather than true loss of repressiveness, as proposed above. There is some indication that instability of episome attachment can indeed be a consequence of genetic exchange, since in the case of *lambda*, the *gal⁻*/ex *gal⁺* syngenotes are reported to segregate

haploid types at a relatively high rate (about 10^{-3} per division; Morse *et al.*, 1956).

The fact of an F agent having acquired a specific affinity for a chromosomal site recalls Richter's report (1957) of the exact converse: a chromosomal locus which acquired a high affinity for the wild-type F agent. Since this "mutation" arose in a stock which had been selected as a typical Hfr strain, we may speculate that genetic exchange was responsible for Richter's observation also; in other words, the "Hfr-3" locus had acquired some material from the episome, giving it the observed affinity.

These considerations led us to ask whether, in strain P4x-1, the chromosome had not likewise acquired some of the episome by reciprocal exchange, forming a locus between *pro* and *lac* having a high affinity for wild-type F. To test this, an F^- strain was prepared by treatment of strain P4x-1 with acridine orange (Hirota and Iijima, 1957). This F^- strain (AB-91) was then converted to maleness with F^1 and F^2, respectively, and the resulting donor strains compared for frequency of transfer of the loci *pro* and *lac*. The results are shown in table 4. It is seen that, unlike the ordinary F^- strain AB-89 (table 3), strain AB-91 acquired a relatively high frequency of oriented chromosome transfer when carrying either the F^1 or the F^2 episome.

To prove the existence of a "sex factor affinity" (*sfa*) locus linked closely to *lac*, strain AB-91(F^-) was crossed with Hfr strain AT-12, selecting for recombinants receiving met^+ from the donor and TL^+ from the F^-. AB-91 is lac^+; AT-12 is lac^-. Three lac^+ and three lac^- recombinants were isolated and purified, separately converted to maleness with F^1 and tested for fertility. The results, presented in table 5, show that two of the three recombinants receiving lac^- from AT-12 have lost the *sfa* locus, becoming low frequency donors by acquisition of F^1 particles. The recombinants which received lac^+ from AB-91, however, retained the high affinity for F^1, becoming high frequency donors of the *pro-TL* region when F^1 was introduced.

DISCUSSION

Our experiments show that genetic variation can take place in the sex factor of *E. coli*. This fact, together with the occurrence of loci having high affinity for sex factors, necessitates some innovations in terminology: separate designations are needed for genetically different sex factors; for chromosomal loci having special affinity for sex factors; and for bacterial strains possessing either or both.

We propose that sex factors with recognizable genetic differences simply be numbered, as is now the practice with temperate phages having different points of prophage attachment to the chromosome (Jacob and Wollman, 1956). We also propose that chromosomal loci exhibiting strong affinity for the sex factor be designated *sfa* (for "sex factor affinity") with a subnumber. The locus in strain AB-91, since it arose simultaneously with F^2, will be designated *sfa-2*.

The designation of bacterial strains carrying different sex factors can be made analogous to the designation of strains carrying different prophages, in which the prophage symbol is placed in parentheses following the strain number. For example, K-12(λ) represents strain K-12 carrying the *lambda* prophage. With respect to sex factor, the F^+ strain of K-12 as isolated from nature can be designated K-12(F^1); acridine treatment may yield K-12(F^-); and introduction of F^2 into the latter may yield K-12(F^2).

The possession of an *sfa* locus does not create any special problems of terminology, since it is already customary simply to give new strain numbers to recombinants or mutants. Thus, strains P4x-1 and AB-91 include in their genotype the loci met^- and *sfa-2*.

There is one further problem of terminology, and that is to reconsider the designations of different types of genetic donors. Until now, only two types have been recognized: F^+ and Hfr. F^+ strains were characterized by possession of autonomous sex factor, randomly oriented chromosome transfer, and low frequency of recombination. Hfr strains were characterized by possession of chromosomal sex factor, uniformly oriented chromosome transfer, and high frequency of recombination. We now see, however, that genetic donors can differ from one another in several different properties of the included sex factor and of the chromosome, all of which can presumably vary independently. Some of these properties are listed in table 6, in which a few known types of donors are represented along with their characteristic behaviors. As shown, any given donor type may be characterized in terms of the fraction of the population acting as donors, the uniformity of chromosome orientation during transfer, and the relative frequency of recombination which results. In addition, dif-

TABLE 6

Some factors determining sexuality in Escherichia coli

Designation of Cell Population			State and Properties of Sex Factor (F)				Properties of Chromosome		Characteristics of Population with Respect to Chromosome Transfer		
Type	No.	Prototype strain	Location and transmission	Probability of attachment to chromosome	Preference for site of attachment	Stability of attachment to chromosome	*sfa* loci present	Origin present	Fraction of population acting as donors	Uniformity of chromosome orientation among donor cells	Relative frequency of recombination[a]
Hfr	—	HfrC[b]	Chromosomal locus	—	—	Stable	—	Yes	All	Uniform	Very high
F^+	I	K-12	Autonomous population	Low	None	—	None	No	Very small	Entire spectrum of types present	Very low
	II	P4x-1	Autonomous population	High	One preferred site	Unstable (?)	One (homologous with F^2)	Yes	Majority	Uniform	Relatively high
	III	AB-1	Autonomous population	High	One preferred site	Unstable (?)	None	Yes	Majority	Uniform	Relatively high
	IV	AB-89(F^2)	Autonomous population	High	One preferred site	Unstable (?)	None	No	Majority	Uniform	Intermediate
	V	AB-91(F^1)	Autonomous population	High	None	Unstable (?)	One	Yes	Majority	Uniform	Relatively high
F^-	I	Y-10[c]	Absent	—	—	—	None	No	None	—	—
	II	♀3[d]	Absent	—	—	—	One	Yes	None	—	—
	III	AB-122	Absent	—	—	—	One	No	None	—	—
	IV	AB-113	Absent	—	—	—	None	Yes	None	—	—

[a] With respect to marker transferred at highest frequency.
[b] Cavalli (1950)
[c] Lederberg *et al.* (1952).
[d] Richter (1957).

ferent types of donors will show different recombination kinetics.

In view of the extremely large number of predictable types of genetic donors, any attempt to give each a name would be futile. The term "Hfr," although frequency of recombination itself is no longer a useful criterion, can still be reserved for donors in which the sex factor is present only as an attached element. The term "F^+," however, if it is to have any meaning at all, can only be used to indicate that the strain harbors an autonomous, transmissible F. It thus becomes a general class designation, and includes a great many clearly distinguishable subtypes. In table 6 we have assigned arbitrary numerals to a few of the known types of F^+ strains, as some sort of notation is convenient for ease of reference. The important point, however, is to recognize that "F^+" is only a generic term and is by itself inadequate to describe the genetic donor behavior of any strain.

Table 6 also shows that F^- strains may also vary in several sexual properties, which are only exhibited when the strain acquires sex factor from a donor. The difference between AB-89(F^-) and AB-91(F^-) has already been shown in tables 3 and 4; the presence of the *sfa-2* locus in AB-91, but not in AB-89, has been discussed. Furthermore, F^- strains may differ in at least one other respect; namely, the presence of origin. We have taken a virgin F^- strain and produced from it a series of F^- recombinants by crossing it with an Hfr, selecting from the latter a marker close to origin. When the virgin and recombinant F^- strains are converted to maleness with a particular sex factor, the latter acquire a significantly higher fertility as genetic donors. This difference is attributable to their incorporation of the origin of the parental Hfr. Thus, at least four types of F^- strains exist, and a much larger number is predictable in view of the possibility of constructing F^- recombinants possessing two or more *sfa* loci.

Variant F^+ strains of type II, of which P4x-1 is the prototype, are apparently of common occurrence. Hirota (1959) having been apprised of the existence of F^2, has screened a large collection of Hfr strains and has found variant clones in most of them and in each case, the F particle has acquired an affinity for the specific point at which it had been attached to the chromosome. Jacob (*personal communication*) has discovered another series of such strains derived from the Hfr cultures in the collection at the Pasteur Institute at Paris.

The possibility of genetic exchange between sex factor and chromosome gives further unity to the episome concept. The known acquisition of the *gal* locus by λ-prophage (Morse *et al.*, 1956) suggests the possibility of a recombinant sex factor acquiring an entire functional locus from the chromosome. This possibility has recently been realized, and is reported elsewhere (Jacob and Adelberg, 1959).

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SUMMARY

It has been shown that genetic recombination occurs between attached sex factor (F) and chromosome in Hfr cells of *Escherichia coli*. Reciprocal exchange is evidenced by the changed properties of both partners: the sex factor acquires a high and specific affinity for the chromosomal site at which it had been attached, while returning to the autonomous state; the chromosome acquires an affinity at that site for either wild-type or recombinant sex factor.

Operationally, the recombinant sex factor is recognized by the fact that it is transmissible to F^- cells as an extrachromosomal particle, converting the recipient cells to donors capable of transferring their chromosome with a specific orientation and with a relatively high frequency. The clone derived from the cell in which the recombination took place can be converted to F^- by treatment with acridine orange. On introduc-

tion of the wild-type F particle of strain K-12, these cells become high frequency donors, unlike ordinary F^- cells which are convertible by wild-type F only to low frequency donors. This property reflects the presence of the *sfa* ("sex factor affinity") locus on the chromosome.

An appropriate terminology is proposed for dealing with genetically varying sex factors, recombinant loci, and strains of bacteria carrying either or both.

REFERENCES

ADELBERG, E. A. AND BURNS, S. N. 1959 A variant sex factor in *Escherichia coli*. Records Genet. Soc. Am., **28**, 57.

CAVALLI, L. L. 1950 La sessualita nei batteri. Boll. ist. sieroterap. Milan, **29**, 1–9.

HAYES, W. 1957 The kinetics of the mating process in *Escherichia coli*. J. Gen. Microbiol., **16**, 97–119.

HIROTA, Y. 1959 Mutants of the F factor in *Escherichia coli* K-12. Records Genet. Soc. Am., **28**, 75.

HIROTA, Y. AND IIJIMA, T. 1957 Acriflavin as an effective agent for eliminating F factor in *Escherichia coli* K-12. Nature, **180**, 655–656.

JACOB, F. AND ADELBERG, E. A. 1959 Transfert de charactères génétiques par incorporation au facteur sexuel d'*Escherichia coli*. Compt. rend., **249**, 189–191.

JACOB, F. AND WOLLMAN, E. L. 1956 Sur les processus de conjugaison et de recombinaison chez *Escherichia coli*. I. L'induction par conjugaison ou induction zygotique. Ann. inst. Pasteur, **91**, 486–510.

JACOB, F. AND WOLLMAN, E. L. 1957 Analyse des groupes de liaison génétique de différentes souches donatrices d'*Escherichia coli* K12. Compt. rend., **245**, 1840–1843.

JACOB, F. AND WOLLMAN, E. L. 1958 Les épisomes, éléments génétiques ajoutés. Compt. rend., **247**, 154–156.

LEDERBERG, J. AND LEDERBERG, E. M. 1952 Replica plating and indirect selection of bacterial mutants. J. Bacteriol., **63**, 399–406.

LEDERBERG, J., CAVALLI, L. L., AND LEDERBERG, E. M. 1952 Sex compatibility in *Escherichia coli*. Genetics, **37**, 720–730.

LURIA, S. E. AND BURROUS, J. W. 1957 Hybridization between *Escherichia coli* and *Shigella*. J. Bacteriol., **74**, 461–476.

MONOD, J., COHEN-BAZIRE, G., AND COHN, M. 1951 Sur la biosynthese de la β-galactosidase (lactase) chez *Escherichia coli*. La specificite de l'induction. Biochim. et Biophys. Acta, **7**, 585–599.

MORSE, M. L., LEDERBERG, E. M. AND LEDERBERG, J. 1956 Transductional heterogenotes in *Escherichia coli*. Genetics, **41**, 758–779.

RICHTER, A. 1957 Complementary determinants of an Hfr phenotype in *E. coli* K-12. Genetics, **42**, 391.

WOLLMAN, E. L. AND JACOB, F. 1955 Sur le mécanisme du transfert de material génétique au cours de la recombinaison chez *Escherichia coli*. Compt. rend., **240**, 2449–2451.